ANTHOCYANIN pigmentation of the aleurone layer in maize endosperm is influenced by the stippled allele of the R locus in two characteristic ways. First, it conditions the irregular distribution of seed pigmentation aptly described by its name (Figure 1). Occasionally the instability is lost in the germ line, yielding a strongly and uniformly pigmented form termed self-colored \((R^c)\). In the nomenclature commonly applied to unstable loci such changes are subsumed under the name mutation. Secondly, stippled reduces the action of sensitive alleles in heterozygotes (Brink 1956). The reduced level of action is heritable, and also potentially reversible. Studies concerning this unusual phenomenon, termed paramutation, have been reviewed by Brink (1964) and by Brink, Styles and Axtell (1968). Rather than its paramutagenic property, the primary consideration of the present article is the chromosomal basis for stippled to self-colored mutation and the relation of mutation to aleurone spotting.

A useful concept for understanding mutable allele behavior is to consider the somatic and germinal instability in a given case as having a common basis. Those changes which occur early during sporophyte development lend the most direct support to this view. Meristems so affected give rise progressively, first to somatic tissues which evidence the change phenotypically, and then to reproductive tissues which transmit the altered form. This view is also supported, though less directly, by correlated levels of somatic variegation and germinal mutability. Such positive correlations have been observed both where the source of variation is genetic, as in the case of marbled aleurone \((R^{mb})\) in maize (Weyers 1961), and where the variation is of environmental origin (Harrison and Fincham 1964, for example). R-stippled, however, is exceptional in this regard. Ashman (1960, 1965) described a modifier of its expression \((M^s)\) which markedly increased aleurone spotting without influencing germinal mutation. Mutation frequency, on the other hand, was affected by R locus composition of the parent sporophyte, being elevated about three fold among \(R^{st}\) gametes from \(R^{st}R^{st}\), as compared with those from \(R^{st}r\) plants. A class of self-colored mutations from both genotypes probably has a basis common to that which underlies aleurone spotting. The present study identifies a further class of mutations from \(R^{st}R^{st}\) and certain \(R^{st}\) heterozygotes whose origin is associated with meiotic recombination.
MATERIALS AND METHODS

The particular accession of R-stippled investigated traces to a stock obtained from R. A. Emerson at Cornell University. The original intensely spotted form reflects interaction of stippled with the modifier $M^{st}$, situated 5.7 recombination units distal to $R$ on the long arm of chromosome 10. Both $R^{st}$ and $M^{st}$ increase spotting in proportion to dosage (Ashman 1960). Pigmentation attributable to $R^{st}$ is confined to the aleurone spots, to irregularly occurring sectors in the scutellum of the embryo, and to occasional streaks in the coleoptile. In self-colored derivatives these tissues are colored uniformly. Those adult plant parts pigmented by action of $R^{r}$ or $r^{r}$ alleles remain acyanic, even upon change of $R^{st}$ to $R^{rc}$. The stippled allele studied is strongly paramutagenic and has been used widely in other experiments as a source of primary paramutagenic activity. Its stability was investigated in the homozygote, and in heterozygous combination with each of the other alleles described below.

$R^{nf}$ (R-Navajo; Cudu source); Kernel crown pigmentation (Figure 1) develops late, beginning approximately 25 days postpollination compared to 15 days for stippled; scutellum pigmentation outlines the embryo axis in mature seed; coleoptiles and especially roots are variably pigmented; and anther pigmentation ranges from weak to moderately strong. No mutations to fully colored aleurone were observed among 29,740 kernel progeny of $R^{nf} R^{nf} 0 \times r^{rf} r^{rf} 0$ matings.

$r^{r}$ (colorless aleurone, colored seedling tissues and anthers): The present study employed the form of $r^{r}$ carried by the commercial inbred line, W22.

$r^{r}$ (I) (near-colorless aleurone, colored seedling tissues and anthers): These crossover derivatives of $R^{r} R^{st}$ heterozygotes combine the red plant feature of $R^{r}$ with the paramutagenic property of $R^{st}$ (Ashman 1960, 1965). Isolates numbered one and two used here behaved similarly, so the results have been pooled. No strongly colored aleurone mutations were observed in 46,150 gametes tested by $r^{r}(I)/r^{r}(I) 0 \times r^{rf} r^{rf} 0$ matings.

