THE PALE GREEN MUTABLE SYSTEM IN MAIZE[1]

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IN 1938 (and 1941) RHOADES reported the case of a mutational event controlled by a specific intranuclear factor (the Dotted gene)[3] which causes the recessive a_1 allele to mutate to the dominant form A_1. Since then additional evidence for specific genic controllers of mutation has been reported by MCCLINTOCK (1950, 1951) and BRINK and NILAN (1952). Their work has shown that mutable genes are governed by accessory factors. These same factors are readily transferable to various locations in the genome and are able to regulate the time and rate of gene mutation. It was further shown that these factors of mutable systems can, when transposed, govern the action and mutability of other loci (MCCLINTOCK 1953).

The current report is an analysis of the pale green mutable gene system and its various components. This system is similar in many ways to previously described systems and, in addition, there is evidence for an even closer relationship of component parts. An abstract of the material in this report has been presented by PETERSON (1953).

MATERIALS AND METHODS

Pale green mutable (pg^m) was found by E. G. ANDERSON among material exposed to the Bikini A-bomb. Mutant plants from segregating ears were crossed to standard inbreds. These F_1 plants were both selfed and outcrossed to standard inbreds again. Such recurrent outcrossing was practiced successively for a number of generations.

Two distinct mutant types, pg^m and pg^s, appear among the F_2 progeny of pale green mutable outcrosses (Figures 1 and 2).

These pg^m seedlings are characterized by numerous dark green stripes on a pale green background. As will be shown later, these stripes represent mutations of pale green to green ($pg \rightarrow Pg$).

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[3] A number of abbreviations used in the text are described:

Dt—Dotted gene that causes the a_1 allele (colorless aleurone) to mutate to A_1 (colored aleurone).

Ac—Activator described by MCCLINTOCK that causes *Ds* (the Dissociator locus) to change.

Spm—Suppressor-mutator described by MCCLINTOCK that suppresses the action of a locus such as a_1 and also causes it to mutate.

v—Refers to the variegated flower locus in Nicotiana.

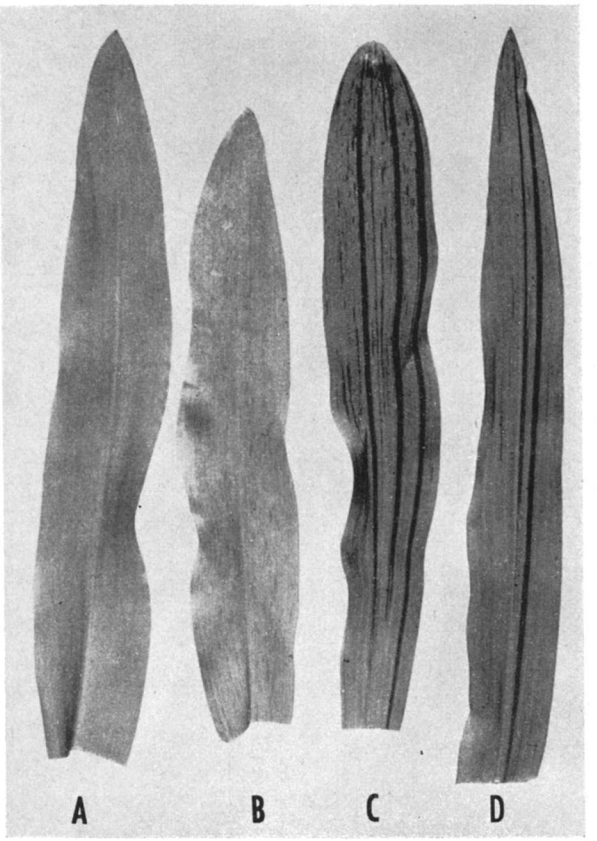

FIGURE 1.—Seedling leaves of mutant plants. A.—*pg*^{s}; B, C and D—*pg*^{m}; B.—very late occurring mutant stripes; C.—early and late occurring mutant stripes; D.—early occurring mutant stripes.

There are various gradations of variegation (Figures 1B, 1C, and 1D) which depend upon the time of the mutation event (early or late in plant development). In most cases these differing patterns are a function of the inherited properties of the allele. In general, intercrosses of early and late pattern types show the former to be dominant. Mutable seedlings grow vigorously in a sand bench and in the field, some reach maturity and produce pollen and ears.

The *pg*^{s} seedlings represent the stable-type pale green allele and possess only an occasional stripe or sector of mutability (Figures 1 and 2). *pg*^{s} plants grow as vigorously as *pg*^{m}.

RESULTS AND DISCUSSION

The three classes of pale green segregating F_2 progenies

The two phenotypes, *pg*^{s} and *pg*^{m}, appear together with the normal green plants in three characteristic ratios in segregating F_2 progenies of outcrosses of

