RECONSTITUTION OF THE VARIEGATED PERICARP ALLELE IN MAIZE BY TRANSPOSITION OF MODULATOR BACK TO THE *P* LOCUS[1]

ELWIN R. ORTON AND R. ALEXANDER BRINK

Department of Horticulture and Forestry, Rutgers—The State University, New Brunswick, New Jersey, and Department of Genetics, University of Wisconsin, Madison

Received July 6, 1965

VARIEGATED pericarp in maize exhibits numerous, irregularly distributed colored stripes or sectors, of varying sizes, on a colorless background. BRINK and NILAN (1952) showed that underlying the collective variegated phenotype is a *P* allele consisting of two different elements. One constituent is the P^{rr} gene which conditions self-colored pigmentation of the pericarp and cob. The other element is a transposable factor termed Modulator (*Mp*). When *Mp* is present at the *P* locus in the short arm of chromosome 1, in conjunction with the P^{rr} gene, the pigment-producing activity of the latter is repressed and colorless pericarp and cob results. Transpositions of *Mp* away from the *P* locus, occurring in the somatic cells at different developmental stages of variegated pericarp plants, permit the expression of P^{rr} as red sectors of diverse sizes on the colorless background.

VAN SCHAIK and BRINK (1959) found that *Mp* transposes to many different sites, but that in a high proportion of cases the new site was near the original position. Secondary, or subsequent, transpositions were found to occur also, seemingly with frequencies comparable to the primary move. Under these conditions it would be predicted that, in red-eared mutants from variegated pericarp plants carrying a transposed Modulator closely linked to *P*, the variegated pericarp allele occasionally would be reconstituted by transposition of *Mp* back to the *P* locus. The present paper is a report on experiments designed to test this prediction.

TERMINOLOGY

The variegated pericarp allele is symbolized by the expression $\overline{P^{rr}Mp}$, which signifies that the two distinct components, P^{rr} and *Mp*, are intimately associated with each other at the *P* locus. EMERSON (1917), in his pioneer studies on variegation, employed another symbol, P^{vv}, that is descriptive of the collective pericarp and cob phenotype, and is more convenient to write. The two expressions will be used interchangeably in the present paper.

Medium variegated pericarp, which is treated here as the standard phenotype, is characterized by a diverse array of kernels, most of which carry several well defined stripes, as illustrated in Figure 1A and C. A single variegated allele, in

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, and Paper No. 1030 from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin.

