R-STIPPLED MAIZE AS A TRANSPOSABLE ELEMENT SYSTEM

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ABSTRACT

The *I-R* element at the *R* locus destabilizes kernel pigmentation giving the variegated pattern known as stippled (*R-st*). In *trans* linkage phase with *R-st* the element was shown to act as a modifier of stippled, intensifying seed spotting in parallel with effects of the dominant linked modifier *M-st*. Presence of *I-R* in the genome was, therefore, shown to be detectable as a modifier of *R-st*. When this test was used, new modifiers resembling *M-st* were often detected following mutations of *R-st* to the stable allele *R-sc*. Such mutations evidently occurred by transposition of *I-R* away from the *R* locus to a site where it was identifiable as a modifier. *M-st* may be such a transposed *I-R*. Analysis of mutations to *R-sc* during the second (sperm-forming) mitosis in pollen grains showed that some of the transposed *I-R* elements were linked with *R*, whereas others assorted independently. Their strengths varied from barely discernible to a level equal to *M-st*. Overreplication frequently accompanied transposition at the sperm-forming mitosis, leading to transposed *I-R* elements in both the mutant and nonmutant sperm.

R STIPPLED is a mutable system in maize formed by interaction between a determinant for intense (self-colored) pigmentation and a closely associated unstable inhibitor designated *I-R* (ASHMAN 1970; KERMICLE 1970). Mutation (reversion) to self-colored (*R-sc*) occurs both in germinal and somatic tissues, the mutation being ascribed to loss of *I-R* inhibition. Somatic mutation in the aleurone layer of the endosperm gives rise to the kernel-spotting pattern known as stippled. Spotting is greatly enhanced in the presence of a dominant modifier, *M-st*, located six units distal to the *R* locus on the long arm of chromosome *10* (ASHMAN 1960). Somatic mutations also occur during development of gametophytes and embryo and rarely during later stages of sporophyte development. *R-st* to *R-sc* mutations near or at meiosis produce single, whole-kernel self-colored derivatives.

BRINK (1958) suggested that *R-st* instability was similar to that of genes in maize known to be under the control of transposable elements. Evidence favoring *I-R* transposition as a mechanism of *R-st* instability was obtained from mutations occurring in the female gametophyte, yielding kernels having only self-colored embryo or endosperm (SATYANARAYANA 1970). Such mutations

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are of mitotic origin and offer the technical advantage that meiotic assortment does not intervene between the mutational event and the subsequent recovery of a mutant gamete. Mutations of this origin sometimes carried in their genomes new modifiers of stippling not found in sib nonmutant lineages. The coincident mutation and appearance of a new modifier was interpreted as evidence for the transposition of *I-R* away from (Sc) to another site where it was identifiable as a modifier of *R-st*.

The present paper presents parallel evidence based on the male gametophyte. The pollen system offers two additional features. The pair of sperm that contribute nuclei to the endosperm and embryo of a given kernel derive from one pollen grain as the immediate products of division of a generative cell. Hence, the paternal contributions to embryo and endosperm are sister cells. Second, mutations from *R-st* to *R-sc* occur relatively frequently at the sperm-forming gametophyte mitosis.

MATERIALS AND METHODS

R alleles: BRINK (1973) has reviewed earlier findings concerning the particular R-stippled stock used in this study. In the absence of *M-st* the aleurone of single-dose *R-st* kernels is lightly spotted with anthocyanin (pictured in ASHMAN 1960). Both *R-st* and *M-st* increase spotting in proportion to dosage. Plants are colorless, *i.e.*, green.

r-g (colorless aleurone, seedling parts and anthers): phenotype equivalent to a deficiency of the *R* region.

r-r (colorless aleurone, colored seedlings and anthers): the inbred W22 source was used.

r-v is an abbreviated symbol for a class of variegated plant-color derivatives first described by R. B. ASHMAN (1965) and designated as unstable *r-g (nc)*. The aleurone is colorless or nearly so and plant color is unstable (flecks of color in seedling tissues and anthers in the presence of *M-st*). Obtained as crossover derivatives from *R-st/R-r* heterozygotes, this allele combines the plant color component (P) from *R-r* with the instability (*I-R*), paramutagenicity and near colorless (Nc) components of *R-st*. Component constitution is consistent with the model (P) (*I-R*) (Nc) (KERMICLE 1970; ASHMAN 1970). Three isolates (*v-236*, *v-245*, *v-248*), lacking *M-st* and with virtually no aleurone pigmentation (colorless except for occasional flecks on a few kernels) were used. The near colorless aleurone and paramutagenic features of *r-v* are not considered further since they have no known effect on the stippled phenotype.