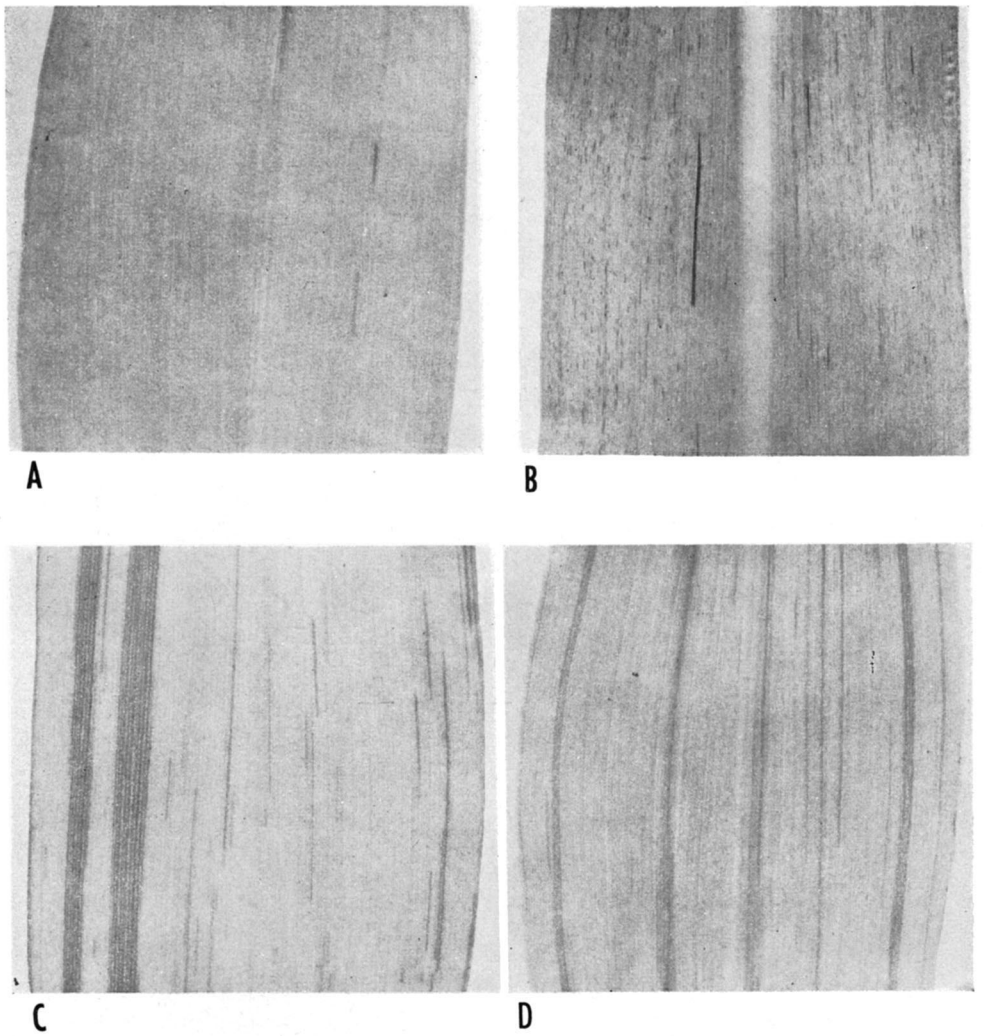

FIGURE 2.—Close-up photographs of mutant seedlings. A.—pg^s with sector of mutability. B, C, and D—pg^m; B.—very late occurring mutant stripes; C.—early and late occurring; D.—early occurring mutant stripes.

heterozygous mutable ($+/pg^m$) plants. These F_2 progenies represent three different classes—the m. m and s, and s (Table 1).

These class designations are based on the proportion of pale green seedlings that are stable. The three classes are as follows:

s = 3 green : 1 pg^s

m and s = 12 green : 3 pg^m : 1 pg^s

m = 3 green : 1 pg^m + low frequency of pg^s

Plants from these three types of F_2 progenies were used in further crosses.

The data to be presented in this paper support the hypothesis that the mutable

TABLE 1

F_2 progenies (1957 527 and 1957 528) arising from the outcross of an autonomous pg^m *allele to a standard line showing the segregation of* pg *that distinguishes m, m and s, and s classes*

Standard × 1956 464–3 → 1957 527; Standard × 1956 464–32 → 1957 528; +/+ × +/pg^m → selfed *pg*-segregating progeny

1957	*Pg*	Total *pg*	pg^s	pg^m	Classes s	m and s	m
527–3 ⊗	69	18	0	18	..	..	1
–4 ⊗	138	37	0	37	..	..	1
–33⊗	134	37	0	37	..	..	1
–34⊗	113	33	2	31	..	..	1
–36⊗	117	28	0	28	..	..	1
–37⊗	122	43	9	34	..	1	..
–60⊗	154	50	3	47	..	..	1
–62⊗	141	46	2	44	..	..	1
–63⊗	59	30	0	30	..	..	1
–64⊗	89	20	1	19	..	..	1
–65⊗	188	42	2	40	..	..	1
–67⊗	91	35	0	35	..	..	1
–71⊗	81	26	0	26	..	..	1
Total	1496	445			0	1	12
Total = 1941		in m classes	10	432			
Percent *pg* = 22.92							

1957	*Pg*	Total *pg*	pg^s	pg^m	Classes s	m and s	m
528–6 ⊗	106	14	0	14	..	..	1
–7 ⊗	64	20	0	20	..	..	1
–8 ⊗	183	34	2	32	..	..	1
–9 ⊗	94	15	0	15	..	..	1
–10⊗	81	19	0	19	..	..	1
–12⊗	59	16	4	12	..	1	..
–13⊗	245	44	10	34	..	1	..
–17⊗	81	14	0	14	..	..	1
–18⊗	82	23	0	23	..	..	1
–26⊗	130	11	0	11	..	..	1
–27⊗	91	9	2	7	..	1	..
–28⊗	78	20	6	14	..	1	..
–29⊗	76	21	0	21	..	..	1
–30⊗	66	23	4	19	..	1	..
–33⊗	115	26	1	25	..	..	1
–36⊗	67	21	21	0	1	..	..
–61⊗	92	15	0	15	..	..	1
–62⊗	210	66	2	64	..	..	1
–65⊗	193	41	11	30	..	1	..
–66⊗	352	39	4	36	..	..	1
–68⊗	128	38	0	38	..	..	1
–69⊗	139	35	0	35	..	..	1
–71⊗	137	45	0	45	..	..	1
–72⊗	137	26	0	26	..	..	1
Total	3006	635			1	6	17
Total = 3641		in m classes	9	453			
Percent *pg* = 17.4							

Pg = green
pg^s = stable pale green
pg^m = mutable pale green
⊗ = selfed

pg system consists of two accessory components. One component, *I*, inhibits the expression of the normal green allele, *Pg*, and causes the pale phenotype to be expressed. The removal of *I* (the mutation event) is controlled by a second factor, Enhancer (*En*), that can be variously located; at the *pg* locus where control is autonomous (m class) and on chromosomes independent of *pg* (m and s class). In the absence of *En*, the *pg* seedlings are stable.

The m and s class: F_2 populations segregating 12 green : 3 pg^m : 1 pg^s, arose from two types of outcrosses: from green plants heterozygous for pg^m (Table 1, 6) and from some homozygous mutable plants (Table 2). (G families listed in Table 2 contain only green seedlings and arise from the mutation of *pg* to *Pg*.) Such a modified two-pair ratio indicates that a factor controlling the mutability of *pg* is assorting independently—when present, *pg* is mutable, when absent, the allele is stable (pg^s). This is similar to the two unit interaction of the Dotted gene (*Dt*) on a_1 (RHOADES 1938, 1941) and of *Ac* on *Ds*—controlled loci (MCCLINTOCK 1950, 1951). In view of its ability to make *pg* mutable, this factor is called Enhancer (*En*). Thus, the F_1 genotype that gave rise to the m and s type class is designated *En*/+, +/*pg*.