$r^{g}$ (colorless aleurone, green seedling tissues and anthers): Bottom recessive of the $R$ allelic series. Phenotype equivalent to that of a deficiency for the $R$ region.

Marker genes: The closest known proximal locus, golden-I ($g_{1}$) was employed in combination with either leaf-color ($Lc$), or the major modifier of stippled ($M^{st}$), or abnormal chromosome 10 ($K10$) distally as linked marker loci. The testcross linkage data below report the $g$-$R$ recombination fraction and the chiasma interference between this region and the $R$-$M^{st}$ interval in the genetic background of inbred strain W22 used for the mutation experiments. Frequent recombination in the $g$-$R$ region, 22.2% here as compared with an average of 14% reported in an early summary of linkage data (Emerson, Beadle and Fraser 1935), lessens the effectiveness of golden as a marker. Strong chiasma interference tends to offset this limitation. Employing the values of 22.2 for $g$-$R$ and 5.7 for $R$-$M^{st}$ in conjunction with coincidence = 0.13, an average of only 1.6 per thousand chromosomes are expected to be recombinant in both regions.

<table>
<thead>
<tr>
<th>Year</th>
<th>Test heterozygote ($\varrho$)</th>
<th>$g$ $R^{r}$ $M^{st}$</th>
<th>$+$ $R^{st}$ $+$</th>
<th>Random sample Fraction</th>
<th>$R$-$M^{st}$ crossovers Fraction</th>
<th>Among $R$-$M^{st}$ Fraction</th>
<th>Coefficient of coincidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>$g$ $R^{r}$ $M^{st}$</td>
<td>69/244</td>
<td>28.3</td>
<td>1/77</td>
<td>1.3</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>$+$ $R^{st}$ $M^{st}$</td>
<td>72/307</td>
<td>23.5</td>
<td>2/61</td>
<td>3.3</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>$g$ $R^{g}$</td>
<td>79/440</td>
<td>18.0</td>
<td>4/108</td>
<td>3.7</td>
<td>.21</td>
<td></td>
</tr>
<tr>
<td>Totals and weighted averages</td>
<td>220/991</td>
<td>22.2</td>
<td>7/246</td>
<td>2.8</td>
<td>.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the late 1960s, the combination tested in 1967 was heterozygous for the more closely linked distal marker leaf-color (Bray 1964), incorporated into the $r^D$ homologue from an Ecuador-$R'$ strain. The position of $Lc$ relative to $R$ and $Mst$ is inferred from its frequency among the 108 $r^D$ gametes, representing 4.7% of 2308 total, that had acquired $Mst$ from the $R'$ homologue. Plants representing the 108 cases were classified for the purple pigmentation characteristic of $Lc$ in leaf blades of young plants, in leaf blades, auricles, and nodes at the time of flowering, and in the pericarp following exposure to light approximately two weeks after pollination. The $Lc$ characteristics were particularly well-developed at each stage in these materials due to prevailing cool temperatures, favorable for anthocyanin formation. Sixty-three individuals showed each characteristic of $Lc$ action whereas 45 showed none. Accordingly $Lc$ is considered to be located 1.95 units (45/2308 × 100) distal to $R$.

An alternative distal marker in the early experiments was abnormal chromosome 10 ($K10$), identified by the distortion of ratios resulting from preferential inclusion of $K10$ in the functional megaspore (Rhoades 1912). In $K10$ heterozygotes recombination distal to $R$ is reduced to about one or two units from a normal minimum of 35. Use of $K10$ as a marker was discontinued when fewer $R_{sc}$ mutations were observed from combinations involving it.

For the study of stippled mutability in male germ cells a strain of $R'_{st}$ developed having white ($y$), and shrunken (sh,) endosperm, and red aleurone ($pr$). These markers, on separate chromosomes from one another and from $R$, served to distinguish the male gametophytes of this strain from others in the breeding nursery, thus permitting exclusion of cases resembling mutation but originating from contaminant pollen.

