

MOVEMENT OF MODULATOR IN MAIZE: A TEST OF AN HYPOTHESIS¹

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ABSTRACT

A model of Modulator movement (GREENBLATT 1968) from one chromosomal site to another requires that all movements produce potential twin mutations within the affected cell lineage. From this model the prediction would be that (1) untwinned red and untwinned light-variegated sectors within the pericarp of medium-variegated maize must occur in equal frequency even though partners become lost, during ear morphogenesis, to the final pericarp tissue, and that (2) among the backcross progeny of a homozygous $P^{rr}Mp$ individual mated with P^{wr}/P^{wr} pollen, red offspring and light-variegated offspring would occur in equal frequency. Both expectations have been realized and herein reported.

IT is now possible to equate the movements of a transposable element from a given chromosomal site, the P locus on chromosome one of maize, to other sites in the chromosome complement with the production of a colored/colorless variegation in the pericarp tissue of the maize ear. In fact, it has come to that point in our understanding where it is possible to suggest an unraveling of the mechanism of the element's movement by the genetic analysis of these variegations within the pericarp tissue. GREENBLATT (1968) has outlined a specific sequence of steps which constitutes the mechanism of movement for transposable elements. It is the purpose of this report to provide additional data which are in direct support of the prior conclusion that all transpositions result in twinned genetic consequences, a key factor in the formulation of the hypothesis which attempts to explain how transpositions occur.

Key prior reports which serve as background to these studies were presented by McCLINTOCK (1951), BRINK and NILAN (1952), GREENBLATT and BRINK (1962) and GREENBLATT (1966; 1968). The major relevant conclusions derived from these reports follow:

A. Medium variegated pericarp is conditioned by the P^{rr} allele at the P locus on chromosome 1 conjoined with the moveable element, Modulator (Mp), and is symbolized $\overline{P^{rr}Mp}$. This compound allele may be homozygous ($\overline{P^{rr}Mp}/\overline{P^{rr}Mp}$) or heterozygous ($\overline{P^{rr}Mp}/P^{wv}$). P^{wv} or P^{wr} are alleles at the P locus which do not produce pigment and are neutral to Mp . While Mp is at the P locus, there is no P^{rr} action. During the development of the plant, the Mp leaves the P locus,

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thereby releasing the pigment producing potential of the P^{rr} gene. The resultant phenotype is a variegated pattern of colored/colorless cells throughout the pericarp tissue; the colorless cells still retain at least the original $\overline{P^{rr}Mp}$ complex while the colored cells have a P^{rr} fully functional and no Mp present at the P locus. The number of colored stripes serves to measure the number of transpositions of Mp from P .

B. Light variegated pericarp is conditioned by the addition of a second Mp element to the $\overline{P^{rr}Mp}$ genome. Since Mp can move to sites other than the P locus, it can recombine either mitotically or meiotically with the P locus and segregate with a $\overline{P^{rr}Mp}$ complex. When the Mp which was originally at the P locus moves away, it is referred to as a transposed Modulator ($tr-Mp$). Thus, a light-variegated phenotype is conditioned by $\overline{P^{rr}Mp} + tr-Mp$. The addition of $tr-Mp$ to a cell with $\overline{P^{rr}Mp}$ reduces the frequency of Mp leaving the P locus throughout development. The phenotypic effect is a significant reduction in the number of red stripes in the pericarp. This genetic constitution is most easily viewed in the heterozygous condition in order to identify a single $tr-Mp$ effect. In the heterozygous condition, when large areas of both medium- and light-variegated occur on the same ear, their recognition and separation are very easily accomplished.

C. A red pericarp cell lineage (occurring within a somatic cell generation or the next plant generation) which mutated from medium-variegated, is interpreted to mean that the Mp , formerly conjoined with P^{rr} , has transposed from the P locus and may be elsewhere within the genome. The effect of $tr-Mp$ on the receptor chromosome sites is of no phenotypic consequence in these pericarp studies.