When these F_1 plants are further outcrossed to normal lines and segregating families subsequently obtained, two classes of *pg* segregating progenies appear—s, and m and s, which occur in a 1:1 ratio ('57 805–10 and 806–18 in Table 3). This ratio shows that one half of the *pg* containing gametes of the F_1 plants possessed *En* and one half were without *En*.

Further confirmation of the presence of such a factor assorting independently of *pg* is obtained by F_3 tests. Results of these crosses (Table 4) confirm the fact that there are two units in this mutable system, the *pg* allele and *En* which when present causes *pg* to be mutable. The progenies of these F_3 tests segregate as fol-

TABLE 2

Summary of F_2 progenies resulting from the outcross to various inbred lines of two mutable plants homozygous for independent En

Pollen parent	F_1 family	Classes of F_2 progenies†			
		G	s	m and s	m
1950 43–54 pg^m/pg^m	51 199	0	0	2	0
	51 226	1	0	2	0
	51 229	3	0	18	2
1950 43–57 pg^m/pg^m	51 200	1	0	1	0
	51 202	2	0	6	1
	51 696	1	0	5	1*
	51 418	10	0	17	0
	Total	18	0	51	4

* This m type was outcrossed and tested further as 1957 530–4 and 530–5 (Table 6).

† The three class designations are based on the proportion of pale green seedlings that are stable from selfed progeny.
s = 3 green : 1 pg^s
m and s = 12 green : 3 pg^m : 1 pg^s
m = 3 green : 1 pg^m : with a low frequency of pg^s
G families contain only green seedlings and arise from the mutation of *pg* to *Pg*.

TABLE 3

Summary of F_2 progenies resulting from outcrosses of plants that give m and s, and s progenies to standard inbreds

Pollen parent Plant	Segregation from self $Pg : pg$	$(pg^s : pg^m)$	Class	F_1 family	G	Classes of F_2 progenies s	m and s	m
Outcross of m and s								
1957 805–10⊗	146 : 31	8 : 23	m and s	1958 1260	29	14	9	0
1957 806–18⊗	107 : 30	9 : 21	m and s	1958 1267	14	7	9	0
Outcross of s								
1957g 9–16⊗	81 : 17	17 : 0	s	1957 804	24	20	0	0

TABLE 4

F_3 progenies obtained from the self-pollination of F_2 green plants occurring in m and s F_2 progenies

F_2 families	Types of pale green segregating among the progeny Only pg^s (++)	pg^m and pg^s (*En* +)	Only pg^m (*En En*)	Total
1951 97–100, 273–274	10	15	7	32
1952 292A	5	8	2	15
1956 431–432	7	12	7	26
	—	—	—	
	22	35	16	

lows: ¼ only pg^m (*En En*):½ ¾ pg^m to ¼ pg^s (*En* +):and ¼ only pg^s (++).

The stable type progenies that give only stable seedlings can become mutable when crossed to *En* bearing stocks (Table 7). *En* has no obvious effect in the absence of *pg*. Furthermore, in appropriate tests *En* was found to be ineffective in activating *Ac* or *Dt*–controlled loci.

Proof for the mutation event—pale green to green—is obtained from the outcross of homozygous mutable plants to inbred lines (Table 2). Among the 73 F_2 progenies, there were 18 not segregating *pg*. These possess the *Pg* allele, a result of the *pg* to *Pg* mutation, and remain stable. The high proportion of *Pg* alleles (18/73) from pg^m/pg^m plants arises from early mutation which in some cases would include the sporogenous tissues. Appropriate genetic markers have been used in these crosses to rule out the possibility of contamination.

The s class: The s class comprises the F_2 progenies that segregate 3 green to 1 pg^s (Table 5). That *pg* is a simply inherited recessive is indicated by the 25 percent of *pg* plants. This s class is observed as an exceptional F_2 progeny type derived from the outcross of $+/pg^m$ plants (Tables 1 and 6). In further outcrosses of such types (57g 9–16 in Table 3) only s type progenies result.

Seedlings of the s class lack *En*. Some pg^m seedlings (1/512) do arise among s type F_2 progenies. This suggests a reversion to the mutable form (Figures 2A and 3). The significance of this event will be discussed in a later section.

The m class—the autonomous Enhancer: In contrast to the above mentioned

TABLE 5

Segregation of pg *seedlings among F_2 progenies of the s class*

	Pg	pg^s	pg^m	Total	Percent *pg*
1951–1952 families	8590	2930	7	11,520	25.5
1957 families	1878	657	0	2,535	25.9
Total		3587	7		

$pg^m = 1/512 = 0.19$ percent.

TABLE 6

The classes of F_2 progenies arising from the first and second generation outcrosses of m-type plants to standard inbreds

Source of pg^m $+/pg^m$	Segregation from self of $+/pg^m$ *Pg*:*pg*	(pg^s:pg^m)	F_2 progeny family	G	Classes of F_2 progenies s	m and s	m*	Total	Freq. of non-m type
1st generation outcrosses 1957									
1956 464–3	175:39	3:36	527	26	0	1	12[a]	13	1
1956 464–32	103:26	0:26	528	18	1	6	17[b]	24	7
1956 464–59	74:15	0:14	529	15	0	2	14[c]	16	2
1956 474–10	146:22	0:22	530	26	2	3	14[d]	19	5
1956 476–16	72:17	0:17	531	15	0	0	8	8	0
1956 464–35	50:20	0:20	535	28	0	3	11	14	3
1956 465–1	126:20	0:20	536	23	1	2	6	9	3
1956 g9–19	70:17	0:17	803	25	0	5	11	16	5
1956 g9–12	89:24	0:24	805	54	6	24	29[e]	59	30
1956 g9–7	84:21	0:21	806	59	6	16	25	47	22
					16	62	147	225	
				Percent	7.11	27.56	65.33		
				Percent expected with 3 *En*	12.56	37.50	50		
2nd generation outcrosses 1958									
1957 527–4	138:37	0:37	1269	23	6	8	11	25	14
1957 527–33	134:37	0:37	1270	30	1	8	11	20	9
1957 527–36	117:28	0:28	1272	29	1	7	9	17	8
1957 528–6	106:14	0:14	1273	37	2	6	12	20	8
1957 528–10	81:19	0:19	1275	33	2	2	9	13	4
1957 528–17	81:14	0:14	1277	16	2	5	7	14	7
1957 528–29	76:21	0:21	1279	20	1	4	18	23	5
1957 528–33	115:26	1:25	1281	25	5	5	12	22	10
1957 529–1	169:30	0:30	1282	17	2	3	8	13	5
1957 529–7	162:21	0:21	1283	26	1	1	9	11	2
1957 529–30	105:17	0:17	1284	34	1	1	13	15	2
					24	50	119	193	
				Percent	12.4	25.9	61.7		
				Percent expected with 3 *En*	12.50	37.50	50		

* Some of the m types of this family were retested and gave rise to a-1269–72, b-1273–81, c-1282–84, d-1285–86 and e-1258–63.