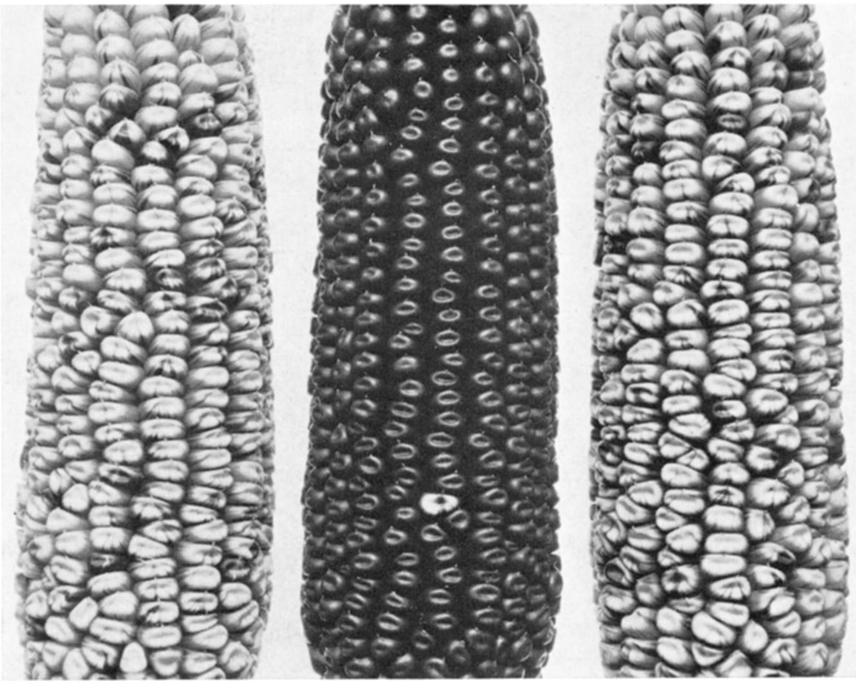

FIGURE 1.—Reconstitution of the variegated pericarp allele in maize. (A) (left)—standard medium variegated in inbred W23. This phenotype is conditioned by the $\overline{P^{rr}Mp}$ complex at the *P locus*. (B) (middle)—unstable self-red mutant derived from (A) by mutation of P^{vv} to P^{rr}. This phenotype is conditioned by the P^{rr} allele and a closely linked *tr-Mp*. (C) (right)—medium variegated mutant derived from a sectored kernel selected from a mutant self-red ear similar to that shown in (B). This phenotype is conditioned by a mutant $\overline{P^{rr}Mp}$ complex at the *P* locus.

heterozygotes with a colorless pericarp gene, underlies this phenotype. Two kinds of colorless pericarp alleles occur: P^{wr} conditions red cob, and P^{ww} gives colorless cob.

The grade of variegation (cf. BRINK 1954) is markedly and progressively reduced in $\overline{P^{rr}Mp}/P^{wr}$ plants by addition to the genome of one or more transposed Modulators (*tr-Mp*) in hemizygous form, as follows:

$\overline{P^{rr}Mp}/P^{wr}$ = Medium variegated (standard form)

$\overline{P^{rr}Mp}/P^{wr}$ plus *tr-Mp*/— = Light variegated

$\overline{P^{rr}Mp}/P^{wr}$ plus *tr-Mp*/— plus *tr-Mp*/— = Very light variegated

Thus, a transposed Modulator tends to stabilize the *Mp* at the *P* locus in a variegated pericarp plant. The addition of a single *tr-Mp* reduces the frequency of transpositions of *Mp* from the P^{rr} gene and, hence, the frequency of red pericarp stripes, and results in the light variegated phenotype. A second *tr-Mp* element in a genome further increases the stability of *Mp* at the *P* locus, giving the very light variegated phenotype.

The effect of a transposed Modulator is on the *Mp* component of the $\overline{P^{rr}Mp}$

complex rather than on P^{rr}. Expression of the P^{rr} gene when alone at the P locus is not altered by *tr-Mp* elsewhere in the genome.

MATERIALS AND METHODS

The experimental stocks used were descendants, in the seventh and later backcross generations ($P^{rr}P^{wr} \times$ W23$P^{wr}P^{wr}$), of two self-colored mutants from medium variegated pericarp plants in a yellow dent inbred strain known as W23 in its commercial form (P^{wr}) into which a single P^{vv} allele had been introduced earlier. No distinction will be made here between the two independently occurring P^{vv} to P^{rr} mutations, as the results obtained with the two respective sets of derivatives were essentially alike.

The $P^{rr}P^{wr}$ ears were examined for aberrant sectors of a variegated, colorless, or orange phenotype that involved more than one fourth of the surface of a kernel. Seeds that exhibited smaller sectors were also selected if the sector extended over the mid-section of the embryo. This selection procedure was based on the observation of ANDERSON and BRINK (1952) that the heritability of mutant types observed in sectors affecting only this limited region is relatively high. Some mutant patches were found on the otherwise self-red $P^{rr}P^{wr}$ backcross ears that encompassed from one to several kernels. An ear carrying a whole-kernel mutant is illustrated in Figure 1B.

A total of 680 sectored kernels were isolated in this manner, from which 576 plants were grown that bore ears which could be scored for pericarp phenotype. Known factors involved in loss of the remaining 104 individuals were nongermination of the seed, mechanical injury to the growing plants and particularly, damage from insect and fungus pests.