D. The red and light-variegated phenotypes often occur as twinned sectors on otherwise medium-variegated pericarp. Their contiguous relationship is considered evidence that both somatic areas trace back, by mitotic descent, to a single cell in which a single transposition of Mp away from P locus occurred. The red co-twins have a $tr-Mp$ 62% of the time. The light-variegated co-twins carry a $tr-Mp$ all of the time. When both co-twins have a $tr-Mp$, it is at the same site in each. Twins of independent origin have $tr-Mp$ at different sites. Individual red and light-variegated plants also occur among the backcross progeny of a medium-variegated parent. Their frequency among the pigmented class of progeny has been measured as 10.22% red and 7.41% light-variegated. This difference from equality is highly significant. Twin somatic sectors have these two phenotypes in equal frequency.

At issue are the events which allow Mp to leave the P locus and take up residence at another site on a chromosome. GREENBLATT (1968) proposed that (1) Modulator moves only within the limited period in the cell replication cycle during which chromosome one is being replicated, (2) the $\overline{P^{rr}Mp}$ complex replicates while other portions of the chromosome are not as yet replicated, and other portions have already replicated, (3) the new copy of Mp , the one just replicated and not conjoined with the original strand, moves, and (4) it moves to receptor sites on the strand still carrying the original $\overline{P^{rr}Mp}$ complex, not to the newly forming strand which donated the Mp , and (5) these receptor sites function as such due to their state of replication, i.e., they are about to be replicated.

This sequence is believed to be the only one possible for transposition. This was deduced from the conclusion that a single movement of Modulator always results, after the completion of the cell cycle, in one daughter cell carrying the light-variegated phenotypic potential and the other daughter carrying a red phenotypic potential. Any other sequence of events would produce red types without concurrent light-variegated types. Such events are thought not to occur.

The data supporting the conclusion that all transpositions result in only potential twin formation were: (1) physical twin spots are frequently found within medium-variegated pericarp tissue; (2) the difference in frequency of red and light-variegated offspring among the backcross progeny of a medium-variegated parent was accounted for by the recombination of many independently occurring *tr-Mp*'s with the *P* locus at the differential mitosis following transposition and at subsequent meiosis which reduces the number of light-variegated types while leaving the number of red types unaltered; (3) measurement of the number of *tr-Mp*-containing red sectors arising as twinned and untwinned spots in the pericarp found them to be the same. That is to say, the distinction of having a light-variegated twin present or absent in the mature pericarp does not serve to separate red sectors into two classes with respect to the presence or absence of a *tr-Mp* element. Such a separation would be the case, hypothetically, if red sectors could be produced by transposition by a sequence of events other than those postulated.

There are two sources of possible dispute regarding the proposed 1 red:1 light-variegated-per-transposition hypothesis which require reconciliation. (1) Untwinned red and light-variegated sectors are found to occur in medium variegated pericarp. If untwinned sectors are brought about by the random loss of the other sector during ear development then loss of either twin would be expected to occur equally frequently. Thus, counts of untwinned red sectors and untwinned light-variegated sectors should show both occurring in equal numbers. The data that follow show that both untwinned types do indeed occur in a 1:1 ratio. (2) Not finding the twinned mutant classes in equal number among the backcross progeny of a medium-variegated parent has been interpreted to mean that *tr-Mp*, when recombining with the *P* locus prior to the test generation, would convert potential light-variegated types into non-mutant medium-variegated while not affecting the number of red types. GREENBLATT (1968) measured the *P* to *tr-Mp* recombination rate indirectly by using a random sample of red types, each of which arose by independent transpositions. The magnitude of the observed recombination rate was sufficient to account for the calculated deficiency of light-variegated types. If *tr-Mp* recombination does account for the difference in red:light-variegated frequency, then studying its recombination among the progeny of a homozygous medium-variegated backcross mated to a colorless pericarp, non-Modulator stock, should erase this inequality. That is, recombination of *tr-Mp* and *P* would not alter the type or number of the mutant classes. Again, the data that follow show red and light-variegated to be equal in frequency among the progeny in such a test mating.

This additional evidence provides support for the key conclusion that all transpositions lead to 1 P^{rr} (red):1 $P^{rr}Mp + tr-Mp$ (light-variegated) chromosome

type. Such a restriction in the consequences of a transposition requires that the replicating strand which donates the *Mp* for transposition not be the strand that serves as the receptor, and it is most probable that the *Mp* which transposes is the new copy and the receptor strand is not the new copy but the original.

