TWIN MUTATIONS IN MEDIUM VARIEGATED PERICARP MAIZE[1]

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THE variegated allele (P^{vv}), conditioning irregular red striping in the pericarp and cob of maize frequently mutates in somatic cells to stable self-red (P^{rr}), and to unstable light variegated (BRINK and NILAN 1952; BRINK 1954; BRAWN 1956). A hypothesis, based on the work of MCCLINTOCK (1950, 1951) and BRINK and associates (1952, 1954), which attempts to explain the high mutability, depicts the P^{vv} allele as a compound structure composed of the P^{rr} gene for red pericarp and cob conjoined with a transposable element, Modulator (*Mp*), which acts as an inhibitor of pigmentation when at the *P* locus. EMERSON's P^{vv} symbol (EMERSON, BEADLE, and FRASER 1935) for variegated pericarp may be equated, therefore, to $\overline{P^{rr}Mp}$. Transposition of *Mp* from the *P* locus results in full expression of the pigment-producing action of the P^{rr} allele. Loss of *Mp* from the *P* locus usually is coincident with acquisition of the element at some other site, often on the same chromosome (VAN SCHAIK and BRINK 1959). Modulator at a new site, i.e., transposed Modulator (*tr-Mp*), plus the variegated allele at the *P* locus, result in the light variegated pericarp phenotype.

Red and light variegated mutations sometimes occur as readily observable twin spots on medium variegated ears (Figure 1). According to an earlier hypothesis

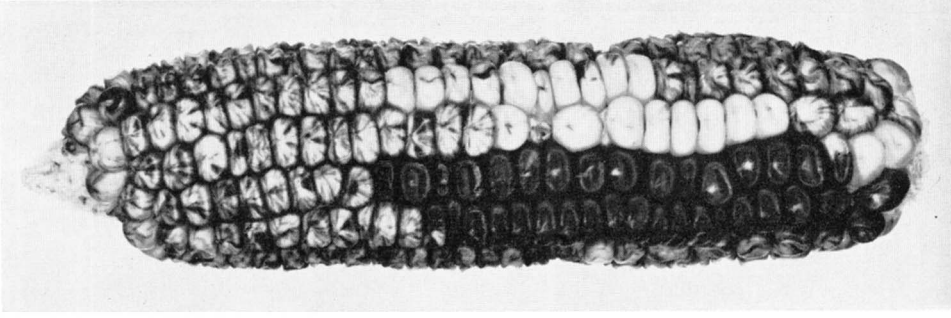

FIGURE 1.—Twin mutations consisting of adjacent patches of red and light variegated kernels on an otherwise medium variegated ear.

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(BRINK and NILAN 1952) a twin spot results from a mitosis that is differential at the *P* locus: the *Mp* element lost from one daughter nucleus (red), as a result of transposition away from P^{rr}, was assumed to be conveyed, along with an unchanged P^{vv} allele, to the other (light variegated) daughter nucleus. Subsequent tests have disclosed, however, that twin spots are not a homogeneous group, but fall into two classes with respect to the presence or absence of *tr-Mp* in the red component (BRINK 1955). The present study is concerned particularly with the class of twin mutations which contains *tr-Mp* in the red sector as well as the adjacent light variegated sector.

It is postulated that this class of twin mutations results from a single transposition of *Mp* during the time of chromosome replication. P^{rr} and its conjoined *Mp* are assumed to replicate at the *P* locus, producing two $\overline{P^{rr}Mp}$ complexes, prior to replication of certain other portions of the chromosome. An *Mp* from one of the two newly formed daughter $\overline{P^{rr}Mp}$ complexes transposes to such an unreplicated site, and then replicates in phase with the chromosome in that region. Completion of the mitotic cycle would result in daughter nuclei of two genotypes: (1) P^{rr} + *tr-Mp*, conditioning red pericarp, and (2) $\overline{P^{rr}Mp}$ + *tr-Mp*, which gives rise to the light variegated phenotype.

If the above interpretation is valid then it would be expected that *tr-Mp* would be situated at the same locus in the red and light variegated co-twins in a given case. Three-point linkage data are presented in this report that confirm the predicted identity of chromosome position of *tr-Mp* in the red and light variegated sectors of thirteen independently occurring twin mutations.

