MUTABLE R-NAVAJO ALLELES OF CYCLIC ORIGIN IN MAIZE

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ABSTRACT

The generation in cyclic fashion of 26 mutable *R*-Navajo (mR^{nj}) alleles in maize involved transposition of a non-specific repressor of gene action, Modulator (*Mp*), first away from, and then back to, the *R* locus represented by the *R*-Navajo (R^{nj}) allele on chromosome 10. The mR^{nj} alleles reconstituted in this way varied widely, and continuously, in mutability to R^{nj}—that is, in transposition of *Mp* away from the *R* locus, thus derepressing the R^{nj} gene. They were alike, or nearly so, however, in activating *Ds* chromosome breakage and in increasing the stability of variegated pericarp, another unstable compound allele comprising *Mp* conjoined with P^{rr} on chromosomal 1. These latter two phenomena are based primarily on loci elsewhere in the genome. It is postulated that the 26 reconstituted mR^{nj} alleles carry a common *Mp* which, however, is intercalated at a different site within each allele. Nucleotide sequence in the regions adjacent to *Mp* is assumed to determine the frequency with which a form of micro-nondisjunction occurs whereby *Mp* is released from a donor site. Transposition to a new site is interpreted in terms of a chromosome model that gives effect to nicking, or single strand breaks, occurring throughout the genome as a prerequisite to unwinding, strand separation, and replication, of the DNA double helix.

MODULATOR (*Mp*) is a transposable repressor of gene action in maize that was identified by BRINK and NILAN (1952) as a component of the unstable variegated pericarp allele, P^{vv}. It is combined in the latter with the stable gene, P^{rr}, conditioning colored pericarp and cob ($P^{rr}\,Mp = P^{vv}$). ORTON and BRINK (1966) showed that, after mutation to stable P^{rr} by transposition of *Mp* away from P^{vv}, the unstable variegated allele could be reconstituted by return of *Mp* to the *P* locus. A significant finding in the study was that, following such return of *Mp* to P^{rr}, only a few of the reconstituted unstable alleles corresponded to the original variegation factor; the majority of the reconstituted alleles conditioned new forms of variegation differing in intensity of the irregular colored striping on a colorless pericarp background.

The present investigation is a restudy of the latter phenomenon with a more favorable experimental system which, however, involves the same transposable element, *Mp*. In the current investigation, Modulator is a component of the unstable *R*-Navajo allele, at the *R* locus on chromosome 10, rather than of the variegated pericarp allele on chromosome 1. Application of the term "repressor" to Modulator in the present article is intended to imply only that *Mp* reduces

action of the gene at the locus at which it is present to, or toward, the null level, without reference to the nature of the underlying processes.

The previously reported properties of Modulator of particular significance in the present investigation may be summarized as follows:

1. *Mp* was recognized by BRINK and NILAN (1952) as a component of the long-known variegated pericarp allele. BARCLAY and BRINK (1954) showed that it corresponds to, and seemingly is identical with, Activator (*Ac*), isolated in another stock by McCLINTOCK (1951).

2. *Mp* represses action of the gene with which it is immediately conjoined, as observed in our laboratory at the *P*, *Wx*, and *R* loci.

3. Modulator is transposable from a given chromosome site to any one of many others. The new sites tend, however, to cluster near the donor locus (VAN SCHAIK and BRINK 1959).

4. The integrity of *Mp* usually is maintained when transposition occurs. KEDHARNATH and BRINK (1958) noted an apparent exception to this rule.

5. A transposed *Mp* in the genome reduces the frequency of *Mp* transpositions from the *P* locus about 60% in the case of medium variegated pericarp on W23 inbred background, thus resulting in the light variegated pericarp phenotype (BRINK 1954).

6. The Dissociation factor (*Ds*) conditioning chromosome breakage, identified by McCLINTOCK (1951) as a member of the *Ac-Ds* mutable allele system, provides a specific test for presence of *Mp*, apparently anywhere in the genome. *Ds* is sensitive also to *Mp* dosage changes.

7. Apparently, there are no sites in the genome to which *Mp* moves where it is undetectable by the usual methods. Supporting this conclusion is GREENBLATT's (1966; 1968) demonstration of equality between the number of transpositions of *Mp* away from the *P* locus and acquisition of *Mp* in parallel fashion elsewhere in the genome.

8. *Mp* is known to occur only at one or another chromosome site and not at all as a cytoplasmic component.

9. *Mp* may become fixed at a locus without undergoing any other detectable change.

10. Mutation of medium variegated pericarp frequently results in twinned sectors of red and light variegated. Light variegated carries the P^{vv} allele, and so is unchanged at the *P* locus; the red sector has lost *Mp* from the *P* locus, thus allowing expression of the P^{rr} gene. Significantly, a transposed *Mp* is found in both members of the twinned pair in about 2/3 of the cases. GREENBLATT and BRINK (1962) demonstrated by linkage tests that, for a given pair of such co-twins, the transposed *Mp* is in the same position in both members. They interpreted this fact to mean that the receptor site of *Mp* is in a chromosome segment which in a majority of cases has not yet divided into chromatids in that mitotic cycle. The receptor site in such cases varies from one twin pair to another.

11. Frequency of *Mp* transposition is under some degree of regional chromosome control. WILLIAMS and BRINK (1972) observed that when an unstable *R*-Navajo allele (mR^{nj}) was located in a K10 chromosome (which carries a

large, heterochromatic knob at least 35 crossover units distal to the *R* locus) mutations to R^{nj} occurred significantly more often than in otherwise closely comparable stocks lacking the knob, or in plants in which the knob was situated in the homologous chromosome.

12. A further aspect of transposable elements not based on the Modulator studies, but deemed to be of basic importance for interpreting the data on reconstituted mutable *R*-Navajo alleles to be presented later in this report, concerns the position of these elements with respect to the locus at which they reside. NELSON (1968) showed that a transposable element whose location in the genome can be stabilized by genetic means may occupy different sites within a given locus. Two of the mutants he studied, wx^{m-1} and wx^{m-6}, belonged to the *Ac-Ds* system (MCCLINTOCK 1951). Each carries the repressor, Dissociation (*Ds*), in conjunction with the gene *Wx* and, in the absence of *Ac*, behaves as a stable waxy mutant. In the presence of *Ac* in a particular form, states of both wx^{m-1} and wx^{m-6} may be selected that vary from very low to high frequencies of change to non-waxy (MCCLINTOCK, personal communication). A third waxy mutant, wx^{m-8}, belonged to the *Spm* system (MCCLINTOCK 1965). It is stable in the absence from the genome of *Spm*. NELSON demonstrated that intralocus recombination occurred, with low frequency, between these repressor elements and also between them and certain waxy mutants of other origins. Position of the repressor within the waxy locus was subterminal, and differed in each case.

It will be assumed that the above-mentioned properties of Modulator are general and apply equally as well to the element as a component of mutable *R*-Navajo alleles under consideration here.

Each of the 26 mutable *R*-Navajo (mR^{nj}) alleles with which the present study is concerned was derived from a different stable *R*-Navajo mutant that had originated previously from one foundation, mutable *R*-Navajo factor. Each mutation cycle thus begins with a common mR^{nj} allele and ends with an mR^{nj} allele; stable *R*-Navajo (R^{nj}) is the intermediate stage in the cycle. Reconstitution of mutable *R*-Navajo in this manner involves transposition of Modulator, first away from, and subsequently back to, the *R* locus, as illustrated in the diagram following in which (R^{nj} *Mp*) is equivalent to mR^{nj}.

($R^{nj}Mp$)

R^{nj} (1)	($R^{nj}Mp$) (1)
R^{nj} (2)	($R^{nj}Mp$) (2)
R^{nj} (3)	($R^{nj}Mp$) (3)
etc.	etc.

The immediate object of the present investigation was to determine the spectrum of phenotypic effects resulting from the 26 spontaneous returns of *Mp* to the *R* locus.

MATERIALS AND METHODS

Origin, composition, and phenotypic expression of mutable R-*Navajo:* The ancestral mutable *R*-Navajo allele from which the cyclic mutants used in the present study were descended was

isolated by I. M. GREENBLATT (personal communication) in a strain carrying both variegated pericarp and a particular stable *R*-Navajo allele, called cudu, on a reciprocally translocated chromosome, T 1-10g. The bipartite *mR^nj* allele (*R^nj Mp*) was generated by transposition of *Mp* from the *P* locus to the *R* locus on this structurally altered chromosome. It was then transferred from T 1-10g to a normal chromosome 10 by crossing over. The foundation *mR^nj* allele is referred to in the text as "standard" and is designated "c" in the tables and figures.

A distinctive effect of the stable *R^nj* allele is formation of anthocyanin in a solid patch in the crown region of the kernel. Lesser amounts of pigment often are distributed rather irregularly elsewhere in the aleurone. *R^nj* embryos, silks, and anthers likewise are colored.