**Genetic background:** The residual inheritance of the several strains was standardized by backcrossing the stocks carrying the desired genes four or more times to the long-term inbred line, $W22$. Multiple combinations and test heterozygotes were synthesized from appropriate intercrosses among lines so derived. The mutations were isolated and also characterized within the background of strain $W22$.

**Mutation verification and calculation of mutation frequencies:** The general procedure in establishing self-colored mutations was to select isolated kernels having $R_{sc}$ features from ears produced in backcross matings of plants carrying stippled to $rgrg$. Selections that proved germinal by progeny test were considered authentic provided that the recovery of $rg$ and the marker genes confirmed the designated parentage. In the study of mutation in female germ cells the selection of $R_{sc}$ kernels occurring singly insures the independent origin of each mutation. In the male, precaution is necessary to avoid clusters of $Rbr$ mutations that could descend from single events which occurred early in development of the sporogenous tissues. To bring such clusters to evidence a sufficient number of crosses was made from individual males to produce 2500 or more progeny. The data from these tests gave no indication of heterogeneity, however, so the individual plant results were pooled.

The populations of gametes tested were estimated by adjusting the number of kernels screened for mutation by the proportion of presumptive mutants successfully analyzed; these ranged from 72% to 100%. The probability of a difference between two mutant fractions (verified mutants/effective population) due to sampling variation was determined from the appropriate binomial expansion when the total mutants numbered fewer than 50. Where there were 50 or more, the mutant fractions were tested for homogeneity by the chi-square method.

**RESULTS**

Table 1 reports the frequency of stippled to self-colored mutation observed for $R_{st}$ and $R_{st}R_{st}$ sibs tested as male through crosses to $rgrg$. The classification for heritability of selections based either on uniformly colored aleurone or on intensely colored scutellum provides the four categories listed under each parent genotype. The number of colored aleurone selections proving not heritable, and the number of colored scutellum but stippled aleurone selections that verified as germinal self-colored mutants were unaffected by parental source of $R$-stippled...
TABLE 1

The frequency and heritability of self-colored endosperm (aleurone) or embryo (scutellum) selections from crosses of Rsstrg and RsrtRs as male to rgrg Q Q

<table>
<thead>
<tr>
<th>Rsst source</th>
<th>Phenotype of kernel selections</th>
<th>Male gametophytes tested (Rs')</th>
<th>Breeding behavior of selections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endosperm</td>
<td>Embryo</td>
<td>Number</td>
</tr>
<tr>
<td>Rsstrg Δ ♂</td>
<td>Self-colored</td>
<td>Unselected</td>
<td>3620</td>
</tr>
<tr>
<td>Stippled</td>
<td>Self-colored</td>
<td>3400</td>
<td>5</td>
</tr>
<tr>
<td>RsrtRs Δ ♂</td>
<td>Self-colored</td>
<td>Unselected</td>
<td>7730</td>
</tr>
<tr>
<td>Stippled</td>
<td>Self-colored</td>
<td>7390</td>
<td>6</td>
</tr>
</tbody>
</table>

(P = 0.3 and 0.5). The average frequencies of the two categories, having in neither case embryo genotype corresponding to endosperm phenotype, are 54.6 and 61.2 × 10^4, respectively. Equivalence of these frequencies is understandable if the two are referable to reciprocal patterns of double fertilization involving one Rsst and one Rsstrg sperm. Accordingly, about one in 86 pollen grains is estimated to be of this composition. The combined estimate is maximal in that the stippled aleurone, colored scutellum class could include certain types of postzygotic mutations. The few selections of this class which proved not to be germinal mutations demonstrate that the change to self-colored in fact can occur during early stages of embryo development.

Only in the colored aleurone, germinally self-colored category is the mutation frequency in the heterozygote (13.8 × 10^4) significantly different (P = .001) from the homozygous frequency (58.2 × 10^-4). To have corresponding endosperm and embryo constitution, such mutations could arise in the reproductive cycle from the stage of tassel differentiation through the first microspore division. A differential influence of parent sporophyte on Rsst microspores, however, would necessitate a delayed effect and therefore is considered improbable. If, on the other hand, Rsst in Rsst Rsst plants were unstable during differentiation of the sporogenous tissue, clusters of mutations would be expected, leading to heterogeneity between the male parents studied. As heterogeneity was not found, subsequent experiments focused on meiosis as a likely time of origin of the genotype-dependent difference.