MATERIALS AND METHODS

All the stocks used to obtain the twin mutations contained a P^{vv} allele from a common source, and were kept heterozygous for one or the other stable colorless pericarp allele, P^{wc} or P^{wr} (white cob and red cob, respectively). The P^{vv} allele had previously been introduced into two highly inbred dent corn lines, 4Co63 and W9, by repeated backcrossing. The light and medium variegated phenotypes are sharply distinguishable on these uniform genetic backgrounds.

Most of the twin mutations studied were obtained from the 4Co63 line, because of the pronounced instability of P^{vv} in this stock. Although about twice as many variegated plants were grown from W9 as from 4Co63 stocks, only two of a total of the 32 twin mutations isolated were derived from the former group. It is not believed that the contrast in rate of twin formation in the two strains reflects a change in stability of the P^{vv} allele itself. The difference in residual heredity is a more likely explanation. Two distinct transposition rates of Modulator were noted previously when the variegated allele in question was introduced into two other inbred lines, W22 and W23 (BRAWN 1956).

The markers utilized in the three-point backcross linkage analysis were *tr-Mp*, *P*, and the breakage point of one or another of the reciprocal translocations (T) listed below (which give a semisterile phenotype when heterozygous). The *P* markers used in plants derived from the red sectors were P^{rr}, P^{wr}, and P^{ww}. The

same markers were employed for the light variegated derivatives, except that P^{vv} replaced P^{rr}. The reciprocal translocations provided marked points either distal (T1-7g, 1S.79-7S.22), or proximal (T1-2b, 1S.43-2S.36; T1-5b, 1S.17-5L.10, LONGLEY 1958) to *P* on the short arm of chromosome 1. The method of detecting the hemizygous *tr-Mp* factor is described below.

In certain cases matings were designed to incorporate the translocation desired as a marker into the two mutant genotypes after twin formation. This usually involved the pollination of a P^{vv}/P^{ww} *c r* stock with a T1-2b/normal P^{wr} *c r* plant, both parents being derived from 4Co63 lines. (*c* and *r* condition colorless aleurone; their dominant alleles, *C* and *R*, are complementary factors for colored aleurone.) Exceptions to this procedure were (1) a single twin spot from T1-7g/+ plant which had been backcrossed to inbred 4Co63, and (2) a twin mutation on a medium variegated ear in W9 background which had been backcrossed to inbred W9. The plants derived from this latter twin spot were in turn pollinated by T1-5b/+ plants.

Plants obtained from seeds in the red and light variegated sectors, and heterozygous for one of the reciprocal translocations, were used as female parents in backcrosses to a stock of the constitution nonsemisterile $P^{ww}wx$ *c r Y*, and known to lack Modulator. The waxy (*wx*) and yellow (*Y*) seed markers permitted detection of contaminants through the pollen. Pollen from the same plants was placed on silks of a "*C-Ds*" line, in order to test specifically for the presence of Modulator. The *C-Ds* line carried, in addition to the dominant, complementary, aleurone color genes *A*, *C*, and *R*, the Dissociation (*Ds*) factor in the standard position proximal to the *C* locus on the short arm of chromosome 9. *Ds*, in the form here used, promotes chromosome breakage with high frequency at its locus only if either Activator (MCCLINTOCK 1951) or Modulator, its counterpart in variegated pericarp strains (BARCLAY and BRINK 1954), is present in the genome. Breakage at *Ds* leads to the loss of the dominant *C* factor, thus producing a colored-colorless aleurone mottling pattern on the tester ears.

Some of the twin mutations originally obtained subsequently proved unusable for the desired three-point linkage studies, because either (1) the red sector did not contain a transposed Modulator (see Table 1), or (2) individuals segregating all three factors, including *tr-Mp*, were not found among the plants obtained from both the usually small, red and light variegated sectors.