The effect of Modulator when present at the *R* locus in conjunction with the *R^nj* gene is to repress completely anthocyanin formation in all these structures. Mutable *R*-Navajo kernels normally show an irregular spotting pattern, however, in the crown and embryo that result from frequent transpositions of *Mp* away from the *R* locus, particularly at late stages in seed development. On *mR^nj* ears pollinated with the stable recessive factor *r^g* single, whole kernel, *R^nj* mutants arise by transposition of *Mp* away from the *R* locus during the few cell generations between the initiation of a floral primordium and the formation of the embryo sac nucleus destined to form the egg nucleus and a polar nucleus. Transposition of *Mp* during certain other stages of embryo sac development will give rise to kernels in which the embryo and endosperm phenotypes are non-concordant, namely *R^nj* endosperm accompanied by a *mR^nj* embryo and vice versa. Patches of stable *R^nj* kernels on an ear otherwise *mR^nj* result from *Mp* transposition from the *R* locus at a somatic mitosis in an ear shoot prior to formation of floral primordia. Such sectors on an ear vary widely in size depending on earliness of the mutation during development. Twinning of an *R^nj* sector with a sector containing lightly spotted, near-colorless or even colorless kernels is frequently observable. Thus the phenotypic expression of *mR^nj* closely parallels that of variegated pericarp (GREENBLATT and BRINK 1962) except that the former involves the seed, whereas pericarp color is a maternal character. The parallel is expected, of course, in view of the fact that the instability of both alleles resides in a Modulator component of common origin.

Aleurone expression of *R^nj*, and also its unstable counterparts, is sensitive to variation in background inheritance. All the stocks carrying these alleles used in the present study, therefore, were incorporated in the uniform, inbred strain, W23.

Symbols: Each *mR^nj* allele was given a distinctive K number which corresponded to that of the particular stable *R^nj* mutant isolated among the descendants of the GREENBLATT foundation *mR^nj* from which it was derived. The different K numbers thus refer to *mR^nj* alleles of independent origin in terms of reassociation of a transposed *Mp* with the *R^nj* gene. The 26 *mR^nj* alleles chosen for detailed study represent an approximately random sample, with respect to collective aleurone phenotype, from a somewhat larger population originally isolated in the cyclic series.

R^sc (self-color) is an *R* allele that gives solidly colored aleurone in one, two, or three doses. It is dominant to *R^nj* and *mR^nj*. *R^g*$_8$ pale results in diluted aleurone pigmentation, a phenotype readily distinguishable both from that of a weakly spotting (i.e., relatively stable) *mR^nj* allele, and colorless aleurone (*r^g*).

Measurement of the frequency of whole kernel mR^nj *to* R^nj *mutations:* Heterozygotes carrying the respective 26 reconstituted *mR^nj* alleles and either *R^g*$_8$ pale or *r^g* were grown in a detasseling plot in which a W23 *r^g r^g* stock served as the pollen parent. Following harvest, a minimum of 28 well-filled ears were chosen for scoring for each *mR^nj* isolate, except in the case of K149 and the standard *mR^nj* allele, of which only 22 and 6 ears, respectively, were available. More than 28 ears were scored in a few instances. K96, a relatively stable *mR^nj* isolate in which a hypermutable *mR^nj* allele appeared in one sub-line, is an example. The number of whole kernel *R^nj* mutants on each ear, and size of the sector in which these seeds occurred, were determined. The seeds involved in this experiment were scored according to endosperm phenotype only. That is, a "whole kernel" mutant in the present context means a seed in which the aleurone showed the *R*-Navajo phenotype. (The embryo may or may not have been concordant with the endosperm in phenotype, in accordance with the results of another experiment to be mentioned later.)

Sector size: The determination of sizes of mutant R^{nj} sectors involving more than a single kernel was rendered somewhat arbitrary by the fact that one-half the kernels on the ears scored were pale or colorless as a result of segregation for $R^g{}_s$ pale or r^g. R^{nj} kernels belonging to a single sector frequently could have been separated from each other, therefore, by seeds of the alternative class. The uniform scoring procedures followed throughout were (1) R^{nj} mutant kernels in contact with each other were counted as members of the same sector and (2) mutant R^{nj} kernels separated by as much as one-half or more of the width of a kernel were recorded as representing different sectors. The sector size value used for each cyclic mR^{nj} mutant was the average of the mean number of whole kernels per mutational event over the sample of 28, or more, ears scored.

Measurement of amount of aleurone spotting: During endosperm development mR^{nj} mutates to R^{nj} with relatively high frequency as compared with the rates in vegetative cells of the growing plant. The result is an irregular patchwork of colored spots of varying sizes on a colorless background on most of the mR^{nj} kernels from $mR^{nj}/R^g{}_s$ *pale* ♀ × r^gr^g ♂ matings. The intensity of pigmentation resulting from the spotting was measured with a reflectometer (Agtron, Model No. F1, manufactured by Magnuson Engineers, San Jose, California). Spotted kernels of the 26 mR^{nj} isolates were sampled in composited groups of 7 ears from $mR^{nj}/R^g{}_s$ ♀ × r^gr^g ♂ matings. Most of the mR^{nj} isolates were represented by the averaged reflectometer readings of four such bulked groups, giving a total of 28 ears sampled. Flat-sided, uniformly-shaped, kernels were mounted in 55 mm petri dishes for the reflectometer readings. The kernels were pressed upright into modeling clay on the bottom of the dish so that the crowns only were exposed. The seeds were packed tightly in order to minimize the effects of shadows between kernels. The reflectometer was adjusted to read zero reflection for normal R^{nj} kernels (fully pigmented at the crown) and 100 for colorless (r^g) kernels. Highly repeatable values for a given cyclic mR^{nj} mutant were obtainable by this procedure. The pigmentation scores entered in Table 1 were obtained by reversing the scale—that is, by subtracting the reflectometer reading from 100.

A Dissociation (Ds) *test for* Mp *transposability:* Chromosome breakage occurs at the *Ds* locus with high frequency in nuclei that also carry *Mp* or its equivalent, Activator (*Ac*), as first reported by McCLINTOCK (1951). The *Ds* stock used in the present investigation carried *I* and *Ds* on the short arm of chromosome 9, with *Ds* in standard position closely linked to waxy. *I* is an inhibitor of aleurone color, dominant to *C*. The *R* factor present, complementary for anthocyanin formation, was R^{sc}, which gives self-color in single dose in the absence of *I*. $mR^{nj}/R^g{}_s$ heterozygotes representing each of the mR^{nj} isolates were pollinated with *I-Ds*, R^{sc} pollen. Two major classes of kernels, termed "dark" and "light", were formed on the resulting ears. The proportion in which these two kinds of seeds occurred provided a basis for measuring *Mp* transposability, as will be explained when the experimental results are presented. The *Ds* test provided a means of scoring the cyclic mR^{nj} mutants for stability that was independent both of the frequency of whole kernel R^{nj} mutants and intensity of aleurone spotting as measured by the reflectometer.

Effect of the cyclic mR^{nj} *isolates on the variegated pericarp phenotype:* Plants heterozygous for the cyclic mR^{nj} isolates and colorless aleurone (mR^{nj}/r^g) were crossed with a W23 strain homozygous for variegated pericarp and colorless aleurone ($P^{vv}P^{vv}$, r^gr^g). The F_1 offspring were grown in a detasseling plot in which a W23 $P^{wr}P^{wr}$, r^gr^g stock served as the pollen source. The harvested ears, as expected, comprised two main groups in about equal numbers (a) light variegated pericarp, segregating mR^{nj} and r^g aleurone, and (b) medium variegated pericarp, colorless aleurone. The group (a) ears, of which 10 were available, on the average, for each cyclic mR^{nj} mutant, were scored against three standard ears ranging in variegated pericarp grade from class 1, which was near-colorless, to class 3, which was lightly striped. The mean variegated pericarp grades thus obtained were used as measures of the effect of the *Mp* components of the respective cyclic mR^{nj} isolates on the frequency of *Mp* transposition from the P^{vv} allele during ear shoot development of the light variegated pericarp plants.