Modifier status of stocks: All stocks in this study lacked *M-st*, unless stated otherwise. Absence of *M-st* is designated *m-st* where necessary for clarity.

Selection of R-sc *mutants*: Following the cross *r-g/r-g* female × *R-st/R-st* male, variant kernels with nonconcordance of embryo (scutellum) and aleurone phenotypes occur with high frequency (approximately 1% of pollen grains, KERMICLE 1970). These fall into two classes: Class A: Stippled aleurone and self-colored embryo (germinally *R-sc/r-g*). Class B: Self-colored aleurone and colorless embryo (germinally *R-st/r-g*).

These nonconcordant embryo-endosperm mutants are attributed to mutation of *R-st* to *R-sc* at the second mitosis in male gametophytes, producing pollen grains in which one sperm nucleus carries *R-sc* and the other *R-st*. Reciprocal patterns of double fertilization then give rise to mutant kernels of the two classes. This interpretation is supported by the approximate equivalence of frequencies of the reciprocal mutant classes.

In the present study, only class A mutants with full scutellum pigmentation were analyzed. Those with the scutellum pigmented only fractionally were discarded as probable postzygotic mutations. All kernels with fully self-colored aleurone were selected. These included class B mutants as well as *R-sc* mutants arising before second pollen grain mitosis (mutant aleurone and embryo). Subsequent progeny tests distinguished these two types.

Scoring of R-st phenotype: Measurements of spotting intensities of *R-st* were made in three ways: (1) On a per kernel basis, using a dissecting microscope equipped with an eyepiece grid reticule

TABLE 1

Average aleurone-spotting scores (scale 1–7) of r-v/r-v/R-st *and* r-r/r-r/R-st *kernels from the cross* r-r/r-v ♀ × R-st/R-st ♂, *indicating a modifying effect of* r-v *on* R-st

	Kernel class[a]	
r-v isolate	*r-v/r-v/R-st*	*r-r/r-r/R-st*
v-236 (4 lines)	4.16 ± 0.08	2.58 ± 0.08
v-245 (4 lines)	4.55 ± 0.11	2.57 ± 0.11
v-248 (5 lines)	4.25 ± 0.10	2.59 ± 0.10

[a] Values based on 25 kernel samples from two ears each of the four or five lines tested.

to determine spot density. At 25× magnification the grid covered an area approximately 4 mm square. All spots occurring within the boundaries of this square were counted with the grid positioned centrally 1 mm from the upper edge of the abgerminal face of a kernel. (2) On a per kernel basis, using a 1- to 7-scale defined by six standard kernels selected to provide a set of stippled patterns grading from a score of 1, almost spotless, to 7, slightly darker than the fairly heavy pattern given by one dose each of *R-st* and *M-st*. (3) On a per ear basis, using a 1- to 5-graded scale of ears. Class 2 was selected to encompass the range of spotting intensities given by single-dose *R-st* in the absence of *M-st*. Class 1 represented lighter phenotypes, whereas classes 3–5 were progressively darker spotting patterns. Class 5 would have included cases carrying *M-st*.

RESULTS

A trans*-effect of* I-R *at the* R *locus*

To test the effect on the stippled phenotype of *I-R* carried in *trans* linkage phase, stocks carrying *I-R* but not *R-st* were utilized. This was accomplished using *r-v*, an allele in which the strong seed color determiner of *R-st* has been replaced by a plant color determiner such that *I-R* induces instability of plant rather than seed color. Crossing of *r-v* with *r-r* generated *r-v/r-r* heterozygotes which when pollinated with *R-st* (*m-st*) produced two classes of stippled kernels. Those with *r-v/r-v/R-st* endosperm carry *I-R* in *trans* position, whereas sib *r-r/r-r/R-st* kernels do not. The two classes are distinguishable by a simple germination test. Coleoptiles and roots of *r-v/R-st* seedlings are flecked with color or are colorless, whereas these tissues are colored uniformly in *r-r/R-st*. In practice, 60–70 random kernels per ear were evaluated for spotting. The first 25 identified by germinating as belonging to each class were used to calculate average scores.