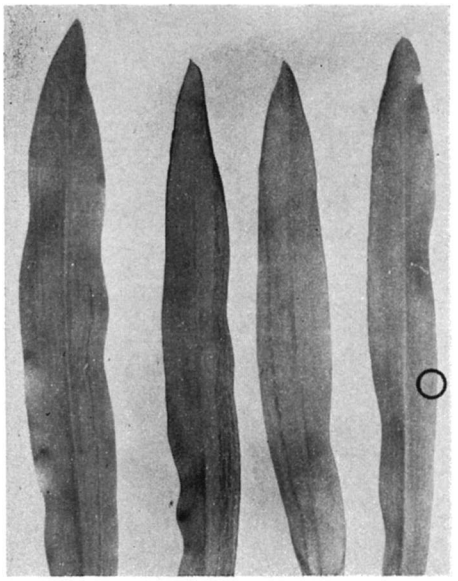

FIGURE 3.—pg^s seedlings which contain some stripes and sectors of mutability. A faint individual stripe that is commonly found in pg^s seedling leaves is circled.

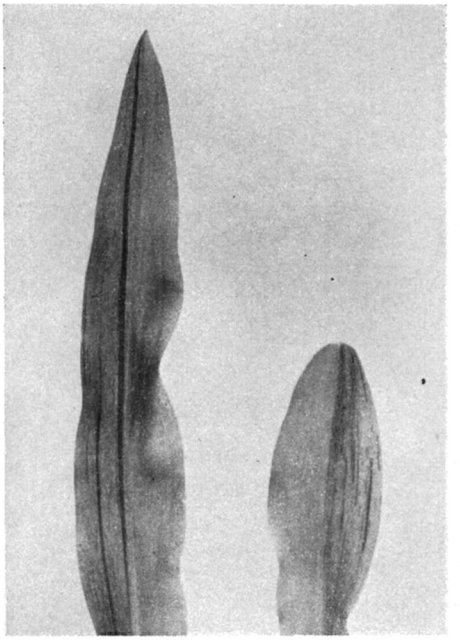

FIGURE 4.—Stable sectors in the leaves of pg^m plants; these may represent the somatic loss of *En*.

s, and m and s type F_2 progenies, a third—the m type—was most frequent (Tables 1 and 6). In these m type F_2 progenies all the pale green seedlings are mutable except for a small percentage (0–10 percent) of stables. Two such families (527 and 528 of Table 6) are shown in Table 1. The m class predominates among the F_2 progenies of second generation outcrosses of m type plants and continues to give rise to some s and m and s type F_2 progenies. In further tests these exceptional types continue to behave as s, and m and s types (Table 3).

The simplest explanation of why in the m class all the *pg* seedlings are mutable is that *En* is located next to the *pg* locus or is a component of it so that mutability is apparently autonomously controlled (designated $\widehat{pgEn}$). The *En* element at the *pg* locus resembles the independent *En* since both control mutability although they are located in different positions.

From the data in Table 6 it can be concluded that the F_2 progenies from outcrossed mutable plants, whose mutability is controlled by autonomous *En*, are predominately m type although s, and m and s type progenies also arise. The frequency of these latter types is interpreted as indicative of a high rate of change from $\widehat{pgEn}$ (m class) to *pg* with independent *En* (m and s class) and to *pg* with no *En* (s class). This is significant since it relates the independent *En* to the autonomous.

There is an alternative that should be considered in attempting to explain the

m class and the origin of the resulting outcross derivatives. Could the low frequency of stables among m type F_2 progeny be explained by the segregation of more than one independently assorting Enhancer as was found by McCLINTOCK (1956) for *Spm*? In the self of a heterozygote for *pg* and *En* (*pg*/+, *En*/+), a 3 mutable to 1 stable ratio is obtained among the pale green seedlings due to the segregation of *En*. If *En* is present in duplicate (*En* at two independent locations) $En_1/+$, $En_2/+$ the frequency of non-*En* pale green or stable seedlings is 1/16. In the triplicate condition, $En_1/+$, $En_2/+$, $En_3/+$, only 1/64 of the resulting pale green seedlings would lack an *En* and be stable. Therefore, the frequency of pg^s under the influence of many Enhancers would simulate the m type progeny and could not under the present crossing procedure be readily recognized in an F_2 progeny.

In order to distinguish between this possibility and the hypothesis of highly transposable autonomous *En*, a recurrent outcross program was begun to test the independent *En* composition of heterozygous *pg* plants (+/*pg*). After one generation of outcrossing to inbreds, heterozygous plants (+/pg^m) that yield m type F_2 progeny were also outcrossed to standard lines and F_2 progenies were subsequently obtained. These F_2 progenies were then classified as s, m and s, and m (Table 6-second generation outcrosses). If *En* were present in duplicate, the outcross of such a genotype to a standard inbred ($En_1/+$, $En_2/+ \times +/+$, $+/+$) with regard only to *En*, would yield F_2 ears in the following ratio: ¼ s (no *En*): ½ m and s (1 *En*): ¼ m (2 *En*). If the m class were due to the presence of *En* in the triplicate condition ($En_1/+$, $En_2/+$, $En_3/+$), the outcross of such a genotype would yield 1/8 +/+, +/+, +/+; 3/8 *En*/+, +/+, +/+; 3/8 *En*/+, *En*/+, +/+ and 1/8 *En*/+, *En*/+, *En*/+. Since the latter two genotypes would yield 1/16 and 1/64 pg^s seedlings respectively, their progeny are grouped into the m class. This results in a combined ratio of 1/8 s: 3/8 m and s: 4/8 m.