Effect of P^{vv} *pollen on aleurone spotting of the cyclic* mR^{nj} *mutants:* The converse of the above test involved application of pollen from W23 plants homozygous for variegated pericarp to individuals heterozygous for the respective 26 cyclic mR^{nj} mutants and colorless aleurone. The ears obtained were scored by matching against standard ears representing four grades of

TABLE 1

Test data on mutable R-*Navajo isolates of cyclic origin. All 26 isolates listed in column (1) are descended from the foundation mutable* R-*Navajo allele designated "Standard"*

(1)* Designation of mutable *R*-Navajo isolate	(2)† Median percent mR^{nj} to R^{nj} mutant kernels	(3)‡ Reflectometer aleurone pigmentation score of isolate	(4)§ Mean R^{nj} mutant sector size	(5)‖ Median *I-Ds* test score for *Mp* transposability	(6)¶ mR^{nj} aleurone spotting score after P^{vv} pollination	(7)** Mean variegated pericarp score of mR^{nj} heterozygote
K10	7.3	64	1.16	2.8	2.4	1.7
K11	3.3	39	1.08	5.3	1.5	1.5
K23	0.4	16	1.00	1.8	1.2	2.1
K36	2.2	42	1.08	0.6	2.2	2.1
K40	17.4	78	1.39	5.4	2.6	2.2
K56	0.4	43	1.05	1.6	1.6	2.0
K62	10.1	76	1.23	3.3	2.6	1.5
K72	10.5	56	1.21	—22.3	2.1	1.9
K74	14.7	80	1.31	6.5	2.6	1.9
K75	3.7	69	1.10	1.3	2.2	1.9
K78	13.2	69	1.29	7.2	1.9	2.1
K87	6.9	59	1.11	4.5	2.5	1.6
K94	6.2	72	1.11	0.9	2.3	2.1
K96	2.4	68	1.04	0.4	3.0	2.1
K101	6.7	61	1.12	0.0	2.6	1.9
K111	3.9	65	1.06	0.9	2.2	2.0
K121	8.4	77	1.19	—0.2	3.4	1.9
K126	10.4	77	1.21	3.8	2.8	2.1
K131	1.4	60	1.03	2.8	2.2	1.7
K132	2.8	42	1.07	4.1	1.6	2.0
K134	0.7	20	1.05	4.6	1.4	1.9
K137	1.4	26	1.05	2.3	1.4	2.2
K147	11.2	32	1.23	41.7	2.1	2.0
K149	0.4	12	1.00	0.8	1.9	1.5
K153	12.0	72	1.27	4.6	2.9	2.0
K158	1.6	35	1.04	3.5	1.4	1.5
Standard	3.8	44	1.00	..	..	..

* Each isolate is represented by a distinct K number.

† Score based on 28 ears.

‡ The scale is from 0 (colorless) to 100 (fully pigmented R^{nj} aleurone).

§ Based on whole kernel mutants only, so minimum score is 1.00 kernel.

‖ Score equals excess over 50% of colorless plus "light" pigmented kernels, following pollination of mR^{nj}/r^g plant with *I-DS*. Median value based on counts from six individual ears.

¶ On the scale used, 1.00 represents colorless (or near-colorless) aleurone and 4.00 represents moderately light spotting.

** Genotype of plants was mR^{nj}/r^g, P^{vv}/P^{wr}; on the same scale medium variegated plants would have scored about 5.00.

aleurone mottling, as follows: class 1, near-colorless; class 2, very light spotting; class 3, light spotting; and class 4, medium light spotting. About 10 ears were scored for each cyclic mR^{nj} mutant.

Sexual transmission difference in the frequency of mR^{nj} *to* R^{nj} *mutation:* Reciprocal crosses were made between r^gr^g plants and mR^{nj}/r^g individuals representing each of the 26 mR^{nj} isolates of cyclic origin. The frequencies of kernels exhibiting mutant R^{nj} endosperms were determined for the two resulting sets of ears. The frequencies were then compared on a paired basis over the 26 mR^{nj} isolates according to female or male transmission of the mR^{nj} allele.

Concordance between embryo and endosperm phenotypes: The R^{nj} allele conditions anthocyanin formation in embryo and endosperm in conformity with the respective genotypes of these two components of the seed. Likewise, mutable *R*-Navajo may be phenotypically expressed autonomously in both structures. Reciprocal crosses were made between $r^g r^g$ and mR^{nj}/r^g plants representing six of the mR^{nj} isolates. The resulting seeds that contained *R*-Navajo mutant endosperms were then scored for embryo phenotype, mutant or non-mutant. The data were summarized in terms of percentage of kernels showing concordance between embryo and endosperm phenotype following female and male transmission of the mR^{nj} allele.

RESULTS

Frequency of kernels with the mutant R-*Navajo aleurone phenotype:* The percent mutant R^{nj} kernels following mR^{nj}/R^g_s pale♀ × $r^g r^g$♂, or mR^{nj}/r^g♀ × $r^g r^g$♂ mating for the 26 mR^{nj} isolates of cyclic origin, and also the standard mR^{nj} allele, are shown in column (2) of Table 1 and in Figure 1. The kernels were

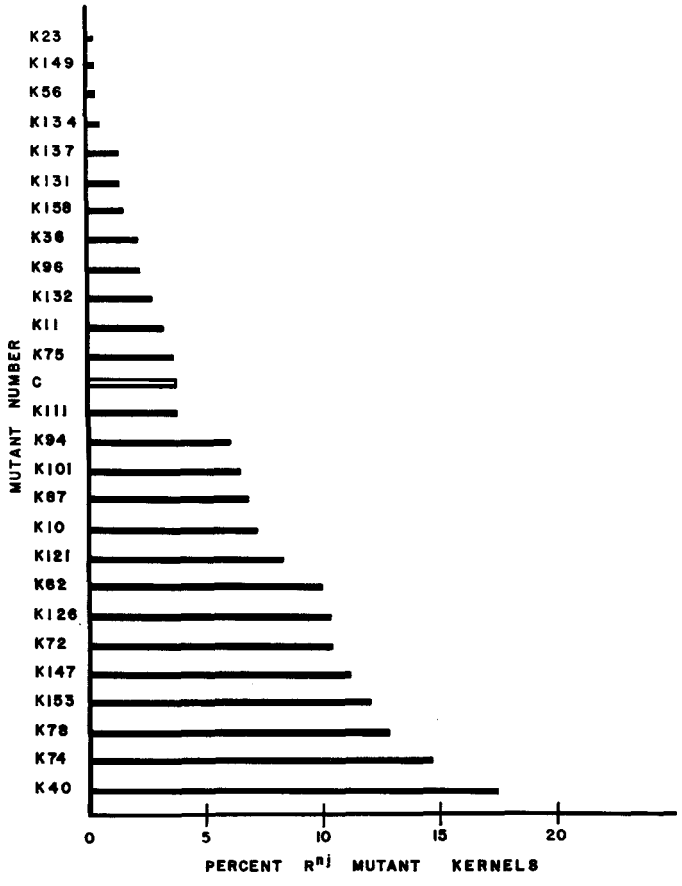

FIGURE 1.—Percent mutant R^{nj} kernels among the colored offspring from mR^{nj}/r^g ♀ × $r^g r^g$♂ matings. The 26 mR^{nj} isolates of cyclic origin, designated by K numbers, are arranged from top to bottom in order of stability. The standard mR^{nj} allele from which the 26 isolates were descended is designated c.

scored according to aleurone phenotype. The values entered are medians based on kernel counts for 28, or more, individual ears, except for K149 and the standard mR^{nj} allele, of which only 22 and 6 ears, respectively, were available. The stock termed standard or (c) represents the foundation mR^{nj} allele ancestral to all the reconstituted mR^{nj} isolates under test.

The reconstituted mR^{nj} alleles varied to an extreme degree in frequency of mutant R^{nj} kernels, as is readily apparent from the Figure 1 bar diagram. The three entries showing the lowest values, K23, K149, and K56, at the top of Figure 1, averaged only 0.4% mutant R^{nj} kernels. In contrast, the three least stable cyclic mR^{nj} isolates, K78, K74, and K40, with the highest values, gave an average of 15.1% mutant R^{nj} kernels. Thus the difference in frequency of mutations from mR^{nj} to R^{nj} between the two groups of three cyclic mR^{nj} isolates at the extremes of the distribution is about 38-fold.

It will be noted from Figure 1 that the R^{nj} mutation values for the 26 reconstituted mR^{nj} alleles vary continuously, and are distributed both above and below the value for the standard, ancestral, mR^{nj} allele.

Inspection of the detailed data on which column (2) in Table 1 is based showed that, in general, the ancestral mR^{nj} allele and the 26 reconstituted alleles did not vary markedly from ear to ear in percentage of mR^{nj} to mutant R^{nj} kernels when allowance is made for sampling effects in populations varying around a mean of about 300 colored kernels per ear. K96, however, was a conspicuous exception to this rule. Two families representing K96, based on sib parents, yielded a homogeneous group of 43 ears with a median percentage of mutant R^{nj} kernels of 2.4, the value entered in Table 1 as typical of the isolate. In contrast, the progeny of a third K96 sib, comprising a homogeneous group of 28 ears, gave a median mutation rate to R^{nj} of 20.2%. Evidently a "change in state' (McCLINTOCK 1951) at the *R* locus has occurred within the K96 stock markedly affecting frequency of *Mp* transposition. The hypermutable K96 derivative is under further study.