Data from three independent isolates of *r-v* are summarized in Table 1. If *I-R* in *trans* arrangement with *R-st* modifies stippled phenotype, kernels giving colorless or flecked seedlings should differ from those giving colored seedlings. The results show that presence in the endosperm of two doses of *I-R* in *trans* resulted in *R-st* spotting 1.6–2.0 units darker on the 7-unit scale. *I-R* in *trans*, therefore, modifies *R-st* expression in the same direction as the linked modifier *M-st*.

A parallel experiment measured the magnitude of the *M-st* effect on *R-st*. An *r-g M-st* stock was crossed with *r-r m-st*, and the resulting *r-g M-st/r-r m-st*

heterozygotes then pollinated with *R-st m-st*. Two principal classes of stippled kernels were generated: *r-g M-st/r-g M-st/R-st m-st* carried *M-st* in *trans* and produced colorless seedlings, and *r-r m-st/r-r m-st/R-st m-st* lacked *M-st* and produced colored seedlings. The aleurone-spotting score (averaged over four lines) of *R-st* kernels that produced colorless seedlings was 5.78 ± 0.14 on the 7-unit scale. Sib kernels, giving colored seedlings, scored 2.80 ± 0.14. The difference of 3.0 units somewhat underestimates the effect of *M-st*. Because of recombination between *r* and *M-st*, 6% of the colorless seedlings should lack *M-st*; similarly, 6% of colored seedlings are expected to carry *M-st*. Despite this underestimation, the effect of *M-st* measures one class higher than the strongest effect of *I-R* in *trans* (Table 1).

Parallel control experiments compared sib *r-g* and *r-r* chromosomes lacking *I-R* and *M-st*. No appreciable differences were found. The results (not presented) confirm the suitability of *r-g* and *r-r* as reference alleles for effects of *I-R* and *M-st* on *R-st*.

Transposition of I-R *at second pollen grain mitosis*

Major de novo *modifiers of* R-st *in mutant kernels of the stippled aleurone but self-colored embryo class*: Kernels either uniformly pigmented over the aleurone or in the scutellum of the embryo were selected following crosses of *R-st R-st* as male to *r-g r-g* ear parents, both lacking *M-st*. Among various *R-sc* mutant classes, the stippled aleurone: *R-sc* embryo combination (class A) is unique in that it provides an opportunity to detect modifiers of *R-st* expression both in the aleurone and in the embryo. If such a modifier were present in the *R-st* sperm contributing to the aleurone, the phenotype of the immediately resulting kernel would be altered. If a modifying gene were also present in the *R-sc* sperm that fertilized the egg, effects of the modifier would be present in progeny and detectable by pollinating with *R-st*. Five *R-st m-st* sublines produced 134 class A mutants in a population of 29,000 kernels. Analysis of a sixth line was discontinued because heterogeneity of aleurone-spotting intensities among plants indicated presence of at least one modifier already in this subline.

In all five lines the germinally mutant seeds had median spot numbers higher than a random sample of nonmutant sib seeds (Table 2). A frequency histogram (Figure 1) of the combined data shows that 30 of the 134 class A mutants had counts of more than 40 spots, whereas only two of the sampled nonmutant kernels reached such a count. However, a large fraction of the mutant kernels (55%) gave counts of less than 20 spots—indicating that a high density of aleurone spotting was not a universal feature of stippled but germinally *R-sc* mutant kernels.

It is apparent that upon mutation of *R-st* to *R-sc* at about the time of second pollen grain mitosis a significant proportion of kernels with *R-st* endosperm shows evidence of a modified aleurone-spotting pattern. This finding is consistent with the hypothesis that mutation from *R-st* to *R-sc* may occur by transposition of the *I-R* element from the *R* locus to another chromosome site. The transposed element is sometimes found within the same sperm nucleus as the

TABLE 2

Median aleurone spot counts for 134 r-g/r-g/R-st *kernels with mutant* (R-sc) *embryos compared with an equal number of sib kernels with nonmutant* (R-st) *embryos. The cross was* r-g/r-g × R-st/R-st.

Subline no.	No. of cases	Embryo class of *r-g/r-g/R-st* kernels[a]	
		Mutant (*R-sc*)	Nonmutant (*R-st*)
1	12	17	8
2	14	20	2
3	28	15	7
4	41	18	7
5	39	8	3
Overall	134	15	5

[a] Median number of spots within a 4-mm square, centered on the abgerminal face of each kernel.