The above ratios were not obtained in the progeny testing of m type parents (Table 6). In the first generation outcrosses some parents such as 1956 464–3, 464–59 and 476–16, gave only a low frequency of non-m types (s, and m and s). Half the gametes of 1956g 9–12 and 9–7 (Table 6) were of the non-m type. In the second generation outcrosses, 1957 plants 528–29, 529–7 and 529–30 gave low frequencies of non-m type F_2 progenies while the other plants gave a higher rate. The general heterogeneity of the data with respect to the frequencies of s, and m and s derived progenies of the different plants may be complicated by the presence of nonautonomous *En* on the same chromosome but not adjacent to *pg*. In any case the expected ratios of the three classes based on the presence of three *En* are given in Table 6 for each generation of outcrossing. These expectations do not agree with the results obtained. It is also obvious that they do not fit a 1 s:2 m and s:1 m ratio which would be expected if *En* were present in two independent positions.

The alternatives can be further tested by comparing the frequency of expected pg^s with the observed in F_2 progeny if three *En* are assorting independently. It was previously mentioned that with three *En* (*En*/+, *En*/+, *En*/+) 4/8 of the F_2

ears should be of the m class. In this case 3/4 of the m class would be *En*/+, *En*/+ and would yield 6.25 percent stables and ¼ would be *En*/+, *En*/+, *En*/+ yielding 1.56 percent stables. The combined yield of stables would therefore be 5.07 percent [i.e., (¾ × 6.25% = 4.08%) + (¼ × 1.56% = 0.39%) = 5.07%]. Two families were chosen to test this. In family 1957 527 the observed occurrence of pg^s is ten [expected 22.6 (5.07% × 442)—P=0.02] while in 1957 528 the observed is nine [expected 22.9 (5.07% × 462)—P = 0.01] (Table 1). Again the observed frequency does not agree with the expected if *En* is assorting independently in triplicate.

Another test can be made to distinguish between the multiple *En* and autonomous *En* alternatives. The non-*pg* segregating plants arising from the outcross of +/$\widehat{pgEn}$ are crossed by a known pale green stable that responds in the presence of *En*. If the m class is due to autonomous *En*, the subsequent F_2 ears should yield only s type progenies. If, however, many *En* are present in the background, m, m and s, and s type classes should arise. The preliminary results from such crosses indicate that *En* must be autonomous since most of the F_2 progenies segregate only stables. This does not exclude the possibility that an occasional independent *En* is present in autonomous stocks. Extensive crosses are underway to test more fully the independent *En* composition of autonomous stocks.

From these experiments it is clear that the m class is not due to the presence of independently assorting Enhancers at two or three loci. The hypothesis that *En* is autonomous is considered to be valid. The occurrence of stables in a low frequency results when *En* moves from the *pg* locus. This transposition of *En* accounts for the appearance of the s, and m and s classes. The s class would result from the exclusion of *En* in a *pg* gamete while the m and s class would result from the inclusion in a gamete of *En*, independent of *pg*. Stable sectors occur somatically which may indicate the loss of *En* (Figure 4).

The possible presence of independent *En* in the background of some autonomous stocks makes difficult the determination of the exact rate of change of pg^m to pg^s. The best estimate can be obtained by analyzing the rate of change in the m class to the s, and m and s types (Table 6). In these tests there are 153 s, and m and s class ears in a total of 419. This rate of change (36.7 percent) is indicative of very high rate of transposition of *En* from the *pg* locus. The rates and direction of change are illustrated in Figure 5. This does not, of course, consider the effect of early mutations upon sporogenous tissue.

The rate of change of $\widehat{\text{pgEn}}$ *to* pg *and* pg + En: As stated above the frequency of s versus m and s type progenies would be difficult to predict if any independent *En* were present in the autonomous stocks. If the change of $\widehat{pgEn}$ to *pg* without *En* is simultaneous with its incorporation into some part of the genome, then one would expect a 3 s: 1 m and s ratio of the two classes; (the chance of the inclusion of transposed *En* with the *pg*-containing cell in somatic mitosis rather than with the $\widehat{pgEn}$-containing cell plus the additional chance of its noninclusion with *pg* during assortment in meiosis would lead to such an expected ratio). There is a higher proportion of the m and s class and this deviation from the expected 3 s : 1

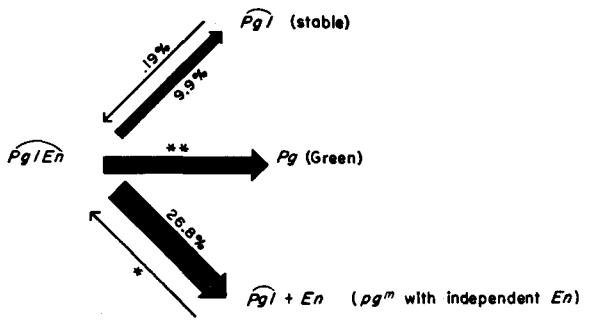

FIGURE 5.—The direction and relative rates of change of $\widehat{Pg/En}$ gametes.

* These exceptional progeny have not all been tested but the rate of change in this direction appears higher than the change from $\widehat{Pg/}$ to $\widehat{Pg/En}$.

** An absolute rate cannot be determined due to early mutations affecting sporogenous tissue.

m and s ratio (Table 6) may indicate that the transposition event occurs late in microsporogenesis so that *En* is included in the same cell with *pg*.

Dual position of En *and the nature of* pg^s

In a preceding section the pale green mutable controlled by an independent Enhancer was discussed. From this type plant, heterozygous both for *pg* and *En*, (*Pg/pg*, *En/+*) a stock segregating only mutable seedlings was obtained (*Pg/pg*, *En/En*). Further selfing yielded a stock not segregating *pg* but homozygous for *En*. This stock, (*Pg/Pg*, *En/En*) was used as an *En* tester and was maintained by selfing for a number of years.

In order to test the effect of this stock on different *pg^s* alleles, stable pale green plants were crossed to this *En* tester stock. If *En* will cause *pg^s* to mutate, all F_2 ears obtained from such crosses (*Pg/Pg*, *En/En* × *pg/pg*, +/+) should segregate 12 green: 3 *pg^m*: 1 *pg^s*, the ratio obtained from m and s type segregation. Out of 78 pale green segregating progenies from this cross, 71 were of the m and s type (Table 7).

Thus it is evident that a factor independent of the *pg* locus, but controlling its mutability, is present in this stock. In similar crosses of *pg^s* to unrelated genetic tester and agronomic lines the presence of *En* was never detected.

In addition to the m and s class, two of the F_2 progenies were of the s class. This suggests the loss of the *En* factor in some of the gametes of the original *Pg/Pg*, *En/En* parent. In further tests *En* proved to be absent.