TABLE 1

Distribution of transposed Modulator in the red sectors of 80 independently occurring red and light variegated twin mutations in four medium variegated stocks

Stock	Transposed Modulator present	Transposed Modulator absent
1	11	7
2	9	5
3	13	6
4	19	10
Total	52	28

Offspring carrying the necessary markers were obtained from seeds in both sectors of 13 twin mutations. These plants were pollinated by P^{ww} *wx c r Y*, as stated above. Families, based on single ears, were then grown out for classification in a detasseling plot in which a P^{ww}, *wx* nonsemisterile, *C-Ds* stock was used as the male parent. Use of a homozygous *C-Ds* line as pollen parent permitted scoring for *tr-Mp* on a plant to plant basis as well as for the *P* marker and semisterility.

In scoring the variegated pericarp classes of plants, the *C* to *c* mottling was not used to determine the presence of *tr-Mp*, but rather the grade of pericarp variegation of each such individual was recorded (i.e., medium variegated = P^{vv}; light variegated = P^{vv} + *tr-Mp*). The necessity for scoring the variegated pericarp plants by this method became evident early in the study, because the expected *C* to *c* mottling was found to be obscure on some light variegated ears. Scoring for medium and light variegated was obligatory when *tr-Mp* was closely linked with *P*. In families in which *tr-Mp* was recombining at random with *P*, or was loosely linked, at least a few kernels on the light variegated ears exhibited readily observable *C* to *c* mottling and so were scorable on this basis. The occurrence of light variegated ears without the expected *C* to *c* pattern is explained by the finding of BARCLAY and BRINK (1954) that increasing dosages of Modulator postpone *Ds* breakage events. Seemingly, mitosis usually is completed in triploid aleurone cells containing four transposed Modulators before the onset of *Ds* action.

All newly arisen red mutant plants from light variegated parents were excluded from the data for the reason that a *tr-Mp*, if present, could be attributed either to the *tr-Mp* being mapped or to an extraneous Modulator newly transposed from the *P* locus. The newly arisen, and comparatively infrequent, very light variegated mutants (P^{vv} + 2 *tr-Mp*) from light variegated parents, however, were included. This decision was based on the assumption that in such cases the original *tr-Mp* was still residing at the site taken at the time of co-twin formation.

χ^2 tests for heterogeneity were employed throughout in evaluation of the recombination data obtained from within a sector and within a twin.

EXPERIMENTAL RESULTS

Tests for Modulator in the red member of co-twins: Data bearing on the frequency with which the red sector in twins taken at random contain a transposed Modulator are found in Table 1. The four groups in the table represent the results obtained on scoring, by the C-Ds test, four separate lots of plants of this origin. It is evident that (1) the different samples of twins give about the same result with respect to the relative frequency of occurrence of the two classes of twin mutations and (2) twin mutations with a *tr-Mp* element present in the red sector are the more frequent class, comprising 65 percent of the total. Most of the red sectors tested were of sufficient size, in terms of seed number, to preclude complete loss of Modulator from hemizygous *tr-Mp* stock due to sampling variation during meiosis in the parent plants. It appears, therefore, that red sectors lacking Modulator must have resulted from exclusion of this element from the

P^{rr} daughter cell at the time the twin was initiated. By the same token, Modulator must have been included in the other class of twin mutations at this stage.

Detection of secondary transpositions: Linkage studies of Modulator are complicated by the occurrence of transposition of the element away from the site first occupied following removal from the *P* locus. VAN SCHAIK and BRINK (1959) found cases in which the sites of *tr-Mp* in subfamilies derived from a single parent, in which the linkage of *tr-Mp* had been determined, differed both from each other and from the stem plant. Attempts were made to identify secondary transpositions in the present study by comparing the recombination values of *tr-Mp* and the closest marker with the corresponding values for sib families derived from seeds within the same mutant sector. If the value found for a particular family exhibited a highly significant deviation from that of most of its sib families, it was regarded as due to secondary transposition, and was then removed from further calculations. The basis for the decision was a χ^2 test for heterogeneity between the outlying value and the next closest observed value. Such tests disclosed seven cases in which *tr-Mp* was at a site different from the one found in sib families (Table 2). These seven instances constitute eight percent of the 92 families tested. The secondary sites thus identified represent only the larger changes in linkage due to secondary transpositions, since short moves of Modulator along the chromosome would not have been detected by the test used. In no instance did the families removed from further calculation constitute a majority of the items available for computing linkage. This treatment is believed to have removed from the linkage data the gross errors due to secondary transposition and, since it was applied to the derivatives of both twin components, did not introduce bias.