The number of mutant individuals found among the 576 fruitful plants was 179, of which 159 exhibited variegated pericarp in one or another form. The remaining 20 mutants had nonvariegated pericarp. The nonmutant offspring consisted of 98 P^{rr} and 299 P^{wr} individuals. Since the sum of the mutants plus P^{rr} plants ($179 + 98 = 277$) approximates the frequency of the P^{wr} class (299), it is evident that the mutants originated at the expense of the self-red pericarp class.

Frequency of transmission to the offspring of the mutant phenotypes represented by the kernel sectors was related to size of the aberrant area, as would be expected from the observations of ANDERSON and BRINK (1952) regarding heritability of the reciprocal mutant type on variegated pericarp kernels. Approximately 40% (in terms of a total based on the sum of all mutant and P^{rr} individuals) of the offspring from kernels with sectors covering less than two thirds of the kernel surface were of the mutant phenotype. However, the value was found to be 92% for kernels exhibiting sectors extending over two thirds or more of the surface of the kernel.

Most of the plants grown from sectored kernels were self-pollinated and also were mated to a standard W23 P^{wr} strain. In addition, pollen from each plant was placed on a *C-Ds* tester stock as a means of determining the presence (Mp^+) or absence (Mp^-) of Modulator in the genome of the parent plant (BARCLAY and BRINK 1954).

RESULTS

Distribution of mutant phenotypes: The 179 mutant plants recovered from the sectored kernels represented 156 separate mutant sectors on the parent ears and hence, the latter number of mutational events. The distribution of mutant pericarp phenotypes resulting from these mutations is shown in Table 1. About 93% (146) of the 156 independently occurring mutants exhibited variegated pericarp and cob. The remaining ten mutants possessed colorless pericarp and either weakly pigmented or colorless cob. Further reference to the latter mutants will not be made here, except to note that seven of them proved to be Mp^+ and three were Mp^-.

TABLE 1

Distribution according to pericarp phenotype of 156 mutants recovered from sectored kernels on W23 $P^{rr}P^{wr} \times P^{wr}P^{wr}$ *ears*

Background color of pericarp	Number of mutants: Variegated			
	Medium	Light	Very light	Nonvariegated
Colorless	30	34	38	10
Orange	19	20	5	. .
Total	49	54	43	10

Two main categories of variegated mutants were found, namely, those without background pigmentation and those in which the dark stripes were superposed on an orange background. The former class of mutants will be referred to as "variegated", and the latter as "orange variegated".

Three general classes of variegated mutants in terms of striping density, namely, medium, light, and very light, occurred with about equal frequency. The distribution of orange variegated mutants was parallel, except that fewer very light variegateds appeared.

Phenotypes of the three classes of mutant variegateds correspond in a general way, but not precisely in all cases, to those of the medium, light, and very light variegated classes of plants referred to in the section on Terminology. Certain mutants classified as medium variegated, for example, exhibited a somewhat darker grade of variegation than that conditioned by the standard P^{vv} allele in W23 background. At the opposite extreme were mutants classified as very light variegated that were somewhat lighter than standard ears of this category. However, this greater amplitude of variation should not obscure the fact that many mutants were indistinguishable in phenotype from their standard counterparts, as observed, for example, among the offspring of ordinary medium variegated plants. The medium variegated ear illustrated in Figure 1C is one such mutant. The plant that bore this ear was derived from an aberrant, sectored kernel like that on the middle ear in Figure 1. This mutant from self-red pericarp coincided in phenotype with that of standard medium variegated in the W23 inbred strain, as illustrated by the ear at the left in Figure 1. Phenotypically, at least, this instance represents the result of a complete cycle of mutation: medium variegated to self-red, and then back to medium variegated.

Progeny tests of variegated mutants: Fifteen mutants, heterozygous for P^{wr} and representing the principal variegated classes observed, were mated to W23 $P^{wr}P^{wr}$ individuals. The offspring were grown and the resulting ears were classified for pericarp phenotype. The results are summarized in Table 2.