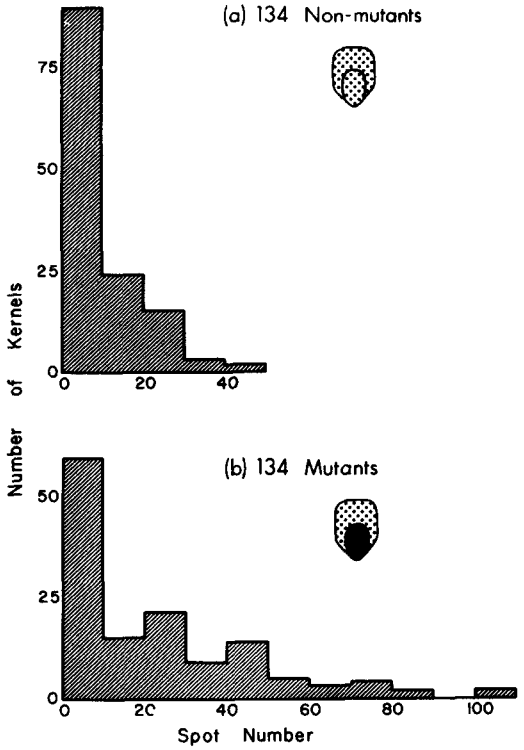

FIGURE 1.—Spot number frequency histograms for the *R-st* endosperm of 134 germinally *R-sc* mutant kernels (lower graph) and 134 nonmutant *R-st* kernels sampled at random (upper graph).

R-st nonmutant chromosome where it is identifiable as a modifier of *R-st* action in the endosperm.

On the basis of this hypothesis a transposed *I-R* element (*tr I-R*) should sometimes be found in the sperm nucleus bearing the *R-sc* mutation. All class

TABLE 3

Independently assorting modifiers of stippled in the progeny of kernels with stippled aleurone but germinally R-sc *embryo*

No. of spots on mutant kernel	No. of cases	
	Total	Modifier assorting in progeny
0–9	59	2
10–19	15	2
20–29	21	5
30–39	9	1
40–49	14	4
50–59	5	2
60–69	3	3
70–79	4	2
80–89	2	1
90–99	0	0
100+	2	0
Totals	134	22

A kernels were planted and pollinated with *R-st/r-r*. One-fourth of the seeds from these crosses should be lightly stippled (*r-g/R-st*) unless a modifier was present, in which case some darker stippled kernels (*r-g, tr I-R/R-st*) would occur. Any modifier assorting independently of *R* would show a 1:1 ratio of light to darker stippled kernels.

Twenty-two of the resulting 134 plants carried an independently assorting, strong modifier of *R-st*. Several other plants appeared to be either segregating for a weak modifier or were carrying a linked modifier, but the necessary detailed tests to analyze these were undertaken only for subline 4 (see following section). Nevertheless, it was evident from 22 clear cases that *R-sc* mutations occurring at the second pollen grain mitosis were sometimes accompanied by generation of a modifier of stippling. This result further supports the hypothesis that these modifiers are transposed *I-R* elements.

Of the 22 identified cases carrying an independently assorting modifier in the *R-sc* embryo, several had also shown evidence for presence of a modifier in the associated endosperm. In particular, eight of the 16 cases that had more than 50 spots in the aleurone (and, therefore, apparently carried a modifier in the endosperm) also segregated for a modifier in the embryo (Table 3).

Modifiers recovered from various mutant and nonmutant classes of R-stippled *subline 4*: One hundred nonmutant *R-st/r-g* kernels were taken at random from subline 4 and subsequently crossed as male parents to *r-r/r-r*. Stippled seeds from the resulting ears were examined for evidence of a modifying element. Examination of this entire control group gave no indication of variability suggesting presence of modifiers. Ten ears were, therefore, chosen at random and from each a 50-kernel sample was scored for *R-st*-spotting intensity on the 1–7 visual scale. The results indicated a mean score of 3.01 ± 0.30 for this representative group, the ten ears ranging in score from 2.58 to 3.56.