The remaining five progenies segregated only a small percentage of *pg^s* (3.8 to 8.1 percent) among the pale green seedlings (Table 8). These ratios are characteristic of duplicate factor ratios (expected 6.25 percent); therefore *En* must be heterozygous at two different and independent locations. The self of such a plant (*En_1/+*, *En_2/+*) would yield a wide array of F_2 types in terms of the *En* factors (Table 9). When these F_2 types are crossed by *pg^s*, the resulting progenies should yield the following ratio with reference to the segregation of *pg^s* among the pale

TABLE 7

F_2 progenies arising from the cross of Pg/Pg,En/En × Pg/pg (*no* En)

	F_2 families			
1956	G	s	m and s	m
433	4	0	3	0
435	4	0	5	2
437	4	0	4	0
441	8	1*	8	0
442	10	0	7	0
443	7	1	10	2†
444	9	0	6	0
445	8	0	9	0
447	6	0	8	1
448	12	0	7	0
450	5	0	4	0
Total		2	71	5

* In further tests *En* found to be absent.
† In further tests two *En* found to be present.

TABLE 8

Exceptional progenies (see Table 7) from the cross Pg/Pg,En/En × Pg/pg^s, +/+

	Pg	*pg*	*pg^s*	*pg^m*	*pg^s*/total *pg* percent
435–5 ⊗	181	56	4	52	7.1
435–12⊗	137	37	3	34	8.1
443–10⊗	132	35	2	33	5.7*
443–13⊗	145	49	3	46	6.1
447–10⊗	131	26	1	25	3.8

* Tested by crossing by *pg^s*.

TABLE 9

Possible genotypes from the self of $En_1/+En_2/+$ *and expected segregation in crosses by* pg^s

Possible genotypes	Segregation in crosses by *pg^s*
1/16 $En_1/En_1, En_2/En_2$ 2/16 $En_1/En_1, En_2/+$ 2/16 $En_1/+/+, En_2/En_2$ 1/16 $En_1/En_1, +/+$ 1/16 $+/+, En_2/En_2$	7/16 would yield only mutable seedlings = Type A
4/16, $En_1/+, En_2/+$	4/16 would yield 3 *pg^m* : 1 *pg^s* = Type B
2/16 $En_1/+, +/+$ 2/16 $+/+, En_2/+$	4/16 would yield 1 *pg^m* : 1 *pg^s* = Type C
1/16 $+/+, +/+$	1/16 would yield only *pg^s* = Type D

Type	A	B	C	D	Total
Observed	11	10	11	4	36
Expected 7:4:4:1 ratio,	15.75	9	9	2.25	36 P = .35

green seedlings: 7/16–no pg^s: 4/16–1/4 pg^s: 4/16–1/2 pg^s: 1/16–all pg^s (Table 9). One of the F_2 progenies (1956 443–10) was tested by crossing a large number of F_2 green plants by pg^s and the ratio of pg^m:pg^s among the resulting progenies was determined. As is evident in Table 9, the actual values agree with those expected when two *En* are assorting independently. This second location may be coincident with the absence of *En* in some gametes (Table 7).

The cross of pg^s by an *En* tester stock also provides information about the nature of the pg^s allele. pg^s is stable in the absence of *En*, but in its presence, mutates from pale green to green at a high rate. Could pg^s be similar to the quasi-stable forms of some mutable systems (McCLINTOCK 1951) that respond to a high dosage of the mutator by causing mutations to occur very late? This lateness results in the absence of observable mutations and gives the appearance of a stable phenotype. Under this mode of action a decrease in the dosage of the mutator results in visible mutability, which is what would be expected in outcrosses of stables in the case of pg^s. However, in such outcrosses, pg^s remains stable. Furthermore, the cross of pg^s to an *En* stock would only accentuate the stable condition among the progeny, if pg^s is due to high dosage of *En*. It does result, however, in mutability and supports the hypothesis that pg^s is due to the absence of *En*.

The pg^{mo} *allele*

Among 22 F_2 ears arising from the outcross of one mutable plant, 21 yielded the usual type of mutable seedlings but the other F_2 ear segregated a strikingly different, mottled-type seedling (Figures 6 and 7). Upon further testing this mutant was found to be allelic to *pg* and has been designated pg^{mo}. The seedlings of pg^{mo} contain very heavy, indistinctly outlined mutant stripes. These contrast with the clear-cut, straight line stripes that are characteristic of pg^m seedlings. The change from pg^{mo} to *Pg* (green areas) causes considerable deviation in the expected 3 *Pg*: 1 pg^{mo} F_2 segregation (Table 10).

In order to test the inheritance pattern of the pg^{mo} allele, pg^{mo} was outcrossed to standard lines and 19 F_2 progenies were obtained. All the pale green seedlings of these F_2 progenies were pg^{mo} which indicates that the control of mutability resides at the *pg* locus and is not determined by an independent factor. This allele was used in other tests.

The heterozygotes (pg^m/pg^{mo}) resulting from crosses of pg^{mo} to mutable plants express the pg^m pattern and show no evidence of the distinctive pg^{mo} type expression. In crosses of pg^{mo} to pg^s, however, all the seedlings are stable (Figure 6B). In the heterozygote, pg^{mo}/pg^s, the pg^s allele in some way completely inhibits the expression of the pg^{mo} allele.

A clue to this inhibition and to the hypothetical nature of the *pg* locus appears in crosses of pg^{mo} with the *En* tester stock. In the F_2 progeny of the cross (*Pg/Pg*, *En/En* × pg^{mo}/pg^{mo}, +/+) the expected ratio of green to pale green occurred, but among the pale green a ratio of 3 pg^s: 1 pg^{mo} resulted (Table 11). In this cross, only one *pg* allele, pg^{mo}, is being tested with *En*. The ratio of 3 stable : 1 pg^{mo} among the pale green seedlings clearly indicates that the *En* factor inhibits the

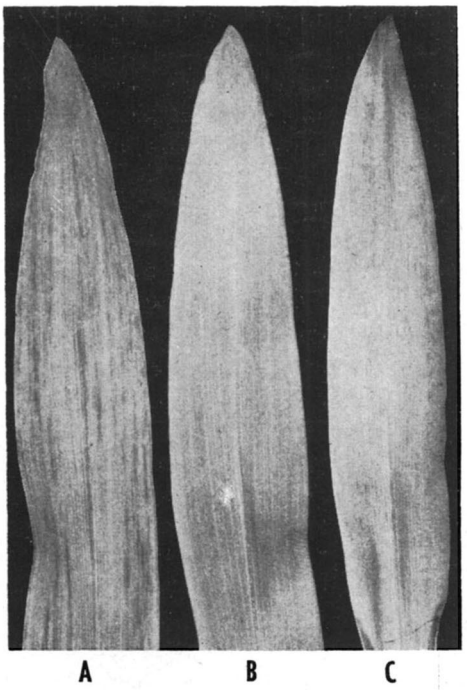

FIGURE 6.—*pg*-mutant seedings. A.—pg^{mo}. B.—pg^{mo} + *En* grown at 65°–70°F; C.—pg^{mo} + *En* grown at 95°F or above.