The same phenotypic classes are represented among the offspring of the medium variegated mutants as were observed by BRINK and NILAN (1952) among the progeny of standard medium variegated plants. However, the frequencies of the self-red and light variegated classes are somewhat greater among the mutants, especially in the case of family 0603. No further data are available bearing on

TABLE 2

Phenotypic distribution of progeny resulting from backcrossing variegated mutants heterozygous for P^{wr} *to W23* $P^{wr}P^{wr}$

Phenotype of mutant parent	Family number	Number of progeny						Total
		Self-red	Medium variegated	Light variegated	Very light variegated	Subliminal* variegated	Colorless (P^{wr})	
Medium variegated								
	0603	26	66	25§	.	.	123	241
	0767	11	20	3	6	.	52	92
	0783	15	34	5	.	.	70	124
	0795	9	28	6	.	.	67	110
Light variegated								
	0763	3	.	45	4	.	45	97
	0765	8	2	47	1	.	62	120
Very light variegated								
	0957‡	.	.	.	65	9	125	199
	0600A	.	.	.	38	2	33	73
	0762	.	.	.	51	3	49	103
	0779	2	1	1	45	1	57	107
	0789–1	1	.	6	44	3	57	111
	0793–1	.	.	.	59	.	62	121
Orange-light variegated								
	0758	6	.	38†	4†	.	50	98
	0775	6	.	52†	.	.	51	109
	0792	2	1	32†	2†	.	66	103

* Scored on basis of weakly pigmented (pink) cob.
† Orange variegated.
‡ Third backcross mating of mutant 0119 to inbred W23.
§ Plus 1 orange-light variegated.

the question whether in some of the reconstituted medium variegateds *Mp* is prone to transpose from the *P* locus with significantly higher frequency than in the case of standard P^{vv}.

The two light variegated mutants that were progeny tested, represented by families 0763 and 0765 in Table 2, bred as would be expected if each carried a transposed Modulator linked closely to the variegated allele (Brink and Nilan 1952).

The low frequency of light and medium variegateds among the progeny of the very light variegated mutants entered in Table 2 could be due to the effect of two transposed Modulators closely linked to P^{vv}. An attempt was made to test this assumption by crossing plants carrying the very light variegated mutant present in family 0957 to standard homozygous W23 medium variegated and observing the effect of the mutant-carrying chromosome on the expression of the standard P^{vv} allele in the resultant offspring. If the basis of the mutant very light variegated phenotype were a $\overline{P^{rr}Mp}/P^{wr}$ constitution at the *P* locus plus two closely linked transposed Modulators in hemizygous condition, it would be predicted from known dosage effects (Brink 1954) that the most frequent class of P^{vv}/P^{vv} offspring following this testcross would show an extremely low, if not subliminal, grade of variegation, i.e., nearly colorless. What was observed, however, were 128 P^{vv}/P^{vv} individuals with a pattern of pericarp striping slightly darker than that of standard light variegated. This result suggests that the P^{vv} chromosome in the very light variegated mutant in question carried only the Modulator present at the *P* locus, and that grade of variegation in this case is affected by a factor in addition to mere number of *Mp* units in the genome. This explanation, however, is little more than a surmise because it does not account for the additional fact that 13 medium variegated plants with variegated cobs were present among the testcross offspring. Such a phenotype was unexpected either on this hypothesis or on the assumption that two transposed Modulators were present in the very light variegated mutant.

Most of the colored offspring of the orange-light variegated mutants represented in Table 2 were orange-light variegated, but a few individuals were either self-red or orange-very light variegated. This breeding behavior is consistent with that observed by VALENTINE (1957) for orange-light variegated plants derived originally from medium variegated individuals.