TABLE 2

Frequency of recognizable secondary transpositions in the progeny derived from the red and light variegated sectors of 13 independent twin mutations

Twin number	Number of secondary transpositions		Number of families analysed
	Red sector	Light variegated sector	
1	1	0	10
2	1	0	7
3	0	0	9
4	2	1	10
5	0	0	4
6	0	0	7
7	0	0	3
8	1	0	5
9	0	0	6
10	0	0	5
11	0	0	6
12	0	1	15
13	0	0	5
Total	5	2	92

Tests for the sites of transposed Modulator in co-twins: The observed percentages of recombination between *P*, *tr-Mp*, and the particular reciprocal translocation (T) involved are summarized in Table 3 for each of the 13 twin mutations analyzed.

The data show that transposed Modulator in the different twin spots varies widely in degree of linkage with the markers used. There are seven twins in which *tr-Mp* is clearly linked to *P*, and five cases in which *tr-Mp* is recombining at random with *P* (twin 5 is an exception—see below). In contrast, the linkage values between *P* and the breakage point of T1-2b are essentially the same for the eleven twins in which this comparison was possible.

Differences in the recombination values between *tr-Mp* and the *P* and T markers for the two sectors of a given twin spot are no greater than those found for the interval *P* to T. This high concordance holds for both the degree of linkage and the linear order of *tr-Mp* with respect to *P* and T in the two sectors of a given twin. As the data obtained from the seven twins in which *tr-Mp* is linked to *P* show, it is thus evident that only a single chromosomal site is involved for the two sectors comprising a given twin spot.

A conclusive statement cannot be made concerning the *tr-Mp* sites in the five twins showing random recombination of *tr-Mp* with the two other markers, because their chromosome location is not disclosed by the available data.

Although a χ^2 test for heterogeneity of the recombination data between *tr-Mp* and the marker found to be closest (*P* or T) does not disclose highly significant differences upon a within-twin analysis, the light variegated sectors consistently show lower values than their corresponding red co-twins. This difference between corresponding sectors is not evident, however, in the five twins in which *tr-Mp* is recombined at random with *P* and in two twins (12 and 13) in which *tr-Mp* showed 20 percent or more recombination with *P*. The possible significance of these findings is discussed in the following section.

The recombination value found for the *P* to *tr-Mp* interval in the red sector of the exceptional twin 5 is 19.19, based on a three-family sample. The light variegated sector, from which there was only one family available for testing, shows that *tr-Mp* recombined at random with both *P* and T. From the standpoint of the experimental results as a whole, a likely explanation for this exception is that the site of *tr-Mp* in one of the sectors was taken as the result of a secondary transposition.

Direction of the site of *tr-Mp* from the *P* locus may be determined from the data in those cases in which linkage occurs. As can be seen from the data in Table 3, the seven twins in which the site is clearly linked to that of *P* (omitting twin 5 because of the difference between the red and light variegated sectors) fall into three groups. *Tr-Mp* is located distally to *P* in five cases, there is no clear distinction as to direction from the *P* locus in the case of twin 11, and in twin 13 *tr-Mp* appears to lie proximal to *P*. The reciprocal translocation present in the case of twin 13 was T1-5b, marking a site close to the centromere. The site of *tr-Mp* was found to be 19 units proximal to the breakage point. It is likely, therefore, that this *tr-Mp* site is on the long arm of chromosome 1.