MATERIALS AND METHODS

A major problem in assessing the presence of light-variegated sectors lying adjacent to medium-variegated areas within the pericarp is that the medium-variegated background confuses the definition of the borders of light-variegated sectors which have fewer stripes. In some genetic backgrounds the stripe rate differences are not distinct enough to allow repeatable detection of the light-variegated sectors. In order to minimize this problem, this study employed the genetic background of 4Co63, an inbred line in which the transposition rate of medium variegated is sufficiently high for the use intended. Light variegated sectors have a stripe rate which is distinctly reduced. (See GREENBLATT and BRINK [1962] for a photograph illustrating this phenotype difference.)

Sectors of less than one kernel in size cannot be clearly defined as to their phenotype. Both red and light-variegated phenotypes have too high a probability of being part of the medium-variegated background. To facilitate recording of data, the sectors were arbitrarily divided into small (1 kernel in size), medium (2-8 kernels in size), and large (9 or more kernels in size). Clear definition of red sectors one kernel and larger is no problem. In order to gain an estimate of the frequency of loss of either member of the twins, a count was also made of twinned sectors. In the same way, small, medium and large sectors were recorded.

Data on the number of red:light-variegated offspring arising from a homozygous variegated parent have already been published by BRAWN (1956). They are presented here and discussed with reference to the behavior of *tr-Mp* after transposition from the *P* locus.

RESULTS

Untwinned red and untwinned light variegated sectors were scored on medium-variegated ears which occurred among the progeny of a heterozygous medium variegated in the Iowa Inbred 4Co63. The mating was 4Co63 $\overline{P}^{rr}Mp/P^{ww} \times 4Co63 P^{ww}/P^{ww}$ and yielded a total of 414 offspring, 217 of which were P^{ww}/P^{ww} segregants and were discarded. Among the 197 colored, 174 were medium-variegated, 13 red, and 10 light-variegated. Only the medium-variegated ears were used for the sector counts. The sectoring rates in this population of ears for untwinned light variegated and untwinned red are presented in Table 1.

TABLE 1

Frequency of sectors larger than one kernel in size on heterozygous medium variegated pericarp ears in the 4Co63 genetic background

Sector phenotype	Number of sectors			Totals
	Small (1 kernel)	Medium (2-8 kernels)*	Large (>9 kernels)*	
Untwinned light-variegated	155	19	2	176
Untwinned red	136	17	6	159
Twinned red/light-variegated	453	278	21	752

* When both sectors occurred together as twins, the larger sector was used to define size of sector.

It can be seen from the totals at the bottom of Table 1 that untwinned reds do not occur more frequently than the untwinned light-variegated sectors. If anything, this sample shows them occurring in lesser numbers. These counts, however, fit an expected 1:1 ratio with $p = .25$.

Table 1 also shows that twin sectors are recovered much more often than the untwinned sectors. In fact, it appears that, on the average, both twin members are found in the pericarp in more than 80 percent of the total. It can also be seen in Table 1 that there is a progressive decrease in the number of sectors, both twinned and untwinned, as the size of the sector increases. That is to say, the transposition rate is lower earlier in ear development than later in development. This finding is in accord with the findings of WOOD and BRINK (1956). In their measurements they recorded sectors of one kernel in size and smaller—later transpositional events than those measured in the present study. These data then serve to extend and confirm their findings.

It is of interest to note that when the size of sector is compared between the twinned and untwinned red-light-variegated sectors, the numbers of each agree very well. The small difference noted would be expected since in the twinned class the larger of the two was used for classification, thus biasing toward increased size the twinned class over the untwinned class. Again, when loss of one sector or the other occurs during morphogenesis of the ear, it seems to occur at all stages of development at a proportional rate.