Presence of modulator in the variegated mutants isolated from sectored kernels: The presence or absence of *Mp* was determined for 487 of the 576 plants grown from sectored kernels by mating each individual to a *C-Ds* tester stock. The results are summarized in Table 3.

These data show that each of the parental self-red lines from which the sectored kernels in question were isolated possessed a transposed Modulator closely linked to *P*. The combined data in Table 3 for all red lines reveal that only six plants among the 82 successfully tested were Mp^-. The parental red pericarp sublines were maintained as $P^{rr}P^{wr}$ heterozygotes by recurrent mating to W23 $P^{wr}P^{wr}$ plants. A closely linked *Mp* occasionally could be eliminated from the genome by segregation at meiosis following crossing over from the P^{rr} to the P^{wr} chromosome or, probably more often, following transposition to a more distant site.

All 140 of the variegated pericarp mutants tested proved to be Mp^+. This is an observation of major importance in support of the thesis that the variegated allele has been reconstituted in these mutants as a result of transposition of *Mp* back to the *P* locus, where it is directly reassociated with the P^{rr} gene. Conversely,

TABLE 3

Modulator constitution and pericarp phenotype of the successfully tested plants grown from aberrant kernels on $P^{rr}P^{wr}$ *ears carrying a transposed Modulator closely linked to* P^{rr}

Phenotype of progeny	Modulator constitution	
	Mp^+	Mp^-
P^{wr}	8	240
P^{rr}	76	6
Mutant		
Variegated	140	0
Nonvariegated	14	3

240 of the 248 $P^{wr}P^{wr}$ segregates derived from mutant kernels were found to be Mp^{-}. As explained in the preceding paragraph, a few Mp^{+} individuals of this pericarp class would be expected as a result either of crossing over of the Mp element linked to P^{rr} or of transposition of Mp to a position in which it could readily assort with P^{wr} at meiosis.

In contrast to the presence of Mp invariably in the variegated pericarp mutants, three individuals occurred among the 17 nonvariegated mutants tested that were Mp^{-}.

Modulator constitution and pericarp phenotype in plants derived from sectors on single ears: Two or more offspring were obtained from kernels of mutant phenotype on individual ears in some of the cases included in Table 3. Brought together in Table 4 are the instances within this group in which at least one colorless pericarp, red cob (P^{wr}) segregate was included in the ear progeny along with at least one variegated mutant and, for both of which, the presence or absence of Modulator was determined. The entries are assembled according to phenotype of the mutant offspring, namely, variegated or orange variegated of a particular grade, or mixed. The table also shows the number of sectors on an ear from which the progeny were derived and, likewise, the frequency with which sectored ears yielding progeny of each particular kind occurred.

The most important single fact documented by the data in Table 4 has been alluded to earlier, namely, that all the variegated mutants tested Mp^{+}. The table also shows that the corresponding P^{wr} segregates, without exception, tested Mp^{-}. Giving effect to the recurrences of the several kinds of sectored ears, as entered in column 2 of the table, there are 83 independent cases in which this relationship was observed. The results of this closely controlled test leave no doubt that the variegated mutant and Mp are regularly transmitted together, as would be expected if they correspond to a common P^{vv} allele.

Under "mixed mutants" three examples are included in Table 4 in which unlike variegated offspring were recovered from different sectors on one ear. The differences between mutants derived from the same ear proved to be of two categories, namely, variation in grade of variegation of a given kind (e.g., light and very light variegated), and differences in kind of variegation (e.g., very light variegated and orange-light variegated). This fact shows that more is involved in the reconstitution of a variegated allele from a self-red mutant than mere return of a transposed Modulator to the P locus. Within a given self-red individual, initially derived from a medium variegated pericarp plant, more than one kind of variegated can be reconstituted, presumably by a single-step process in each case. Any hypothesis concerning reconstitution of the variegated pericarp allele must account for this diversity of mutants from a common red pericarp stock.