TABLE 3

Mean percent recombination between P, tr-Mp, *and the chromosome 1 break point of three reciprocal translocations for the red and light variegated sectors of 13 independent twin mutations*

Twin number	Sector phenotype	No. of families scored	No. of individuals	Mean percent recombination		
				P and *tr-Mp*	*P* and T	T and *tr-Mp*
			T = T1–2b (1S.43 2S.36)			
1	red	6	764	3.66(.5)†	2.49(.5)	5.63
	lt. var.	4	542	2.21(.25)	3.51(.25)	4.61
	Total	10	1306	3.06(.25)	2.91(.5)	5.21
2	red	4	517	48.94(.5)	2.90(.5)	48.74
	lt. var.	3	353	46.46(.5)	4.53(.1)	47.59
	Total	7	870	47.93(.75)	3.56(.25)	48.28
3	red	4	463	46.87(.5)	3.67(.75)	47.08
	lt. var.	5	595	47.39(.5)	3.53(.95)	46.38
	Total	9	1058	47.16(.5)	3.59(.95)	46.69
4	red	5	633	8.21(.25)	3.16(.01)*	9.95
	lt. var.	5	402	6.43(.1)	3.57(.5)	9.45
	Total	10	1035	7.50(.25)	3.32(.05)	9.59
5	red	3	271	19.19(.025)*	4.80(.5)	23.25
	lt. var.	1	107	50.47	10.28	45.79
	Total	4	378	28.04(.01)**	6.35(.1)	29.63
6	red	2	137	13.87(.1)	5.84(.5)	19.71
	lt. var.	5	581	9.29(.9)	4.82(.1)	13.08
	Total	7	718	10.17(.25)	5.01(.25)	14.35
7	red	1	105	48.57	10.48	43.81
	lt. var.	2	213	46.95(.5)	4.23(.25)	47.41
	Total	3	318	47.48(.75)	6.29(.05)	46.22
8	red	3	269	45.35(.25)	5.20(.25)	46.84
	lt. var.	2	262	48.85(.25)	4.96(.1)	51.52
	Total	5	531	47.08(.25)	5.08(.25)	49.15
9	red	5	466	7.08(.75)	4.51(.05)	10.73
	lt. var.	1	121	4.13	1.65	4.13
	Total	6	587	6.47(.5)	3.92(.025)*	9.37
10	red	2	284	52.81(.1)	5.28(.25)	52.46
	lt. var.	3	261	46.74(.25)	3.83(.5)	46.74
	Total	5	545	49.91(.1)	4.59(.5)	49.72
11	red	3	322	12.42(.25)	3.11(.25)	11.18
	lt. var.	3	228	4.39(.5)	1.75(.75)	4.39
	Total	6	550	9.09(.01)*	2.55(.5)	8.36
			T = T1–7g (1S.79 7S.22)			
12	red	10	957	19.33	17.76(.025)*	3.45(.5)
	lt. var.	5	473	21.14	16.91(.5)	5.07(.1)
	Total	15	1430	19.93	17.48(.1)	3.99(.25)
			T = T1–5b (1S.17 5L.10)			
13	red	1	136	29.41	25.00	19.12
	lt. var.	4	480	32.29	27.50(.5)	18.96(.025)*
	Total	5	616	31.66	26.95(.5)	18.99(.05)

† Probability level (a within sector and within twin χ^2 analysis of heterogeneity).
* Significant at 5% level.
** Significant at 1% level.

Additional information concerning the direction from *P* in which Modulator transposes initially is derivable from the data obtained from a light variegated sector whose red co-twin was not available for the three-point analysis due to lack of suitably marked plants. The site of *tr-Mp* in this light variegated sector was distal to *P*, and showed 6.76 percent recombination with *P*, based on a sample of 148 plants. It thus appears that in the class of twin mutations which contains a *tr-Mp* element in both co-twins on the short arm of chromosome 1, Modulator is distal, relative to the site of origin, in the six cases studied.

DISCUSSION

The results presented for the seven twin mutations in which transposed Modulator was found to be located on chromosome 1, and was present in the red, as well as in the light variegated, sectors show that, for a given twin, the chromosomal site is the same in the two cases. The sites of *tr-Mp*, however, differ from twin to twin.

In the five cases in which *tr-Mp* was found to recombine at random with *P* and T the location in the genome of *tr-Mp* remains unknown. This does not necessitate qualification, however, of the conclusion that the *tr-Mp* element in each sector of a given twin is at the same location on the chromosome because the data in question do not serve to test this point.