The second way of analyzing the consequences of a transposition is analysis of the phenotypic classes among progeny. As noted above, among the offspring of a $\overline{P^{rr}Mp}/P^{wr}$ heterozygote mated to a P^{wr}/P^{wr} non-Modulator stock, the frequency of reds significantly exceeds the number of light-variegated types. The argument developed by GREENBLATT (1968) was that the recombination of $tr-Mp$ with the P locus after transposition would reduce the number of realized light-variegated offspring but not reduce the number of red offspring. An experiment which ascertains the frequency of red and light-variegated ears among the progeny of homozygous $\overline{P^{rr}Mp}/\overline{P^{rr}Mp}$ plants pollinated by a P^{wr}/P^{wr} stock would make this recombination loss of transposed Modulator meaningless since if it segregates from one $\overline{P^{rr}Mp}$ complex, it will then segregate with the other $\overline{P^{rr}Mp}$ complex at meiosis. Thus, the numbers of red and light-variegated mutant types should be equal in spite of $tr-Mp$ recombination with P .

Such results have already been reported in an unpublished doctoral thesis by BRAWN (1956) but they were not interpreted in this context. They are reprinted here in Table 2. From the data it can be seen that the red class and light-variegated class are occurring in essentially equal frequencies. Thus, when counts of red and light-variegated class frequencies are made directly in a homozygous variegated progeny and as somatic sectors on medium variegated ears, red and light-variegated classes occur in equal frequency. Couple these findings with the previously published results and interpretations from heterozygous medium-variegated progeny (GREENBLATT 1968); the conclusion that all transpositions lead to both mutant classes in equal frequency is inescapable.

TABLE 2

Segregation of major colored pericarp phenotypes among the offspring from the mating $W23 \overline{P^{rr}Mp}/\overline{P^{rr}Mp} \times P^{wr}/P^{wr}$

Pericarp phenotype	Number of ears	Percent colored
Medium-variegated	6114	90.53
Red	302	4.47
Light- and very light-variegated	337	4.99
Total	6753	100.00

Data extracted from BRAWN (1956).

* The relatively rare orange variegated and colorless pericarp classes are omitted for reason of clarity.

DISCUSSION

The model of Modulator transposition outlined in Figure 1 provides for only potential twin mutations resulting from *Mp* transposition. GREENBLATT (1968) has already shown that alternative hypotheses by which Modulator could transpose provide for an excess of the red mutant class over the light-variegated class. The equality in frequency of these two mutant consequences of transposition then becomes a key issue in unravelling how Modulator moves.

With the data presented in this and prior reports (GREENBLATT 1966, 1968; and GREENBLATT and BRINK 1962), it seems clear that the red and light-variegated classes are being formed at the time of transposition in equal frequency. The direct evidence for this conclusion comes from: (1) twinned red-light-variegated sectors in pericarp occurring in significant numbers. There is no question that twinned sectors come from a single transpositional event. Not only are these two phenotypic classes contiguous in the pericarp, but the *tr-Mp* element found in 62% of the red co-twins resides at the same chromosomal site in their light-variegated co-twins (GREENBLATT 1966). (2) The number of red sectors which have no co-twin within medium-variegated pericarp occur equally as frequently as the untwinned light-variegated sectors. Based on the random loss of sectors due to the uncertain morphogenesis of the ear, this is the result expected if red and light-variegated types are produced in equal frequency at the time of transposition. The loss of one of the two members of a twin mutation in the pericarp seems to be minor in the 4Co63 inbred. Table 1, considering those transpositions which have occurred early enough to result in a spot within the pericarp of 2 kernels or larger, approximately 87 percent are recognizable as twin spots. The loss rate is higher in the later transpositions resulting in spots one kernel in size (only 61 percent are recognized as twins). (3) When the backcross progeny of homozygous $\overline{P^{rr}Mp}$ individuals is used to ascertain the red:light-variegated ratio, the frequencies are found to be equal (Table 2). The segregational losses of light-variegated noted by GREENBLATT (1968) are eliminated so that even though recovery of these phenotypic classes is in the subsequent generation from the one in which the transposition occurred, red mutants and light-variegated mutants are still found in equal frequency. Among the backcross progeny of heterozygous medium-variegated individuals the excess of reds over