DISCUSSION

The experimental results afford a positive answer to the question whether the variegated pericarp allele occasionally may be reconstituted in red pericarp plants derived from variegated individuals that carry a transposed Modulator closely

TABLE 4

Distribution of Modulator among offspring of different pericarp phenotypes from sectors on individual ears

No. sectors on ear	Number of such plants	Phenotypes of progeny	Modulator score of progeny
Variegated mutants			
1	2	Medium variegated	+
		P^{wr}	—
2	4	Medium variegated	+
		P^{wr}	—
3	1	Medium variegated	+
		P^{wr} (2)	—
4	1	Medium variegated	+
		P^{wr} (3)	—
2	7	Light variegated	+
		P^{wr}	—
3	1	Light variegated	+
		P^{wr} (2)	—
1	2	Very light var.	+
		P^{wr}	—
2	3	Very light var.	+
		P^{wr}	—
3	1	Very light var.	+
		P^{wr} (2)	—
Orange variegated mutants			
2	4	Medium variegated	+
		P^{wr}	—
3	1	Medium variegated	+
		P^{wr}	—
2	5	Light variegated	+
		P^{wr}	—
3	1	Light variegated	+
		P^{wr} (2)	—
Mixed mutants			
4	1	Light variegated	+
		Very light var.	+
		P^{wr} (2)	—
5	1	Medium variegated	+
		Light variegated	(no test)
		Orange-medium var. (2)	+
		P^{wr}	—
3	1	Very light var.	+
		Orange-light var.	+
		P^{wr}	—

linked to P^{rr}. Such reconstitution evidently involves the same process as that by which primary self-colored pericarp mutants arise in variegated plants, namely, transposition of Modulator. The direction of the transposition in the reconstitution of the variegated allele, however, is to, rather than away from, the P locus. Tests of 140 variegated pericarp mutants isolated from $P^{rr}P^{wr}$ plants carrying a P^{rr}-

linked *Mp* showed in each case that the variegated individual gave a positive test for Modulator and that variegated assorted as a *P* allele.

The foundation self-red pericarp plants whose descendants were the source of the variegated mutants were themselves derived originally as mutants from standard medium variegated pericarp individuals. Some of the reconstituted variegated mutants were indistinguishable from the standard medium variegated class of plants. They duplicated the latter in density of striping, and in breeding behavior in that most of their colored offspring following $P^{vv}P^{wr} \times P^{wr}P^{wr}$ matings, were medium variegated, but with a few self-red and light variegated plants. The occurrence of self-reds as mutants in these progenies shows that the *Mp* in the reconstituted P^{vv} allele may transpose again from the *P* locus, as in standard medium variegated plants. Likewise, the appearance of a few light variegated individuals as mutants in the same progenies demonstrates that the transposing *Mp* element in these cases has the same modifying effect on the variegated phenotype as a transposed Modulator derived from the standard P^{vv} allele (BRINK and NILAN 1952). These are the results expected of a reconstituted variegated allele that corresponds in detail to standard medium variegated.

However, fewer than 20% of the variegated mutants in question were medium variegated, and of this fraction only a part coincided with standard medium variegated, as just discussed. The remainder of the mutants were orange variegateds, light or very light variegateds, or medium variegateds, similar to but not identical with, standard medium variegated. What is the basis of these variegated mutants which seemingly involve a return by transposition of *Mp* to the *P* locus but which condition pericarp phenotypes different than that associated with the standard P^{vv} allele? The available data do not afford a conclusive answer to this question, but three possibilities may be considered, as follows:

(1) The results of the progeny tests entered in Table 2 are consistent with the view, based on a study of the dosage effects of *Mp* (BRINK 1954), that light variegateds and orange light variegateds differ from their respective medium variegated counterparts in possessing a transposed Modulator closely linked to the *P* locus. The very light variegated could be supposed to carry two closely linked *Mp* units. This explanation proved to be untenable, however, in the single instance in which it was tested. The effect on expression of a standard P^{vv} allele of one of the very light variegated mutants in a heterozygote was found to be that of one *Mp* unit, rather than three *Mp* units.