The following sequences of events are postulated to account for the two classes of twin spots and the linkage data presented:

Twins in which the red sector lacks tr-Mp:

(1) During a mitotic division the chromosome carrying the single variegated allele in a plant heterozygous for colorless pericarp duplicates at the *P* locus to form two daughter chromatids for this region.

(2) *Mp* is separated from the *P* locus in one of the chromatids at the same time, thus changing the variegated allele to the red allele.

(3) The separated *Mp* element is transposed to a chromatid site elsewhere in the genome.

(4) The receptor chromatid then passes to the daughter nucleus which also receives the chromatid carrying the unchanged variegated allele.

(5) The respective "P^{rr}" and "$\overline{P^{rr}Mp}$ + *tr-Mp*" daughter cells then become progenitors of adjacent cell lineages of two distinct kinds, which are manifest in the mature ear as the red and light variegated sectors of a twin mutation.

Twins in which the red sector carries tr-Mp (*see Figure 2*): The differences from the above sequence are assumed to be as follows:

(1) Following loss from the *P* locus in one of the daughter chromatids *tr-Mp* is inserted, during the same mitotic cycle, at a new site at which the chromosome has not yet duplicated.

(2) Subsequent chromosome duplication at the receptor site yields two daughter *tr-Mp* chromatids, one of which accompanies the (mutant) P^{rr} allele to one daughter nucleus, and the other of which passes to the same daughter nucleus as the unchanged $\overline{P^{rr}Mp}$ complex.

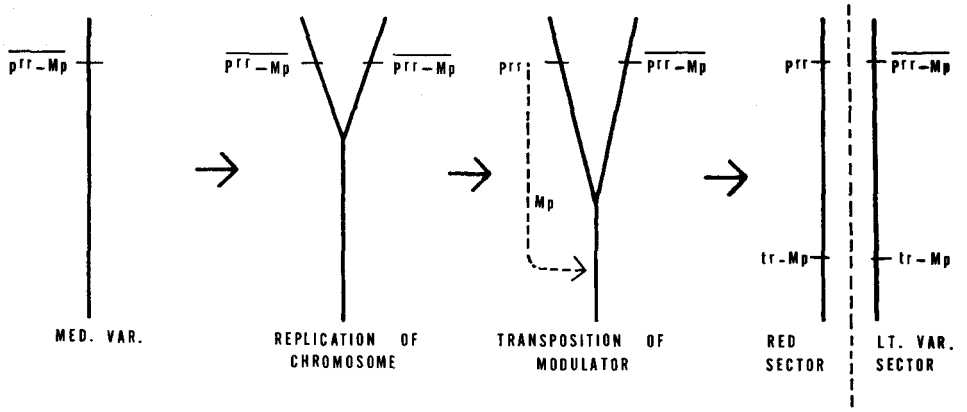

FIGURE 2.—Diagrams illustrating the postulated sequence of events in a heterozygous medium variegated plant heterozygous for colorless leading to the origin of a red-light variegated twin mutation which contains a transposed Modulator in both sectors. (Note: The stable p^{wr} homologue conditioning colorless pericarp is not shown.)

This hypothesis explains the origin of twinned mutations, and also accounts for the common site of the transposed Modulators in co-twins. Likewise the presence of two transposed Modulators in the mutant sectors, whereas only a single Modulator has been lost from the *P* locus, is understandable on this basis.

Basic to this hypothesis is the assumption that a single transposition of Modulator from the *P* locus underlies the development of a twin mutation. Another explanation based on two successive transpositions of Modulator can also be considered. Such an alternative would be as follows: A twin spot forms as postulated by BRINK and NILAN (1952) by passage of the transposed Modulator from one chromatid in a differential mitotic division to the daughter nucleus which receives the unchanged variegated allele, but the recipient "red" cell does not divide so that this potential sector is not realized during development of the ear. A twinned red sector could then arise following a second transposition of *Mp* away from the *P* locus in the presumptive light variegated cell lineage at the next, or an early, mitosis after the primary transposition. Such a red sector would have a transposed Modulator at the same site as in the light variegated co-twin. This second transposition is postulated to occur in a cell carrying $\overline{P^{rr}Mp}$ plus a transposed Modulator, an event which has a probability of occurrence about one third that of the original transposition in heterozygous medium variegated tissue (WOOD and BRINK 1956). On the basis of this reasoning one would expect that the red component in twin mutations would only rarely contain a *tr-Mp*. The findings reported here (Table 1) show, however, that approximately two thirds of the 80 twins scored contained *tr-Mp* in the red member.