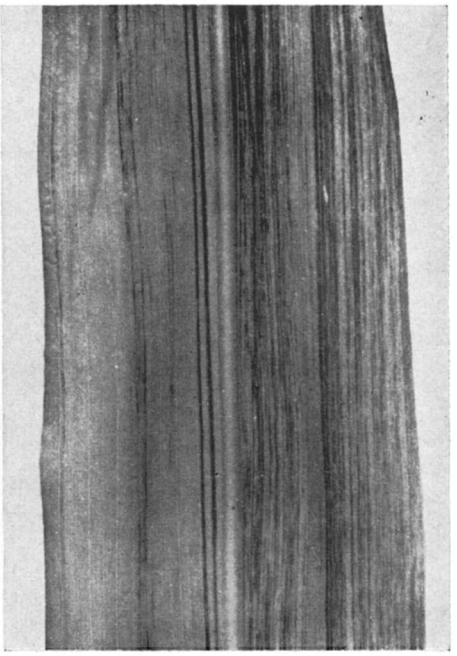

FIGURE 7.—Close-up view of a pg^{mo} seedling leaf.

TABLE 10

Some sample segregations in F_2 progenies arising from the outcross of pg^{mo} *to standard lines*

	Green	pg^{mo}
1956 559–1⊗	42	3
–2⊗	77	11
–3⊗	21	6
–6⊗	38	12

TABLE 11

F_2 progenies arising from the cross pg^{mo}/pg^{mo} *and* Pg/Pg,En/En

1957	*Pg*	*pg*	pg^s	pg^{mo}
567–6 ⊗	39	13	9	4
567–11⊗	48	10	8	2
569–5 ⊗	42	14	12	2
569–8 ⊗	64	18	13	5

expression of the pg^{mo} allele ($\frac{3}{4}En/-,pg^{mo}/pg^{mo}$) (Figure 6B), while in the absence of *En* it is fully expressed ($\frac{1}{4}$ +/+, pg^{mo}/pg^{mo}).

The inhibition of pg^{mo} expression by *En* is not absolute. In mature field plants or in seedlings that are grown at 95–110°F, very tiny late stripes appear in the leaves (Figure 6C). Thus under certain conditions, perhaps when *En* is affected, mutation can occur.

The pg *locus—a hypothesis*

It has been demonstrated that the mutation event, *pg* to *Pg*, is activated by a factor, Enhancer (*En*), that may be located at the *pg* locus or elsewhere in the chromosomal complement. In the presence of *En* mutable seedlings arise while in its absence the *pg* allele is stable. It has also been demonstrated that independent *En* arises by transposition from the *pg* locus. In addition, *En* could be present on more than one non-homologous chromosome.

It is assumed that the pale green mutant allele is an inhibited dominant green and that the mutation process is an uncovering-type event in which the inhibiting factor, *I*, is removed from its location at the *pg* locus. The removal of the inhibitor, which results in green tissue, is controlled by the Enhancer.

On the basis of these considerations the *pg* locus may be pictured as in Table 13.

There is some suggestion that *I* and *En* are more closely related than previously supposed. This is inferred from the observation of individual stripes and sectors of mutability in pg^s plants. These mutable sectors in stable seedlings (Figures 2A and 3) indicate that mutability has been induced at the otherwise stable locus. Such a possibility is strengthened by the isolation in stable stocks of a newly arisen mutable allele. In tests, this new mutable was found to be of the autonomous type. It follows, therefore, that in this case, *En* arose at the locus. Perhaps the *En* factor arises from the *I* factor that is associated with the pg^s allele.

New m type F_2 progenies have also originated in outcrosses of pg^m stocks containing independent *En*. Four m type F_2 progenies were observed in such outcrosses (Table 2) and two were used in further tests (Table 12). It is proposed that *En* arose from *I* material at the *pg* locus.

Other evidence supporting the relationship between *I* and *En* is seen in the

TABLE 12

Summary of F_2 progenies resulting from the outcross to various inbred lines of two m type progenies derived from the outcross of independent En

1957 source of pg^m	*pg* segregation of 1957 plant				1958 family	Classes of F_2 progenies				
	Pg	*pg*	pg^s	pg^m		G	s	m and s	m	Total
530–4	148 :	30	4	26	1285	17	2	3	5	10
530–5	118 :	27	3	24	1286	11	5	4	5	14

TABLE 13

The characteristics and hypothetical designation of each of the phenotypes associated with the pg *locus*

Seedling phenotypes	Genetic symbol	Hypothetical designation of the alleles	Nature of mutability
pale green stable	pg^s	*PgI*	few *PgI* → *Pg* somatic changes; *PgI* → *PgIEn* 1/512
pale green mutable	pg^m	$\widehat{PgIEn}$	Enhancer at the *pg* locus. Many $\widehat{PgIEn}$ → *Pg* somatic changes, $\widehat{PgIEn}$ → *PgI* and *PgI* + *En* in more than 35% of gametes
pale green mutable	pg^m	*PgI* + *En*	Enhancer at an independent location Many $\widehat{PgI}$ to *Pg* changes under influence of *En; PgI* to $\widehat{PgIEn}$ under influence of *En*
normal green	*Pg*	*Pg*	
pale green mottled	pg^{mo}	*PgI'*	Mutates to *Pg* autonomously

interaction effect of pg^s and *En* upon pg^{mo}. In the hybrid, pg^{mo}/pg^s, the seedlings are stable. The characteristic pg^{mo} expression is inhibited. Likewise, the independent Enhancer causes pg^{mo} to appear stable. Thus the *I* element at the pg^s locus and independent *En* act in a similar manner upon the pg^{mo} allele.