(2) The Modulators at the *P* locus associated with the different pericarp phenotypes may be qualitatively unlike. Significant evidence bearing on this possibility is not now at hand. The idea is directly testable, however, in so far as *Ds* chromosome breakage, which *Mp* promotes, would reflect qualitative differences in *Mp*.

(3) Position of Modulator at, or in the immediate vicinity of, the P^{rr} gene differs from one reconstituted variegated allele to another. This appears to be a reasonable explanation of the variations in phenotype associated with return of transposed Modulator to the *P* locus. A major experimental difficulty standing in the way of testing the hypothesis is that the phenotypes arising from crossing over and transposition of *Mp* are confounded with each other. Small differences in position of *Mp* relative to the P^{rr} gene, therefore, would not be ascertainable.

The general conclusion to which this study leads is that a variegated pericarp allele indistinguishable from that associated with the standard medium variegated phenotype can, in fact, be reconstituted in self-red plants carrying a closely linked Modulator by transposition of the latter back to the *P* locus. A variegated pericarp allele of this kind, however, is not a unique result of such transposition. Other kinds of variegated alleles also arise from the same process. The basis of the variation is unknown.

SUMMARY

An experiment was made to determine whether the process whereby self-red pericarp (P^{rr}) mutants in maize arise in medium variegated pericarp plants ($\overline{P^{rr}Mp}/P^{wr}$) by transposition of Modulator (*Mp*) away from the P^{rr} gene to another chromosome site could be reversed so as to reconstitute the variegated allele ($\overline{P^{rr}Mp}$) by transposition of *Mp* back to the *P* locus. Self-red pericarp, highly inbred plants, heterozygous for colorless pericarp ($P^{rr}P^{wr}$) in which the P^{rr} gene had been derived originally by mutation from variegated pericarp, and was closely linked to a transposed Modulator, served as the foundation stock. A total of 156 mutants were isolated by selecting sectored kernels on the $P^{rr}P^{wr}$ ears, and 146 of them were found to exhibit variegated pericarp. All the variegated mutants proved to be Mp^{+} and behaved as *P* alleles, as would be expected if *Mp* had been transposed back to the *P* locus. Some of the mutants were indistinguishable both in phenotypic effect and breeding behavior from the standard medium-variegated allele from which the foundation P^{rr} stock initially was derived. These mutants are considered to be examples of reconstitution of the variegated pericarp allele in its original, or standard, form. The majority of mutants isolated from the $P^{rr}P^{wr}$ plants, however, conditioned other variegated pericarp phenotypes. An untested explanation of these cases is that the position of *Mp* relative to that of the P^{rr} gene with which *Mp* has become reassociated is slightly different than in the standard medium-variegated pericarp allele.

LITERATURE CITED

ANDERSON, R. E., and R. A. BRINK, 1952 Kernel pattern in variegated pericarp maize and the frequency of self-colored offspring. Am. J. Botany **39**: 637–644.

BARCLAY, P. C., and R. A. BRINK, 1954 The relation between Modulator and Activator in maize. Proc. Natl. Acad. Sci. U.S. **40**: 1118–1126.

BRINK, R. A., 1954 Very light variegated pericarp in maize. Genetics **39**: 724–740.

BRINK, R. A., and R. A. NILAN, 1952 The relation between light variegated and medium variegated pericarp in maize. Genetics **37**: 519–544.

EMERSON, R. A., 1917 Genetical studies on variegated pericarp in maize. Genetics **2**: 1–35.

VALENTINE, F. A., 1957 Orange variegated, a mutant originating from variegated pericarp in maize. Unpublished Ph.D. thesis, University of Wisconsin Library, Madison.

VAN SCHAIK, N. W., and R. A. BRINK, 1959 Transpositions of Modulator, a component of the variegated pericarp allele in maize. Genetics **44**: 725–738.