A priori it might be supposed, when Modulator transposes from the *P* locus and becomes inserted elsewhere in the genome, there are so few other sites that can receive the element that the new positions would coincide by chance in appreciable proportion of co-twins. The several chromosome locations at which transposed Modulator was found, however, invalidates this explanation.

The chromosomal sites at which *tr-Mp* was found in the present analysis correspond with those reported by VAN SCHAIK and BRINK (1959) in that a high proportion were located on chromosome 1 and linked with *P*. In this connection it should be noted that VAN SCHAIK and BRINK selected the majority of the independent light variegated mutants studied as single kernels on otherwise medium variegated ears or as single ears (presumably a single kernel on the parent ear) in the progeny of a medium variegated plant. A bias with reference to the position of transposed Modulator is introduced by this procedure that was not recognized at the time. A meiosis intervening between transposition of *Mp* from the *P* locus and the realized light variegated plant provides opportunity for loss from the genome of *tr-Mp* by segregation in a significant proportion of the cases. As the distance increases between *P* and *tr-Mp* the more frequently will *tr-Mp* and $\overline{P^{rr}Mp}$ be assorted to different cells at meiosis. Thus, the results VAN SCHAIK and BRINK obtained would minimize the number of distant sites, and maximize the number of closely linked sites identified. Even with estimates of a theoretical maximum set for such a bias it still does not invalidate their conclusion, however, that the sites of *tr-Mp* are much more often linked to the original position at the *P* locus than would be expected by chance alone. Only the proportion of closely linked sites over more distant ones comes into question.

Recombination between Modulator and chromosomal markers may result from crossing-over and reassortment during meiosis, or transposition in somatic cells and possibly during meiosis also. That transposition of *Mp* in somatic cells leads to recombination is suggested by the data in Table 3 relating to the five twins in which close linkage of *Mp* was indicated. The percent recombination between *tr-Mp* and *P* in the light variegated class in four of the five cases was found to be regularly below that obtained for the corresponding twinned red sectors. In twin 4 the difference is significant at the five percent level, but not at the one percent level. The lower values of recombination between *tr-Mp* and *P* shown by the light variegated class is what would be expected if transposition led to recombination and the process occurred more frequently in red than in light variegated twin mutants. The red sector contains only a single *tr-Mp* element, whereas the light variegated sector contains a *tr-Mp* in addition to the Modulator resident at the *P* locus. WOOD and BRINK (1956) showed that one Modulator strongly suppresses the transposition rate of another Modulator elsewhere in the same genome.

Transposition of *Mp* from *P*, when *P* is carried on a reciprocally translocated chromosome, apparently is not affected by the discontinuity associated with the breakage point. In twin mutation 12 (Table 3), which occurred on a semisterile ear carrying T1–7g, the primary transposition of *tr-Mp* was to the chromosome 7 side of the break. Information concerning the other two translocations (T1–2b, T1–5b) on transposition of *Mp* is unavailable since the translocation markers were introduced after the twin mutation occurred.

Two classes of twin mutations were observed in the present study. The red component of one class carries a transposed Modulator, whereas the other class lacks this element in the red sector. The observed numbers of the two respective kinds of twins were 58 and 28. The meaning to be attached to the difference in