The three mutation experiments reported in Table 2 again employ the mating RsstRsst × rgrg, but with RsstRsst now as female parent and with the R region marked by heterozygous linked loci. The germinal mutation frequencies of 18.0 and 23.0 per 10^4 gametes, based on colored aleurone selections, correspond well with the earlier determination of 20.9 (ASHMAN 1965) and with an average of 25.2 from 62 mutations observed presently in control experiments with various RsstRsst foundation stocks homozygous for the linked marker genes. These frequencies compare with 58.2 × 10^-4 for the male germ line of RsstRsst plants observed in the preceding experiment. The third experiment, with a mutation frequency of
TABLE 2

Progeny phenotype and strand constitution of self-colored kernels selected following mating of Rst-Rst as female to rgrg 8

<table>
<thead>
<tr>
<th>Outside marker class</th>
<th>Parental</th>
<th>Recombinant</th>
<th>Mutation frequency (×10⁻⁴)</th>
<th>Recombinant Rst fraction</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rst source</td>
<td>g Rst +</td>
<td>g G</td>
<td>5  6</td>
<td>7  2</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>G Rst Mst</td>
<td>6  8</td>
<td>2  0</td>
<td></td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>g Rst Le+</td>
<td>55 63</td>
<td>38 33</td>
<td></td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>G Rst +</td>
<td>60 97</td>
<td>24 10</td>
<td></td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>g Rst K</td>
<td>5  2</td>
<td>0  0</td>
<td></td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>G Rst k</td>
<td>G Rst</td>
<td>7  4</td>
<td>2  0</td>
<td>19.3</td>
</tr>
</tbody>
</table>

* The probability of a wider split between the two recombinant fractions attributable to sampling variation.

† 10⁴ tertiary trisomics of translocation B-10a.

10.4 × 10⁻⁴, appears to be exceptional and therefore will be considered separately.

Also given in Table 2 is the result of classifying the colored aleurone selections according to linked marker composition. The nine among 20 germinal self-colored mutations borne on strands recombinant for golden and Mst in the first test compares with two among 16 in the nongerminal class. Similar proportions occurred in the second test where Le was distal marker, with recombinant fractions of 71/189 in the mutant and 34/191 in the nonmutant classes. Whereas there is no evidence from these two experiments for coincidence between recombination and occurrence of the nonheritable class, approximately twice as many of the heritable selections are carried on recombinant chromosomes as expected on a random basis.

Abnormal chromosome 10 (K10) marked the region distal to R in the third RstRst trial. The reduction in mutation frequency from an average of 22.4 × 10⁻⁴ in the preceding two experiments to 10.4 × 10⁻⁴ is of marginal statistical significance (P = .04). Adding to the evidence for some peculiarity of the K10 heterozygotes is the fact that none of the seven Rst mutations was carried by a recombinant chromosome. The difference between these results and those of the preceding two RstRst combinations probably is to be understood in terms of the strongly reduced recombination in the R region of K10 heterozygotes.

Parallel experiments with stippled combined in heterozygotes with alleles known not to mutate to Rst provide separate tests for coincidence of mutation with recombination of the distal, and of the proximal, marker. In combination with Rst or r'(I) (Table 3, upper part) Rst frequency per Rst gamete is found not to differ appreciably from RstRst tested similarly. None of the 34 mutations was carried on a chromosome having the parental marker combination of Rm1 or r'(I), whereas eighteen were marked as the parental Rst homologue. Of the 16 crossovers, six carry the allele of golden and 10 that of Mst introduced by Rst or r'(I).
The six instances of exchange in the 22 unit proximal segment accord well with expectation based on random coincidence of \( R^{sc} \) mutation and \( g_1-R \) recombination. The excess recombinants involve the 5.7 unit distal region, with ten cases compared to an expectation of two.