Aleurone spotting patterns: The variation in stability of the reconstituted mR^{nj} alleles is strikingly shown in the aleurone layer of the crown portion of kernels following mR^{nj}/r^g ♀ × $r^g r^g$ ♂ matings. Among the 26 mR^{nj} isolates the spotting patterns ranged from a profusion of irregularly distributed patches of pigmented cells differing widely in size, for the most unstable examples, to only occasional small colored spots on a predominantly colorless background, for the relatively stable cases. A score for the collective aleurone phenotype of each reconstituted mR^{nj} allele was obtained by measuring the light reflected from the crowns of uniformly-mounted samples of spotted kernels under standard conditions, as described under MATERIALS AND METHODS. The pigmentation values (100—the reflectometer reading) obtained for the 26 reconstituted alleles and the control stock, carrying the ancestral mR^{nj} factor, are entered in column (3) of Table 1. On a scale of 0–100, the scores vary from 12 for the least spotted K149, to 80 for K74. The standard (c) mR^{nj} allele gave a value of 44.

The aleurone spotting scores, based on reflectometer readings, are plotted against the median percent mutant R^{nj} kernels for the 26 reconstituted mR^{nj} al-

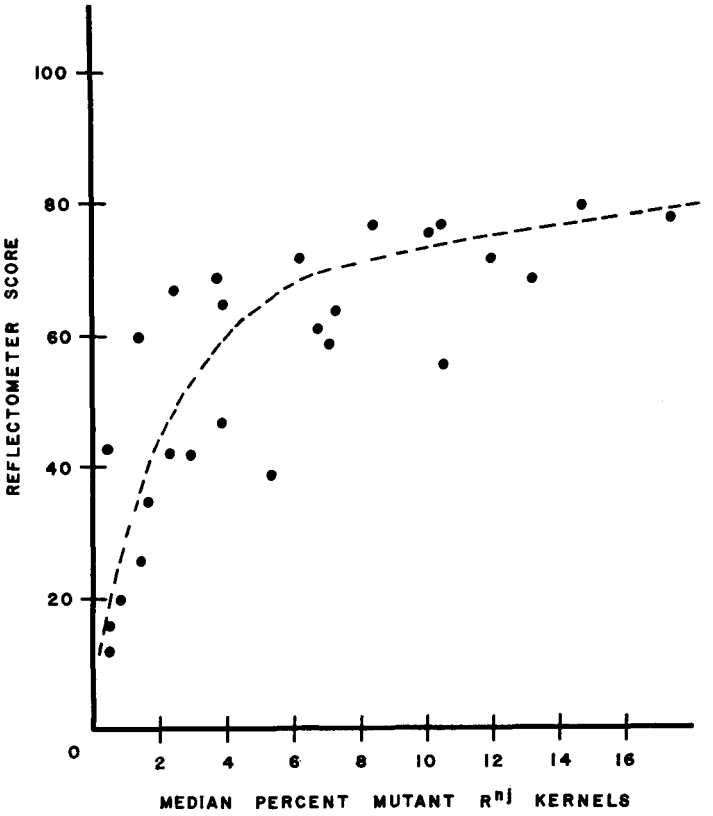

FIGURE 2.—The relation between relatively late occurring and earlier occurring mutations of mR^{nj} to R^{nj} among the 26 isolates. The reflectometer score varies with the intensity of aleurone spotting, and so gives a measure of late mutations. Median percent whole kernel R^{nj} mutants is a measure of earlier occurring mutations.

leles in Figure 2. The two sets of values show a highly significant correlation ($r = 0.76$).

It is evident from the shape of the dotted line drawn by inspection through the individual entries in Figure 2 that regression between the two variables is not linear. The reason for curvature of the line is not known, but it may be a result of greater sensitivity of the reflectometer to low anthocyanin pigment levels. However this may be, the positive correlation shown between median percent of whole kernel mutants and the intensity of aleurone spotting points to the conclusion that Modulator transposition is affected in parallel fashion by factors in the nuclear environment operating before and after fertilization. The rate of transposition of *Mp* on a cellular basis, however, is much higher for all 26 reconstituted mR^{nj} alleles at the later stages of endosperm formation than at any preceding stage.

Mutations of mR^{nj} to R^{nj} in the embryo: The embryos, as well as the endosperms, in the colored kernels resulting from mR^{nj}/r^g ♀ × $r^g r^g$ ♂ matings usually

are spotted. No attempt was made to score the 26 mR^{nj} isolates for embryo spotting. Inspection showed that, in general, the intensities of anthocyanin spotting in embryo and endosperm over the 26 mR^{nj} isolates were positively correlated. It is interesting to note, however, that frequent spotting of the embryo is not reflected by correspondingly high numbers of whole ear R^{nj} mutations or by numerous ears with large mutant R^{nj} sectors among the plants grown from the spotted embryos. This fact shows that the mR^{nj} to R^{nj} mutations in the embryo rarely involve the apical meristem of the shoot. Evidently most such mutations are confined to structures such as the scutellum and coleoptile that mature in the seed or seedling.

Sector size: Most of the mutations to R^{nj} of the 26 reconstituted mR^{nj} alleles that are expressed in the aleurone give rise to patches of colored tissue covering much less than one kernel. The smallest spots are the most numerous, indicating that the highest mutation rates occur at late stages in endosperm development. The diversity of patterns of colored and colorless aleurone areas is so great that a precise enumeration of mutations affecting less than a whole kernel is not practicable. Only an overall measure of spotting was obtained by the use of a reflectometer, as earlier explained. The reflectometer scores, as entered in column (3), Table 1, express the collective aleurone phenotypes of the respective cyclic mR^{nj} mutants, but they afford no evidence concerning the distribution of sector sizes below the one kernel level. The data whereby the several cyclic mR^{nj} mutants may be compared with each other in terms of sector size are limited, therefore, to the extreme upper range of the sector size scale, starting at the one kernel point.

The mean mutant R^{nj} sector size for sectors of one or more kernels is shown for each of the 26 cyclic mR^{nj} mutants in column (4), Table 1. The values vary from 1.00 to 1.39. In Figure 3, the data are plotted against aleurone spotting, based on the reflectometer readings (column (3), Table 1). The two respective sets of independently ascertained values are measures of relatively early-, and relatively late-, occurring mR^{nj} to R^{nj} mutations. The graph shows that there is a strong positive relation between the two variables. The non-linearity may possibly result from greater sensitivity of the reflectometer to low pigment levels, as previously discussed in the section on aleurone spotting patterns.

The evidence on sector size, although not definitive, indicates that all the mR^{nj} isolates exhibit the same type of relationship between mutation frequency and time of mutation throughout the period between initiation of ear shoot development and kernel maturity. No isolate shows an unusually high frequency of early mutations combined with a low frequency of late mutations. Evidently production of large sectors of one or more whole kernels is governed by the same stability relationships of *Mp* to the *R* locus as produce the small sectors composing collective kernel phenotypes.

A Dissociation (Ds) test for Mp transposability: For each of the 26 reconstituted mR^{nj} alleles the numbers of "dark" and "light" kernels were recorded for six ears resulting from the application of *I-Ds*, R^{sc} pollen to mR^{nj}/R^{g}_{pale} plants. The "dark" class of kernels was characterized by numerous patches of pigmented aleurone (*I* to *C* changes) resulting from losses of the color inhibiting gene, *I*, on

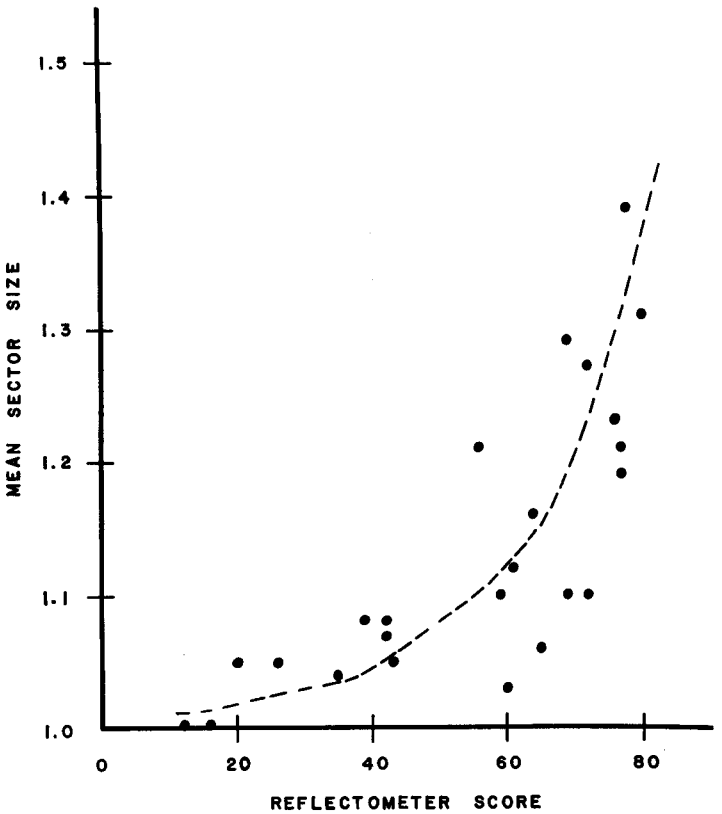

FIGURE 3.—Relation between mean size of sectors involving one or more kernels, as a measure of relatively early mR^{nj} to R^{nj} mutations, and reflectometer score which measures later mutations.