Twenty-five colorless (*r-g/r-r*) kernels from each of the 100 ears were grown and pollinated in a detasseling plot with an *R-st* stock as pollinator which in tests of sib plants on *r-r/r-r* females gave no indication of modifiers. The resulting ears were scored for spotting intensity on a 1–5 basis, a line being considered adequately tested if it produced seven or more ears for scoring. Ninety-three progenies were so tested. Of these, 92 showed no evidence of a modifying element, but one did produce three ears (out of 11) of class 3 apparently segregating for slightly darker stippled kernels. Germination of the darker stippled seeds from these ears yielded 1:1 red and green seedlings, indicating that the effect was not associated preferentially with either the *r-g* or *r-r* chromosome.

It was apparent that the control materials were free from any regular variation that would indicate presence of modifiers in the subline 4 parental stock. The presence of slightly darker stippled kernels in one of 93 progenies suggests that modifiers can arise independently of visible mutation to *R-sc* or else the normal range of *R-st* expression in the absence of modifiers includes some slightly darker stippled cases.

All 41 class A mutants (stippled aleurone, *R-sc/r-g* embryo) in subline 4 were pollinated with *R-st/r-r*. Plants grown from the resulting colorless (*r-g/r-r*) kernels from each source were pollinated in a detasseling plot with *R-st*. Ears were scored for spotting intensity in the 40 lines that gave seven or more ears for scoring. Eighteen lines produced light and darker stippled ears in a ratio not significantly different from 1:1, indicating presence of a modifier assorting independently of *R*. Germination of the more heavily spotted kernels from the darker stippled ears produced 1:1 ratios of red and green seedlings, confirming independent assortment. Three other lines gave one to three ears of class 3 (slightly darker than normal). In only one of these did the darker kernels give predominantly green seedlings, indicating the presence of a modifier linked to the *R* locus. The other two cases could represent either the extreme dark end of the normal expression of *R-st* in the absence of modifiers or the presence of a weak independently assorting modifier identifiable only by an occasional darkly stippled kernel. If these two cases are not included among those with identified modifiers, then 19 of 39 progenies, or 48.7%, were carrying detectable modifying elements.

Thirty-five self-colored aleurone but germinally *R-st/r-g* cases (class B) from subline 4 were crossed as males to *r-r/r-r* and 50 stippled kernels then scored on a 1–7 visual scale (first generation test.) In addition, 25 of the *r-g/r-r* seeds produced from each cross were grown and the plants pollinated with *R-st/R-st* in a detasseling plot. Thirty-two cases produced seven or more ears for scoring the intensity of stippling (second generation test.) Results of both tests are given in Table 4.

Among the 32 cases successfully tested, the first generation test (Table 4, left side) showed 11 to have mean scores ranging from 4.1 to 5.2. These were clearly outside the limits of expectation of the control population (3.0 ± 0.3) and, therefore, reflect the presence of modifiers. Six were confirmed by the second generation test (Table 4, right side). Five of these carried a modifier

TABLE 4

R-st *modifiers in first and second generation progenies of kernels selected as having* R-sc *endosperm but yielding* R-st/r-g *progeny*

First generation: mean kernel scores (1–7 scale) following *r-r r-r* × *R-st/r-g* crosses	Second generation: No. of ears with indicated ear scores following the cross *r-g/r-r* × *R-st/R-st*					
	Ear class					Difference from 1:1 ratio[a]
	1	2	3	4	5	
(a) Cases with independently assorting modifier:						
4.8		4		6	3	NS
4.5		4	1	2	6	NS
4.4		8	3	8		NS
4.3		5	4	3	1	NS
4.2		7	1	6	2	NS
3.8		8	7			NS
3.5		13		5		NS
3.3		13	4	1		NS
(b) Cases with linked modifier:						
5.2		15				*
4.6		15				*
4.4		10				*
4.3		12	2			*
4.2		13				*
4.1		16				*
(c) Cases lacking a definite modifier:[b]						
3.6		14	4[c]			*
3.2		20	1[c]			*
2.7		13	3[c]			*
1.9		13				*
1.4		11				*

[a] χ^2 test for 1:1 ratio of class 2 to other classes. NS: Not significantly different from 1:1. *: significantly different from 1:1 ($P < 0.05$).

[b] Thirteen additional lines gave mean kernel scores from 2.5 to 3.5 and produced 209 class 2 ears only.