I and *En* therefore appear to be related although they differ in activity. *I* inhibits the expression of the normal allele (*Pg*) whereas *En* inactivates or causes the removal of *I*. Whether the difference in activity of the two elements is a question of position or chemical composition can only be conjectured.

The inhibitor of the pg^{mo} allele is designated *I'* and the reason is as follows: the pattern of the pg^{mo} allele (Figures 6 and 7), is suggestive of only partial inhibition of the normal green allele. The inhibiting effect of *I'* seems to be weaker than the effect of *I*. *I* and *I'* may differ only in the strength of their effect on *Pg*.

GENERAL DISCUSSION

Many properties not previously associated with genes have been found in analyzing mutable gene systems in corn. These include the transposition of gene elements from one position to another in the genome, the mutation event at a locus due to the removal of the inhibitor of dominant gene expression, the modification of gene activity, the control of the rates of the reversion process and the induction of mutability at previously stable loci.

Evidence for the transposition of factors, a feature not commonly exhibited by genetic units, comes from linkage studies (McCLINTOCK 1956) as well as from the observation in the *pg-En* and other systems that elements are relocated (a shift from autonomous to independent control of mutability). In addition, the different phenotypes of mutable pericarp represent another instance of a transposition event (BRINK and NILAN 1952; BRINK 1954).

McCLINTOCK (1956) and BRINK and NILAN (1952) and BRINK (1954) have presented critical evidence for the presence in mutable gene systems of particulate inhibitors that cause inhibition of the normal functioning of the gene. Twin sectors of altered phenotypes are interpreted as the result of simultaneous events involving both the loss of a factor (the inhibitor), and its transposition to a new location. These correlated events are instances of undeniable proof that mutation is an uncovering-type action and they attest to the concept of transposition of factors. The nature of the origin of twin sectors obviates any possibility that elements already present are activated.

Additional evidence for the inhibition phenomena comes from a study of the origin of new mutables. In most cases these new mutables arise from previous dominants. New mutables have arisen in *Ac* stocks and respond to *Ac* control (McCLINTOCK 1953).

One new mutable discovered in an *En* stock (PETERSON 1953, 1957), a_1^m, has now been found to be controlled by *En*, but it does not respond to *Ac* or *Dt*. This type of evidence shows that the transposable parts of a mutable system can become associated with other loci and, in addition, that the specificity of such units indicates an origin from an existing mutable system.

The mechanism of transposition is not readily analyzable. It is clear, however, that it is not associated with crossing over events since the mutation occurs in somatic tissue as well as in plants that are hypoploid (derived from crosses with B-translocation stocks—ROMAN 1947) for the chromosome bearing the mutant gene.

Among the various problems associated with mutable genes in corn, two are relevant to the following discussion and they deal with the two units of the mutable system. First, there is the problem of the control of gene action by an accessory element, an inhibitor, adjacent to the locus, and the second problem involves the mechanism of removal of this inhibitor.

With reference to the nature of the control of gene action, McCLINTOCK (1951) favors the view that the presence of heterochromatic elements interferes with the ordinary functioning of a gene without changing the gene itself. BRINK (1958) has recently advanced a modification of this concept in which he suggests that accessory elements interfere with the structural reproduction of the normal genetic units. The subsequent disarrangement of these genetic units would result in their malfunctioning. The proposal by SMITH and SAND (1957) that mutability at the *v* locus in Nicotiana is associated with cell heterochromatization coincides with the above mutable gene hypotheses. In the *Spm* system (McCLINTOCK 1956), inhibition of genic functioning is controlled from an independent position and is not caused as in other systems by the proximity of the inhibitor to the locus. Most evidence obtained from phenotypic reversions with mutable genes supports the view that there is an interference in gene function while the gene itself remains unaltered. This is obvious from the fact that removal of the accessory element results in the immediate normal expression of the

gene. The origin of new mutables suggests that the inhibiting factor is nonspecific and can interfere with the functioning of a great many loci.

In any interpretation of the characteristics of different mutable genes, consideration must be given to the origin and specificity of two–part mutable systems (such as a_1-*Dt*, a_1-*Spm*, *Ac-Ds*, *En-I*). The nature of the specificity is evident in a review of the numerous systems associated with the a_1 locus—RHOADES (1941); MCCLINTOCK (1951, 1956); NUFFER (1955); RICHARDSON (1956); PETERSON (1957). It is significant that although the locus and the phenotype are identical, removal of the specific inhibitor (*I* or *Ds*) is accomplished only by its specific controller (*En* or *Ac* respectively). Furthermore, in the case of the pale green mutable system *En* appears to arise in the presence of *I* and this may be a clue to the individual specificity of systems. Both the specificity of control and the origin of the second member of the system are reminiscent of antibody-antigen relationships.

The mechanism of removal of the inhibitor (*I* or *Ds*) by the activating element (*En*, *Ac*, *Spm* or *Dt*) is a subject of speculation. It appears to be influenced by changes in temperature, by the presence of B chromosomes and by the age of the tissue. The possibility that these elements (*En*, *Ac*, *Dt*) exert a chemical control on the cell environment (RHOADES 1941) is strengthened by two different types of experiments: (1) STADLER's (1944) failure to remove the inhibition with X-rays and (2) BRINK's (1959) recent demonstration that a physical association is not necessary to effect the mutation event.

In conclusion, it can be stated that mutation in the mutable gene systems studied involves the operation of two related units within the cell environment. The mechanism of their operation as well as the modifications they invoke within the cell remain speculative.

SUMMARY

A mutable pale green gene that appeared among the progeny of maize kernels exposed to irradiation at the Bikini tests is herein analyzed.

Two distinct seedling phenotypes, pg^m and pg^s, occur in addition to the normal green plants.

pg^m is the mutable form and is controlled by a factor, Enhancer (*En*), which may be located at the locus (autonomous control) or apart from the locus (independent control).

pg^s is the stable form and lacks *En*.

It is hypothesized that there are two components in the pale green mutable system: *I* (Inhibitor of genic activity) located at the *pg* locus, and *En*, which can be located in various positions, and which causes the removal of *I*.

The pattern of mutability closely parallels MCCLINTOCK's mutable gene scheme; inhibition of normal genic elements in no way structurally alters the elements themselves.

Numerous experiments which illustrate the relationship between *I* and *En* are

discussed as well as the transposition of these components and the induction of mutability at previously stable loci.

A new mutable, pg^{mo}, and its relationship to *En* is also analyzed.

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