an acentric fragment, following chromosome breakage at *Ds* in a proximal position on the same arm of chromosome 9. Presence of *Mp* in the nucleus is a condition of *Ds* chromosome breakage, so all "dark" kernels contain one *Mp* element. The "light" class of kernels is compound. It comprises: (1) the $R^{g}{}_{8}$ pale segregates that lack *Mp*, (2) the mutants of mR^{nj} to R^{nj} that have lost transposed *Mp* from the genome, and (3) the kernels that have inherited a transposed *Mp* as well as an mR^{nj} allele, thus doubling *Mp* dosage in the aleurone. Types (1) and (2) are colorless, and type (3) kernels show very occasional small dots of aleurone color as a result of infrequent *Ds* chromosome breakage late in endosperm development. The occurrence of seeds of types (2) and (3) which are derived from the mR^{nj} segregation class would be expected to raise the frequency of the "light" class of kernels above the 50% expected if both mR^{nj} and $R^{g}{}_{8}$ pale assorted as conventional stable genes. The amount of the excess should vary with the frequency with which *Mp* transposes from the *R* locus, and thus should be correlated with mutation rate to R^{nj} of the respective mR^{nj} isolates.

Scores for *Mp transposability*, derived as the median excess of the "light" class

above 50%, are entered in column (5) of Table 1. The two exceptional values in the list—namely, those for K72 (−22.3) and K147 (41.7)—are probably due to extraneous circumstances. The marked deficiency of light kernels for K72 is the result expected if the ears were borne on plants trisomic for chromosome 10 in which the extra chromosome also carried mR^{nj}. Four of the six ears scored for K147 showed 44.4%, 39.2%, 49.2%, and 44.2% excess light kernels; the two remaining ears gave values of 2.6% and 1.0%, respectively. Type (3) kernels showing very occasional small pigmented spots were conspicuous among the excessive "light" kernels of the former four ears, and it is a plausible assumption that these four very high values reflect the action of a transposed *Mp* units linked with the mR^{nj} allele in question. The markedly deviant scores for K72 and K147 were not considered in the further analysis of the *I-Ds* test data.

Of the 24 remaining cyclic mR^{nj} isolates, 22 show percentages of "light" kernels in excess of 50%, one gave a zero value, and one isolate (K121) gave a deficiency of 0.2%. The positive values vary over a wide range from 0.40 to 7.20, as expected if the frequency of Modulator transposition differs from one to an-

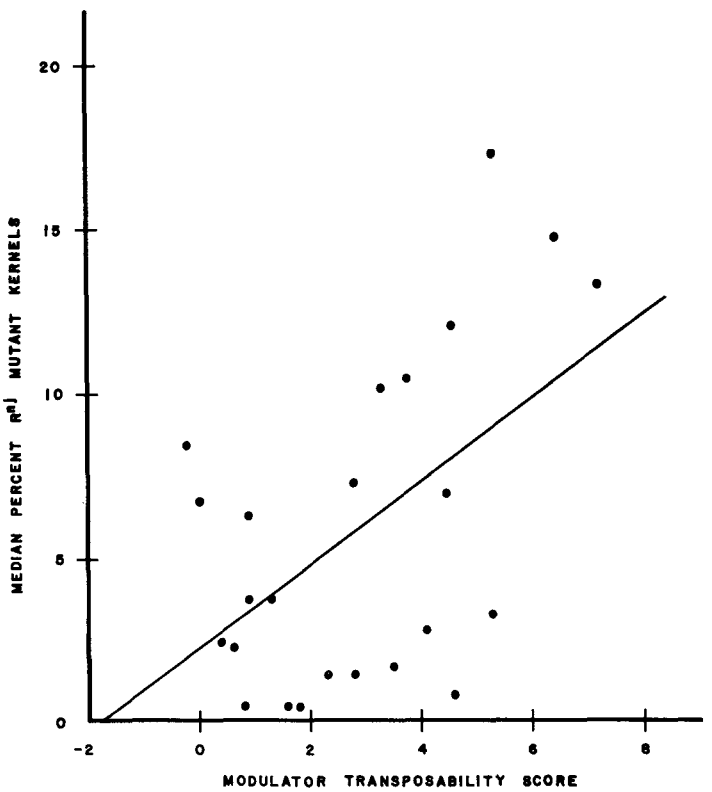

FIGURE 4.—The correlation ($r = 0.53$) between median percent of R^{nj} mutant kernels, and *Mp* transposability score from *I-Ds* tests. The correlation is expected since the two sets of values are theoretically alternative measures of the extent to which *Mp* transposes from the reconstituted mR^{nj} alleles.

other of the reconstituted mR^{nj} alleles. That is, in fact, the basis of the variation in percentage of "light" kernels over 50, is supported by the positive correlation ($r = 0.53$) between these 24 values and the median percentages of R^{nj} mutants kernels for the mR^{nj} isolates (Figure 4).

The phenotypes of the "dark" kernel class were compared with each other by visual inspection. They were found to be closely similar for all the cyclic mR^{nj} alleles. The latter fact shows that all 26 isolates of *Mp* at the R^{nj} locus were alike, or nearly so, in their distant interaction with *Ds* on chromosome 9.

Interaction of the Mp component of variegated pericarp with that of the reconstituted mR^{nj} alleles: The variegated pericarp allele (P^{vv}) and the series of mR^{nj} alleles under study here carry Modulator as a common component. The independently-assorting P^{vv} and mR^{nj} factors interact with each other in cells carrying ing both, so that the frequency of transposition of *Mp* away from the two respective unstable loci is reduced. The effect is parallel to that of a transposed *Mp* in a plant heterozygous for variegated pericarp (P^{vv}/P^{wr}) in which the collective pericarp phenotype is changed from medium variegated to light variegated, as described by BRINK and NILAN (1952). The question to which an answer was sought in the present investigation was whether the mutual interaction was the same between a given P^{vv} allele and all 26 reconstituted mR^{nj} factors.

The mean aleurone spotting scores for the 26 cyclic mR^{nj} isolates following pollination with a W23 stock homozygous for variegated pericarp and colorless aleurone ($P^{vv}P^{vv}$, r^gr^g) are shown in column (6) of Table 1. As noted in the section on MATERIALS AND METHODS, the standard ears against which the testcross ears were matched varied from class 1, near-colorless, to class 4, medium light spotting. On a comparable scale, the ears from the same array of mR^{nj}/r^g plants pollinated by colorless pericarp, colorless aleurone ($P^{wr}P^{wr}$, r^gr^g) would have been distributed over classes 4 to 6. The effect on aleurone phenotype of introducing a single *Mp* factor through the pollen via the P^{vv} allele is to reduce greatly the amount of anthocyanin spotting, as illustrated in Figure 5.

There is a highly significant correlation ($r = 0.81$) between mean grade of aleurone spotting after pollination with P^{vv} and amount of aleurone pigment in the mR^{nj} kernels, as measured by the reflectometer, following $mR^{nj}/R^g{}_s$ pale ♀ × r^gr^g, $P^{wr}P^{wr}$ ♂ control matings (Figure 6). This result shows that the *Mp* component of the variegated pericarp allele at the *P* locus acts to a similar extent on all mR^{nj} isolates to reduce *Mp* transposition from the *R* locus to a level closely parallel to the inherent stability of the respective mR^{nj} alleles.

A parallel test using pollen from a homozygous W23 P^{vv} *— 1* stock, carrying two, rather than only one *Mp* unit at the *P* locus resulted in nearly colorless aleurone uniformly over the 26 reconstituted mR^{nj} alleles. All the latter are similarly sensitive, therefore, to increasing dosages of *Mp*.

The reciprocal effect of *Mp*—namely, that of the factor carried by the respective mR^{nj} isolates on the expression of variegated pericarp at the *P* locus—was measured in F_1 plants between the 26 cyclic mR^{nj} isolates and a uniform W23 $P^{vv}P^{vv}$ stock.