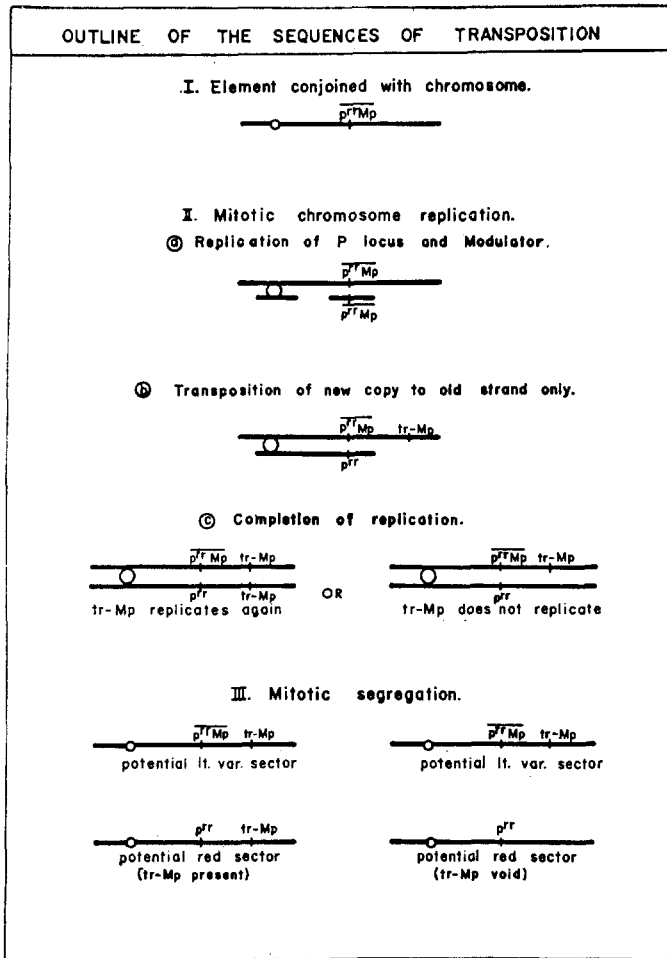


FIGURE 1.—An outline of the hypothesis under test. No statement is implied as to whether Modulator is “in” or “on”, the locus of *P*.

light-variegateds was accounted for by the recombination of *tr-Mp* with the *P* locus. Recombinational loss from the red types does not affect their number, but recombination in a potential light-variegated converts it genetically so that medium-variegated results in the pericarp.

Interestingly, there is an expected recombination of *tr-Mp* with *P* from the red sectors in homozygous medium-variegated which tends to increase the number of light-variegated types without reducing the number of reds. The extent of this change can be calculated from the following points: 62% of the reds resulting from independent transposition are expected to carry a *tr-Mp*. Within this grouping the recombination rate of *tr-Mp* and *P* has yielded a mean value of 17.1% (GREENBLATT 1968). All the *tr-Mp* elements which segregate from *P^{rr}* will segregate with $\overline{P^{rr}Mp}$. Only half of such $\overline{P^{rr}Mp} + tr-Mp$ complexes would be included in a functional megaspore. Thus, $.62 \times .171 \times .50$ equals an increase of

5.3% light-variegated over reds. The percent found using the data in Table 2 is 5.4% more light-variegateds than reds. This increase does not, however, show as a statistically significant deviation from 1:1 ratio as noted above.

The conclusion is that all transpositions lead to an equal number of potential red and light-variegated sectors. The model of the sequences of transposition (Figure 1) suggests that the Modulator element which is transposed from the *P* locus is the one that is newly replicated and not yet connected to the linear replicating strand. It is also seen that the *Mp* does not come from the strand functioning as the receptor. By such a mechanism of transposition an equal number of red:light-variegated types arise, taking into account that 38 percent of these events do not result in *tr-Mp* replicating a second time. As pointed out by GREENBLATT (1968), if *tr-Mp* were to transpose to the newly forming strand and not replicate again, a twin mutation would not result. The $\overline{P^{rr}Mp}$ strand is thus referred to as the *receiver* or *old strand* and the *P^{rr}* strand is referred to as the *donator* or *new strand*.

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