[c] Germination of the dark kernels from these ears gave 1:1 red to green seedlings.

assorting independently of *R* and one was shown by germination analysis to be linked. The remaining five, characterized by the presence of a modifier in the first but not the second generation test, were evidently cases in which the modifier was tightly linked to *R-st* and, thus, was not transmitted at detectable frequency with *r-g* to the second generation.

Three cases were shown by the second generation test to be carrying independently assorting modifiers despite having scores lower than 4.0 in the first generation. Also listed in Table 4 are two cases giving first generation stippling scores of 1.4 and 1.9, values that were significantly lower than the controls. The nature of this variation is unknown.

Subline 4 produced 30 kernels with both embryo and endosperm self-colored, representing mutations preceding the second pollen grain mitosis. The *r-g/r-r* offspring produced from mating these *R-sc/r-g* plants to *r-r/r-r* were pollinated with *R-st* in a detasseling plot. Only one progeny of the 30 showed

evidence of a modifier. In interpreting this apparently low value, consideration must be given to the possibility that a major proportion of these germinal self-colors may have arisen by meiotic crossing over. KERMICLE (1970) showed that *R-st/R-st* as male parent gave *R-sc* mutants with frequency 58.2×10^{-4}, whereas *R-st/r-g* males gave only $13.8/10^4$ *R-sc* gametes. Because *R-st/r-g* did not yield crossover self-colors, it was inferred that, in *R-st* homozygotes as male parents, as many as three-quarters of the *R-sc* mutants originating before the second pollen grain mitosis may be of crossover origin.

DISCUSSION

A modifier of R-stippled not present in the parent plant frequently is recovered in the genomes of female and male gametophytes in which *R-st* mutates to *R-sc*. Transposition of the instability factor *I-R* from *R-st* to a new site accounts for the coincident stabilization of *R* phenotype and origination of the modifier (STAYANARAYANA 1970; present work). A basis for this interpretation is provided by the finding that extra doses of *I-R* carried at *R* in *trans* combination with *R-st* result in a modified *R-st*-spotting pattern.

The finding that an element transposed from an unstable gene serves to modify the level or timing of instability is not without precedent. Thus, extra doses of Activator (*Ac*) delay the timing of reversion in the endosperm of instabilities belonging to the Activator/Dissociator family (MCCLINTOCK 1951). Dosage of Modulator, another member of the *Ac* family of elements, affects level of pericarp coloration. *Mp* inhibits coloration irregularly when in *cis* association with *P-rr*, producing striped kernels. Accumulation of *Mp* elements elsewhere in the chromosome complement reduces the grade of variegation stepwise from medium to light (one transposed *Mp*) to very light (two transposed *Mp*s) (BRINK and NILAN 1952; BRINK 1954). Increased dosage of *Ac* or *Mp* decreases mutability, whereas extra doses of *I-R* increase mutability. This opposite effect underlines the parallel yet essentially unique behavior of different maize transposable element systems.

Transposed *I-R* elements were identified in nearly 50% of the mutations from *R-st* to *R-sc* at the second pollen grain mitosis. Even in the most thoroughly studied material (subline 4) some instances of *I-R* transposition undoubtedly were not detected. Analysis of the stippled aleurone but germinally *R-sc/r-g* group (class A) served to characterize adequately only modifiers that segregated independently of *R* or that were loosely linked. It is probable that a number of closely linked cases went undetected. Transposed *I-R* elements identified from both class A and class B events (self-colored aleurone; colorless germinally *R-st/r-g*) varied quantitatively in strength of modifying effect, ranging from equivalent to *M-st* down to the threshold of detectability in these experiments. Some cases probably were expressed below the level of detectability. Thus, the present procedures give minimal estimates of the presence of transposed *I-R* following mutation.

Transposable elements have been shown to occur in different "states" which result in various qualitative or quantitative differences in expression. These states are often characterized by large and clear-cut differences in strength of

the effect, as in the example of *Mp* (KEDHARNATH and BRINK 1958), *Ac* (MCCLINTOCK 1965) and *En* (GONELLA and PETERSON 1977). However, in the case of transposed *I-R* a graded series of effects was suggested rather than a separation of distinct classes. This is reminiscent of the range of strengths of *Ac* action implied by MCCLINTOCK (1948). It is not known whether *I-r*'s range of expression reflects changes in the element, variation in copy number or some other influence, such as a distance-related position effect.