The mean variegated pericarp scores resulting from the action on a common

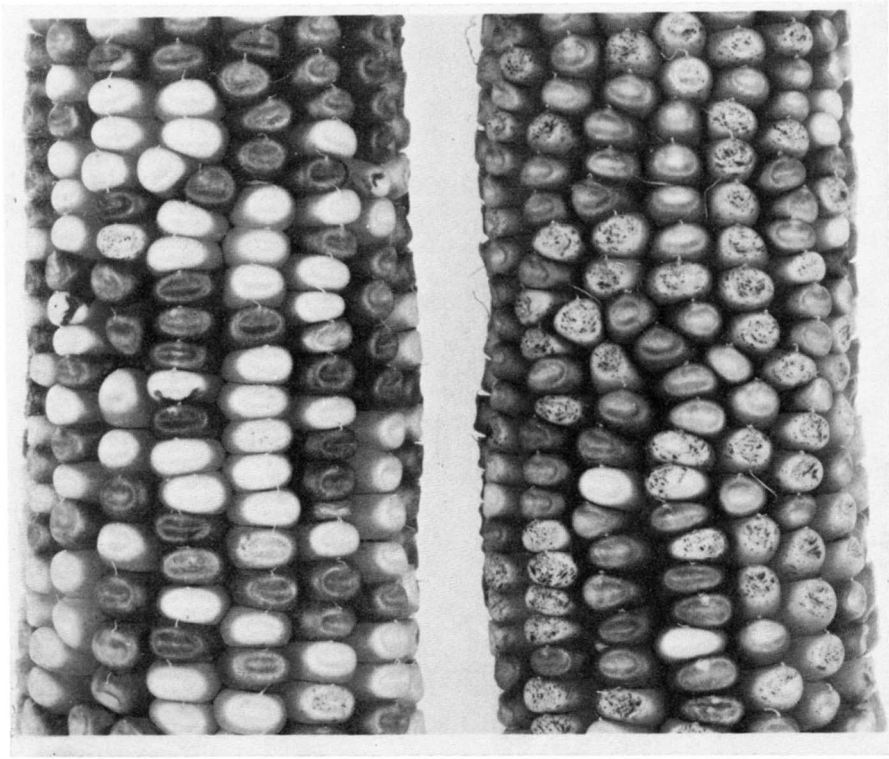

FIGURE 5.—The effect on aleurone spotting (late mR^{nj} to R^{nj} mutations) of pollinating mR^{nj}/R^{g}_{s} pale heterozygotes with $r^{g}r^{g}$ $P^{wr}P^{wr}$, colorless aleurone, colorless pericarp (right), and with $r^{g}r^{g}$ $P^{vv}P^{vv}$, colorless aleurone, medium variegated pericarp (left). The uniformly dark kernels are the unaffected R^{g}_{s} pale segregates. The ear on the left demonstrates the repression of mR^{nj} to R^{nj} mutations by a second dose of *Mp* in the genome.

P^{vv} factor of the 26 respective mR^{nj} alleles are entered in column (7) of Table 1. All mR^{nj} alleles strongly repressed P^{vv} variegation. The values corresponding to the different mR^{nj} isolates varied within narrow limits; furthermore, there was no significant correlation ($r = 0.17$; $p = > 0.4$) between the variegated pericarp scores and intensity of the mR^{nj} aleurone spotting phenotypes measured by reflectometer. This shows that the effects of the *Mp* components of the 26 mR^{nj} isolates acting at a distance in the genome on a common P^{vv} allele are essentially alike, and are independent of their local action at the *R* locus on expression of the R^{nj} gene.

Effect of sexual differentiation on frequency of mutant R^{nj} kernels: The data summarized in Table 2 show that following reciprocal crosses between mR^{nj}/r^{g} and $r^{g}r^{g}$ plants about 2.3 times as many colored kernels are of the mutant R^{nj} phenotype when the heterozygote is used as the male rather than the female parent. The mean values are 13.05 and 5.6, respectively, following male and female transmission, and the difference is in the same direction for all 26 cyclic mR^{nj} isolates. The difference in average frequency of mutant R^{nj} kernels is com-

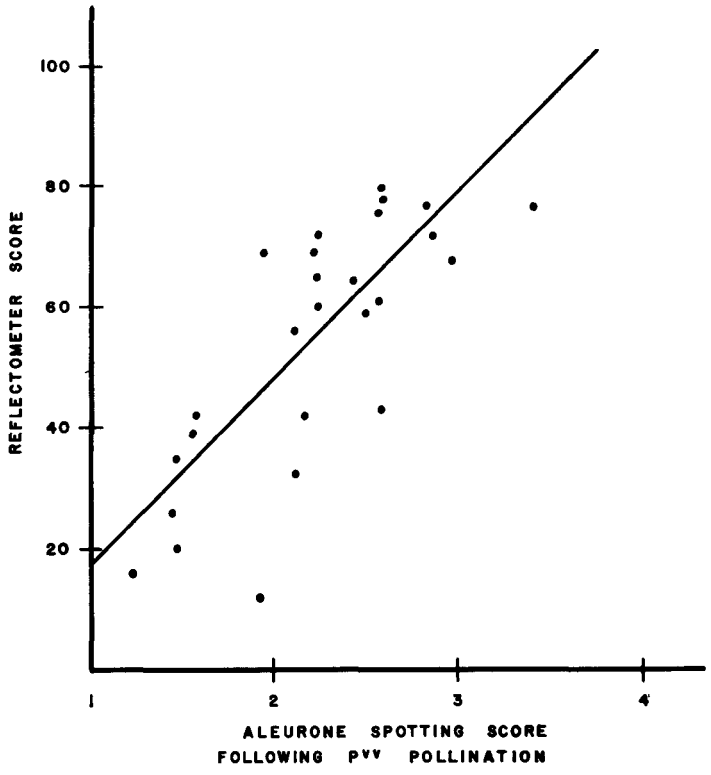

FIGURE 6.—The correlation ($r = 0.81$) between intensity of aleurone spotting following mR^{nj}/r^g ♀ × $r^g r^g$ ♂ matings, in which no *Mp* unit was introduced through the male parent, and mR^{nj}/r^g ♀ × $r^g r^g$ $P^{vv}P^{vv}$ ♂ matings in which an additional *Mp* element was introduced into the endosperm genotype with the variegated pericarp (P^{vv}) allele. Aleurone pigmentation was measured in terms of reflectometer scores for the first set of matings and by matching ears representing each of the 26 mR^{nj} isolates against a set of standard ears after the pollinations with P^{vv}.

parable in direction and amount to that which WILLIAMS (1972) observed for non-waxy (*Wx*) mutants following reciprocal matings between mutable waxy plants heterozygous for stable waxy, (*Wx Mp*)/*wx*, and stable waxy individuals, *wx wx*. The close similarity between the two cases doubtless is attributable to the transposable repressor element, Modulator, as a common component of the respective unstable loci. In neither instance, however, does the evidence show whether the difference is due to a higher *Mp* transposition rate during male development or to a constant transposition rate that extends over a greater number of mitotic cell cycles during tassel differentiation as compared to ear shoot development.

Concordance between embryo and endosperm phenotypes: The evidence on degree of concordance in phenotype between embryo and endosperm following reciprocal crosses between $mR^{nj}/R^g{}_s$ pale and $r^g r^g$ plants, in which six of the reconstituted mR^{nj} alleles were represented, is assembled in Table 3. Only kernels

TABLE 2

Mean frequency of mutant R-*Navajo kernels following reciprocal crosses between* mR^{nj}/r^g *heterozygotes carrying the respective mutable* R-*Navajo alleles listed in the left-hand column, and* r^gr^g *plants*

Mutable *R*-Navajo isolate	Total colored kernels scored		Percent mutant R^{nj} kernels	
	Female transmission	Male transmission	Female transmission	Male transmission
K10	409	466	11.5	26.2
K11	482	446	2.3	9.3
K23	430	447	0.3	1.4
K36	349	408	1.4	7.7
K40	244	446	19.5	29.4
K56	555	765	0.3	1.8
K62	519	796	8.3	16.7
K72	988	646	4.9	13.2
K74	336	520	8.6	28.7
K75	446	581	3.8	19.3
K78	517	618	9.2	19.2
K87	370	462	5.2	18.5
K94	342	502	5.6	20.2
K96	548	665	2.0	2.3
K101	406	472	9.8	20.8
K111	361	506	4.1	5.9
K121	316	409	6.1	19.1
K126	366	364	9.2	18.6
K131	456	629	2.5	3.4
K132	560	489	2.7	3.8
K134	667	591	0.7	1.2
K137	422	421	1.4	4.2
K147	386	362	8.9	19.5
K140	409	392	0.7	2.7
K153	312	515	15.5	24.9
K158	370	250	0.5	1.2
Average			5.6	13.05

possessing a mutant R^{nj} endosperm were scored for concordance. The data show that, on the average, the percentage of non-concordant seeds following female transmission of the mR^{nj} allele is 24.4, as compared with 5.7 for male transmission. These respective values are of the same order of magnitude as those which WILLIAMS (1972) observed for non-concordance following reciprocal crosses between stable waxy (*wx wx*) plants and unstable waxy heterozygotes in which the non-waxy gene was repressed by Modulator (*Wx Mp/wx*). Williams calculated that non-concordant seeds with non-waxy endosperm (*Wx*) and unstable waxy embryo (*Wx Mp*) should be four times as frequent through the female as through the male gametophyte, on the assumptions that (1) the *Mp* transposition rate per mitotic cycle is constant, (2) the same for male and female gametophytes, and (3) the two synergids of the embryo sac contain sister nuclei. It is evident that, on the average, the concordance data for mutable R^{nj} conform to expectation on this basis.