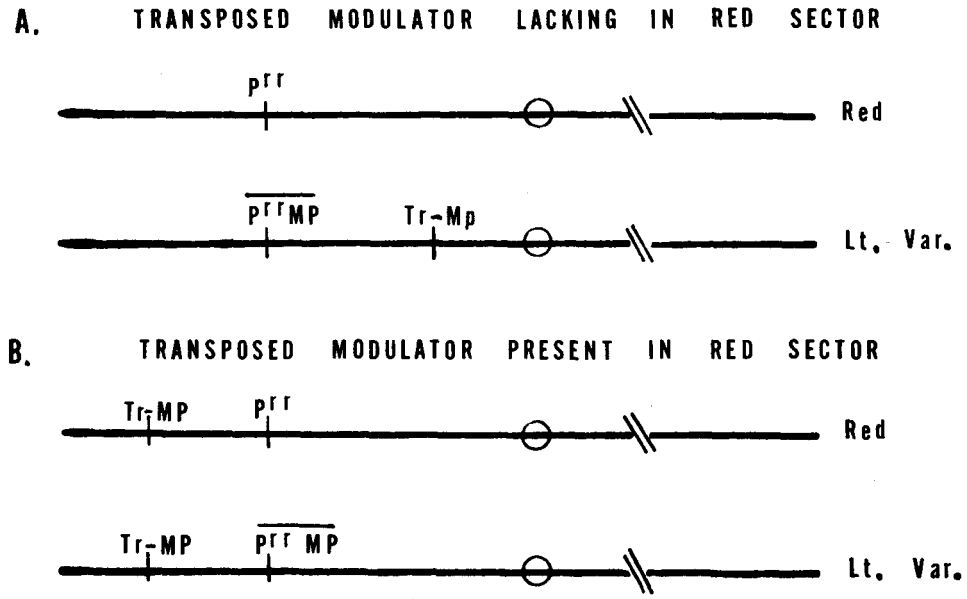

FIGURE 3.—Diagrams illustrating the chromosome constitution found in the two classes of red-light variegated twin mutations. (A) transposed Modulator absent from the red sector, (B) transposed Modulator present in the red sector.

frequency is problematical because it is not certain that the sample studied is representative of red and light variegated twins in general.

There are two main reasons why the total frequency of red-light variegated twin mutations cannot be reliably determined. The first is that the mutated tissue is three-dimensional, whereas only the two-dimensional, surface configuration of a mutant region can be observed. The proportion of twinned mutations in which one component is entirely interior to the other in the ear remains unknown. Secondly, as ANDERSON and EYSTER (1928) originally showed, the frequency of mutation of the unstable, colorless, P^{vv} allele to red increases rapidly as development of the plant proceeds. Most red mutations on a medium variegated ear are expressed, therefore, as small stripes, grading down to areas of microscopic size. It is usually impossible to determine whether those small colored areas are, or are not, twinned with a light variegated sector; the latter, when small, are not identifiable against the medium variegated background, because the contrast between the phenotypes (two intensities of striping) disappears at the lower end of the size scale. The present study is based on early occurring and, hence, large twins. It affords no data on the question whether, as the mutation rate to red rises steeply at later ontogenetic stages, the twinning process also is affected.

GREENBLATT (unpublished data) has observed that approximately one half of *apparently* untwinned large red sectors on medium variegated pericarp ears carried a transposed Modulator. The meaning of this value also is in doubt on the grounds that the classification is uncertain for the reason stated above, and the

representativeness of the sample tested is unknown. It may be pointed out, however, that if during the postulated differential mitosis underlying twin formation, transposed Modulator assorts with the P^{rr} allele but is not duplicated a second time in the same cycle, then the result would be an untwinned red sector containing a *tr-Mp* element.

SUMMARY

Studies of twinned red-light variegated somatic sectors on otherwise medium variegated ears have disclosed the following: (1) There are two classes of twin spots with respect to their Modulator composition. Sixty-five percent of the red sectors were found to contain a Modulator while the remainder were void of *tr-Mp* (the light variegated sector regularly carries two *Mp* elements). The pericarp phenotypes of these two classes of twin spots are indistinguishable. (2) A three point linkage analysis of the *tr-Mp* element found in the red sector and its co-twinned light variegated sector shows the *tr-Mp* element to be located at the same chromosomal site in both sectors of a given twin. The sites found for the *tr-Mp* element in twins of independent origin were, however, not the same. Of the 13 cases studied, seven twins showed *tr-Mp* linked with the markers *P* and Translocation break point and five cases in which *tr-Mp* was recombining at random with these same fixed markers. An hypothesis is presented which explains both the presence of *Mp* in the red co-twin and the concordance in linkage results.

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