The strongest of the transposed *I-R* cases simulates the effect of *M-st*, the standard modifier located six crossover units distal to *R*. It is possible that *M-st* originated as a transposed *I-R* and is relatively stable in that position. WILLIAMS (1972) found each of six modifier-carrying stocks of *R-st* obtained from Peru, Bolivia, Argentina and Chile to carry *M-st* in the standard location. Somatic sectors, suggesting transpositional loss of *M-st* function, nevertheless occur. Recent evidence of structural homology between *I-R* and *M-st* provides additional support for an ancestral relationship between *I-R* and *M-st* (KERMICLE 1984).

The fact that *M-st* is linked to *R* adds circumstantial evidence favoring its origination from *R-st*. Both Modulator and Enhancer transpose preferentially to linked sites (VAN SCHAIK and BRINK 1959; NOWICK and PETERSON 1981). The class B mutants reported in Table 4 represent a small but seemingly unbiased sample for asking whether *I-R* shows a similar preference. This test revealed 11 cases with clearly discernable modifiers of which six were linked to *R*.

By analogy with the behavior of Modulator in variegated pericarp (ORTON and BRINK 1966) it is expected that *R-st* would be reconstituted occasionally in *R-sc* plants by transposition of *I-R* back to *R*. MCWHIRTER and BRINK (1962) screened more than a million gametes from *R-sc/R-sc* plants with negative results. Later work showed that loss of *I-R* may occur by recombination at meiosis (KERMICLE 1970), and it is likely that some *R-sc* stocks lacking *I-R* were used. Subsequently, an *R-sc* allele has been found that reverts with high frequency (12×10^{-4}) to *R-st* (SATYANARAYANA 1970). This isolate (*R-sc:n1747*) originated at a second pollen grain mitosis. Its reversion is not crossover-associated and may occur during somatic development to yield a sector of *R-st* kernels. Such behavior suggests that *I-R* is located nearby, where it frequently transposes back to *R*. However, other explanations such as a change of state from inactive to an active form of *I-R* while remaining at the *R* locus cannot be ruled out. The stippled revertants are variable in expression, some having a diffuse anthocyanin background pigmentation in addition to the characteristic spotting of *R-st*. Only two cases of 17 tested responded to *M-st*. Reversion of *R-sc:n1747* to an unstable form clearly involves more than a return of *I-R* to its precise former position and activity.

In the present study, evidence was obtained for recovery of transposed *I-R* elements in both sperm following a mutational event at the second pollen grain mitosis. This result may be compared with that found by GREENBLATT and BRINK (1962) for *Mp* at the *P* locus. In 65% of somatic transpositions resulting in adjacent red and light-variegated sectors, a transposed *Mp* element was

recoverable from both. Furthermore, the transposed Modulators of given twin spots mapped to homologous positions. GREENBLATT (1974) presented a model of *Mp* transposition that involves regular overreplication of the transposing *Mp* element, with incorporation of homologous copies of *Mp* into both daughter cells at the division following transposition. A similar mechanism may be occurring in association with transposition of *I-R*, resulting in copies of *I-R* in both sperm following mutation at the second pollen grain mitosis. In the present experiment, 16 class A mutant kernels had more than 50 spots (Table 3) and, therefore, probably carried a transposed *I-R* element in the aleurone. Of these, eight embryos (50%) carried a new modifier of sufficient strength and distance removed from *R-sc* to be readily detected. Because the aleurone is a terminal tissue, no test of homologous location was possible in this material.

The case for *R-st* as an instability based on a transposable element rests on analogous behavior with established transposable systems. Functional homology with other systems has not been demonstrated. Thus, tests for the ability of *R-st* to substitute for the regulatory elements of Dotted (RHOADES 1938), Activator/Modulator (MCCLINTOCK 1948; BRINK and NILAN 1952) and Suppressor-Mutator/Enhancer (MCCLINTOCK 1953; PETERSON 1960) have proved negative (GAVAZZI 1967; WILLIAMS 1972). Comparable tests for *Fcu* (GONELLA and PETERSON 1977) and the mutable opaque-2 systems reported by SALAMINI (1980) have not been reported. The most striking behaviors that *R-st* shares with one or more of the transposable systems is the origin of a modifier concurrent with a mutational event, overreplication of the resulting modifier and linkage of the modifier with the unstable locus. These criteria may be useful in implicating transposable elements as the basis for genetic instabilities in other organisms.

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