TABLE 3

Concordance between endosperm and embryo phenotypes of kernels with mutant R^{nj} *endosperm resulting from reciprocal matings between* $r^g r^g$ *plants and* mR^{nj}/r^g *individuals carrying different reconstituted mutable* R^{nj}*-alleles*

Mutable *R*-Navajo isolate	♀ transmission kernels with mutant *R*-Navajo endosperm			♂ transmission kernels with mutant *R*-Navajo endosperm		
		With non-concordant embryo			With non-concordant embryo	
	Total	Number	Percent	Total	Number	Percent
K10	46	6 (2)*	13.0	129	1	0.8
K40	37	7	18.9	132	10 (4)	7.6
K74	30	8 (3)	26.7	157	15 (2)	9.5
K78	54	16	29.6	123	4 (1)	3.2
R87	20	1	5.0	90	5 (1)	5.5
K101	26	14	53.8	49	4	8.2
Total	213	52 (5)	24.4	680	39 (8)	5.7

* Number of kernels of doubtful embryo phenotype, and not included in the computations.

DISCUSSION

The most significant general finding in the present study is the dichotomous character of the experimental results. The several reconstituted mutable *R*-Navajo alleles proved to be widely diverse in terms of action at the *R* locus only, and were alike, or nearly alike, in their modifying effect on phenomena controlled primarily by loci elsewhere in the genome. This dichotomy of effects suggests that the Modulator components of the different mR^{nj} alleles are the same in constitution but vary in some other way with respect to the *R* locus. It is a plausible assumption that the differences at *R* are in location of a common *Mp* unit within the R^{nj} gene.

The 26 reconstituted alleles varied greatly in stability as measured in four ways: (1) In terms of the frequency of mutant R^{nj} kernels there was a 38-fold difference between the least stable and most stable isolates; (2) Reflectometer scores for aleurone spotting ranged from 12 to 80 on a 0–100 scale, indicating a wide variation in the frequency of *Mp* transposition during endosperm development; (3) Considerable variation also was obtained among estimates of *Mp* transposability derived by a technique involving *Ds* chromosome breakage; (4) The mean size of somatic ear sectors involving one or more mutant R^{nj} kernels also varied among the mR^{nj} isolates. That these several measurements reflect the same stability relationships among the 26 reconstituted alleles is indicated by positive correlations between the data of (1) and (2), (1) and (3), and (2) and (4).

The variation in stability of the 26 mR^{nj} alleles appeared to be continuous. The foundation mR^{nj} allele from which the reconstituted factors were descended occupied an intermediate position in the distribution.

In contrast to the pronounced diversity in stability, the 26 mR^{nj} isolates were relatively uniform in their effects on frequency of transposition of *Mp* from the variegated pericarp allele located elsewhere in the genome. No correlation was

observed between the variegated pericarp scores for P^{vv} plants carrying the 26 respective mR^{nj} isolates and their aleurone spotting intensity as measured by the reflectometer. A similar uniform response resulted when the mR^{nj} factors were tested for their degree of activation of *Ds* breakage in another chromosome. These two results show that the effects of the *Mp* components of the 26 mR^{nj} isolates acting at a distance in the genome are relatively uniform and are independent of ther local action at the *R* locus on expression of the R^{nj} gene. Such results are expected if all the mR^{nj} isolates carry a Modulator element of the same constitution. Further evidence supporting a common constitution for all the *Mp* elements is the parallel reduction of the instability of all the mR^{nj} alleles by the addition of a uniform second dose of *Mp* after pollination with P^{vv}. All mR^{nj} isolates showed a reduced intensity of aleurone spotting, but the order of their stability remained essentially unchanged, to give a strong positive correlation between grades of aleurone spotting in the presence and absence of P^{vv}. All these results show that the phenotypic diversity of the reconstituted mR^{nj} alleles does not rest on differences in constitution of the *Mp* elements conjoined with the R^{nj} gene in the 26 respective cases.

As mentioned earlier, NELSON (1968) showed that conditionally transposable elements may occupy different positions within a locus in maize. Two of the mutants tested, wx^{m-1} and wx^{m-6}, carried the *Ds* repressor, but at different sites within the waxy locus. These mutants differ from each other in the frequency with which *Ds* transposes when Activator (*Ac*) is also present in the genome (MCCLINTOCK, personal communication). It may be inferred from such evidence that differential stability of independent *Mp* isolates might also be associated with the occupation of different sites within an affected gene locus.

A working hypothesis is formulated in the following paragraphs which attempts to account for instability and its variation among the reconstituted mR^{nj} alleles under study. Four basic assumptions are made: (1) Frequency of mutation of mR^{nj} is affected by the position of *Mp* within the *R* locus of each specific mR^{nj} isolate. (2) Transposition is a non-reciprocal, one-way change which occurs in somatic cells and which, in a majority of cases, transfers the element from a chromatid donor site to a recipient site in an undivided chromosome segment. (3) There is no necessity for extensive homology between donor and recipient sites. If contact between these sites prior to transposition were dependent on homology it would be necessary to assume that whereas the transposable element at the donor locus was an active repressor of gene action, its homologous counterpart at the recipient locus was regularly inactive in this respect. Furthermore, on this basis, multiple homologs (even more than one within a single locus) throughout the genome would have to be postulated for a given element to be transposable to numerous different sites. (4) Transposition of *Mp* is related to DNA breaks that are prerequisite to unwinding and replication of the DNA double helix and to separation without entanglement of the daughter chromatids in mitosis.

A way in which the concept of single-strand nicking as a prerequisite to unwinding of the double helix and DNA replication may be applied to nonreciprocal relocation of an element like Modulator at any one of numerous non-

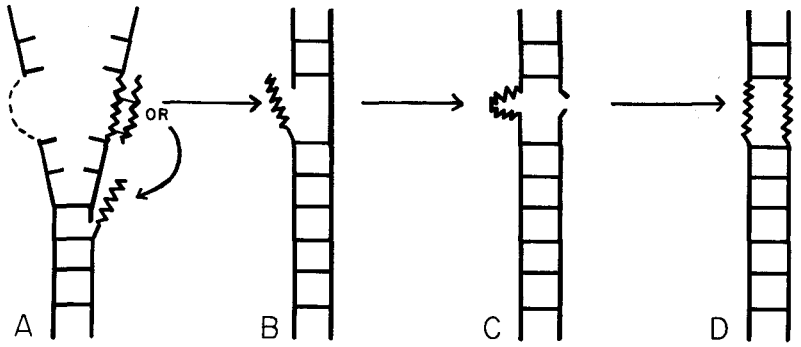

FIGURE 7.—A chromosomal model illustrating transposition of Modulator, represented by a zigzag segment, from one site to another. (A) When strand separation occurs at a replication fork in the DNA double helix at the *Mp* donor site, *Mp* is excised from one strand as a result of single strand breaks at its junctions with the two neighboring nucleotide sequences. It remains attached to the complementary strand (micro-nondisjunction) as the strands move apart. The excised *Mp* is transferred at an overlap (not shown) with a single strand break, or nick, at a chromosome site just ahead of the replication fork or, less often, (B) somewhere else in the genome. It is incorporated at the new site initially in a single strand. (C) The tension resulting from incorporation of *Mp* in one strand causes breakage of the other strand at the same level. (D) Repair occurs whereby *Mp* becomes represented in both complementary strands, before replication, in most, but not all instances of transposition.

homologous positions is illustrated in Figure 7. (See WATSON 1970, Chapter 9, for a discussion in molecular terms of DNA replication in prokaryotes.) The model is to be regarded as speculative, and the underlying postulates may be summarized as follows:

1. Nicking occurs at several sites near a DNA replication fork in the parental helix, and facilitates unwinding of the two strands.

2. The single strand breaks remain open for a short time following separation of the two complementary strands.

3. Nicks may be randomly distributed with reference to nucleotide sequences, or they may be initiated by particular "nicking codons". They may occur preferentially at the two ends of a transposable element in the donor position, thus tending to maintain unity of the latter during transfer to a new site.

4. A form of micro-nondisjunction may occur during separation of the strands at the *Mp* donor site. *Mp* is lost from one strand at this stage by remaining attached to its counterpart in the other strand. In one strand of the two-stranded *Mp* segment the ends are free. The gap in the other strand caused by excision of *Mp* is closed by union of the broken ends (A in Figure 7).

5. Transfer of the single-stranded *Mp* segment with free and cohesive ends occurs at an overlap of the segment with another nick, as illustrated in A and B of Figure 7. The receptor site may be in the region of the same replication fork, or near a replication fork elsewhere in the genome. If such an overlap with a single strand break at another site fails to occur, the *Mp* segment with free ends is reincorporated *in situ* during synthesis of a complementary strand.

6. The distortion caused by the initial incorporation of the transferred *Mp* seg-

ment into only one strand at the receptor site often leads to breakage of the other strand at the same level (C in Figure 7).

7. Repair occurs at the new break by synthesis and incorporation of a complementary *Mp* segment, as shown in D of Figure 7. Separation of the two strands of the double helix, and DNA replication may now follow.

8. If distortion incident to incorporation of a transposed *Mp* segment into only one strand at the new site does not result in breakage of the complementary strand, followed by repair, then after DNA replication, transposed *Mp* will be represented in only one rather than both daughter chromatids.

This model accounts for two well-established facts concerning *Mp* transposition. One is that *Mp* transposes from a chromatid to a chromosome site that has not yet replicated in that mitotic cycle in about two--thirds of the cases (GREENBLATT and BRINK 1962; 1963). Secondly, as VAN SCHAIK and BRINK (1959) showed, most but not all receptor sites are near the donor site. It would be expected on mechanical grounds that most overlaps resulting from looping and folding of the DNA double helix would involve points near each other in the same chromosome. The assumption of several single strand breaks at different sites near one replication fork is in accord with this requirement and also provides an explanation for the sudden alterations in stability, termed "changes of state", which characterize mutable alleles controlled by transposable elements. The formation of a hypermutable variant allele within the K96 family of the present work is an example. In terms of Figure 7A, the insertion of the *Mp* segment with free ends into the right-hand strand a short distance ahead of the replication fork, but within the same gene, after completion of replication, would be capable of giving on the right hand chromatid a locus with an extra dose of *Mp*, and on the left hand chromatid, (following breakage and repair as in Figure 7C and D) a locus carrying a single *Mp* element at an altered position.

Transposition of *Mp* to a site remote from the donor locus would involve overlap between two different replication forks. These could be in the same chromosome or in different chromosomes.

The postulate that nicking sites are numerous throughout the genome is in accord with the observation that *Mp* transposes to many unrelated loci, and also with NELSON's (1968) observation that the repressors in three potentially unstable waxy alleles occupied different sites within the same locus.

MCCLINTOCK (1961) and, more recently, PETERSON (1970) have reviewed the evidence from mutable loci in maize in terms of parallels with the control of gene expression in bacteria. PETERSON gave particular attention to bacterial episomes one of which, the temperate phage designated Mu-1, is of special interest in the present context because of the seemingly random distribution of sites at which it is integrated into the *Escherichia coli* chromosome. The extensively-investigated lambdoid phages possess unique sites of integration into the *E. coli* host chromosome (for review, see DOVE 1968). It was demonstrated by TAYLOR (1963) that, in contrast, Mu-1 is inserted at numerous different sites, at which it inactivates the respective genes present. PETERSON noted that the non-specific insertion of Mu-1 is paralleled by the non-specific insertion of transposable elements in

maize. He postulated that gene activity is affected in the same way in both kinds of organisms—namely, by interruption of the reading frame by the intercalated element. PETERSON also suggested that variations in phenotype could result from insertion of such elements at different sites within a cistron.

In a recent study, BUKHARI and ZIPSER (1972) isolated 76 mutations that involved insertion of Mu-1 into the *Z* gene of the *Lac* operon conditioning β-galactosidase activity in *Escherichia coli*. They demonstrated that the prophage mapped at different sites within the locus in all, or most, of the cases. Insertion apparently was at random. BUKHARI and ZIPSER concluded that since a particular extended run of bases probably would not be repeated 76 times within the *Z* gene, integration of Mu-1 did not involve recognition of an extensive host nucleotide sequence. As an alternative explanation, one could suppose that, in accord with the hypothesis applied here to the mutable *R*-Navajo variants, the sites at which Mu-1 enters the *Z* gene are random single strand breaks, or nicks, in the DNA double helix formed prior to unwinding and replication.

Modulator may involve a unique nucleotide sequence that tends to delay strand separation of the DNA double helix at the *Mp* site, relative to the immediately adjoining sequences, in nearly all positions throughout the genome. Such delay could underlie preferential single strand breakage at the two ends of the *Mp* segment, micro-nondisjunction of *Mp* leading frequently to the occurrence of twin mutations and indeed, the predisposition of *Mp* to transpose from one chromosome location to another. It is significant in this context that heterochromatin, long associated with V-type position effects, is characteristically a late replicating category of DNA (LIMA-DE-FARIA 1959; LIMA-DE-FARIA and JAWORSKA 1972). Furthermore, as BARR, VALENCIA and PLAUT (1968), PLAUT (1969), and VALENCIA and PLAUT (1970) have demonstrated by pulse labelling of the polytene chromosomes of *Drosophila melanogaster* with H^3-thymidine, the control of timing of DNA replication resides within small chromosomal regions. RUDKIN (1969) observed that during polytenization of salivary gland chromosomes in *D. melanogaster* heterochromatic segments replicated fewer times than euchromatic segments. This circumstance would be expected to create breaks in DNA continuity and free ends at the euchromatic-heterochromatic junctions during replication.

WILLIAMS and BRINK (1972) have suggested that non-specific transposable elements such as *Mp* represent small displaced fragments of a chromosomal component normally concerned primarily with the regional control of processes at the chromosomal level rather than at the level of individual genes. Direct evidence concerning the processes involved is not at hand. It appears not unlikely, however, that the elements are concerned with the regulation of one or another step in DNA replication. Repression of gene action at the locus at which it is inserted appears to be a side effect of Modulator. In a foreign position *Mp* overrides the locus specific mechanism controlling gene expression (BRINK 1964) probably by altering the reading frame so that normal transcription does not occur, as PETERSON (1970) has suggested.

The assumption that timing of strand separation in the double helix prior to

DNA replication varies over intragenic distances could account for the wide variation found in the present study in frequency of mutation to *R*-Navajo among the reconstituted mutable *R*-Navajo alleles. The latter could differ in stability according to the degree to which the timing of strand separation characteristic of *Mp* agreed with that of the neighboring sequences within the R^{nj} gene between which *Mp* had been intercalated. The greater the difference in timing between strand separation over the *Mp* nucleotide sequence and that of the neighboring sequences, the greater the likelihood of single strand breakage at the *Mp* boundaries, micro-nondisjunction of a single stranded *Mp* segment, and transposition of *Mp*.

Paramutation, a distinctive form of instability at a locus that is strongly affected by certain alleles in the homologous chromosome can also be brought under this point of view. The phenomenon will be briefly adverted to in order to illustrate how the mechanism postulated to account for the reconstituted *R*-Navajo alleles may operate at diverse unstable loci. The standard R^r allele in maize, following passage through a heterozygote with R-stippled (R^{st}), regularly is reduced heritably in aleurone pigmenting action. The change appears to result from an increase in number of elements comprising an assumed repressor segment adjacent to the R^r gene (BRINK 1964; SASTRY, COOPER and BRINK 1965). It may be supposed that the increase occurs in the manner illustrated in Figure 7A, as the replication fork passes through the repressor segment. A repressor element that has undergone micro-nondisjunction just behind the fork is inserted, following overlap, at a nick immediately ahead of the fork, where it is again replicated, as illustrated in Figure 7, C and D. The result is one strand of the DNA double helix resembling the donor except for a slight shift in position of the repressor element, and a complementary strand carrying two, instead of one, repressor elements. Segments containing various multiples of the repressor element would be built up by repetition of this process in different somatic mitoses.

A further significant property of *R* paramutation is that sensitivity of R^r to heritable change in heterozygotes with a strongly paramutagenic allele, like R^{st}, is retarded but not blocked by coupling of the allele with reciprocal translocations involving breaks either proximal or distal to the locus, and well removed from the latter, or insertion of R^r into a chromosome 10 carrying a large, terminal heterochromatic knob also distant from the locus. (BRINK 1961, 1969; BRINK and BLACKWOOD 1961; BRINK and NOTANI 1961). The effects of these relatively remote structural changes lead to the conclusion that although the multiple elements at the *R* locus may be acting directly on the gene to reduce its expression, their own unstable over-replication is affected by a control system which pervades the entire chromosomal region and is sensitive to gross structural alterations. Such a system might be concerned with the timing and coordination of DNA replication.

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