No colorless seed mutations were verified among selections from the \( R^{nj}R^{st}\) to \( r^{gr}r^{gr} \) matings. A second class of seed color mutations was encountered, however, having stippled-like spots confined to the region of the crown normally pigmented by \( R^{nj} \) (Figure 1). Fifteen independent cases similar to one another and strongly resembling a case described by Brink (1960) were established from a population of 31,410 \( R^{st} \) gametes. Remarkably, each was borne on a recombinant chromosome having the normal allele of golden from the \( R^{nj} \) homologue and the \( M^{st} \) allele from the parental \( R^{st} \) homologue, the marker combination complementary to the \( R^{sc} \) excess class. The new complex is designated \( R^{nj}:st \). It forms pigment concurrently with \( R^{nj} \) about 25 days post-pollination and some 10 days after pigmentation associated with \( R^{st} \) or \( R^{sc} \) is first visible.

The central section of Table 3 reports the marker composition of self-color mutations originating from combination of \( R^{st} \) with the derived allele \( R^{nj}:st \). The feature unique to this heterozygote is that both alleles carry the instability property but differ in time of formation and distribution of pigment. All but one of the 28 \( R^{sc} \) mutations carried the recessive golden allele marking the \( R^{st} \)

### Table 3

<table>
<thead>
<tr>
<th>Source</th>
<th>( R^{st} ) gametes tested</th>
<th>Mutations Number ((\times10^4))</th>
<th>Frequency (g)</th>
<th>Frequency (G)</th>
<th>Parental (g)</th>
<th>Parental (G)</th>
<th>Recombinant (g)</th>
<th>Recombinant (G)</th>
<th>Expected (\times10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( gR^{st}M^{st} )</td>
<td>7,020</td>
<td>13</td>
<td>18.5</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( G R^{nj}+ )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( g R^{st}+ )</td>
<td>10,500</td>
<td>21</td>
<td>20.0</td>
<td>12</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( G r^{r}(I) M^{st} )</td>
<td>17,520</td>
<td>34</td>
<td>19.4</td>
<td>18</td>
<td>0</td>
<td>10</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6</td>
<td>0.06</td>
<td>1.9</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>( gR^{st}L^{c} )</td>
<td>14,130</td>
<td>28</td>
<td>19.8</td>
<td>16</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( G R^{nj}+ )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3</td>
<td>0.02</td>
<td>0.5</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>( G R^{st}M^{st} )</td>
<td>7,430</td>
<td>12</td>
<td>16.2</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( g R^{st}+ )</td>
<td>5,740</td>
<td>4</td>
<td>7.0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expectation if \( R^{sc} \) mutations derive from the \( R^{st} \) homologue and originate independently of recombination.
chromosome proximally whereas 12 bore lc, the closely linked distal marker of Rn1:st. The distribution of linked markers among seven Rn1 revertants from this heterozygote was similarly nonrandom. Each carried the G allele of the Rn1 chromosome; however, three carried the distal Lc marker of Rst. The three in seven proportion is the same as the 12 in 28 Rsc cases having the lc marker of Rn1:st. Very probably, therefore, Rsc and Rn1 derivatives from this heterozygote originate in similar ways.

The control data given in the lower portion of Table 3 show no evidence of coincidence between recombination and Rsc mutation in Rst combinations with r' (Inbred W22 source). The mutation frequencies are reduced slightly relative to similarly marked combinations of homozygous Rst Rst.

DISCUSSION

The instability of R-stippled, expressed both as somatic spotting and as germinal mutability to self-colored, represents interaction of components of a functional complex. McWHIRTER and BRINK (1962) have discussed in general terms the significance of self-colored mutations for this conclusion. To be considered here primarily is the implication of the new finding that a class of Rsc mutations occurs coincident with recombination of markers flanking R. Crossovers that underlie this coincidence apparently fractionate the Rst complex, partitioning between the two homologues components previously associated in coupling phase.

The evidence for crossover-dependent Rsc mutations derives from Rst homozygotes and certain Rst heterozygotes with elevated Rsc rates relative to particular Rst r combinations studied earlier. Utilizing Rst r', ASHMAN (1960) found recombination in the proximal g1-R region to occur at random in respect to Rsc mutation, and McWHIRTER and BRINK, using Rst r, found each of seven Rsc cases to carry the distal marker K of the Rst chromosome. The rationale for the further investigation was that the increment of increased mutation in the high-frequency combinations might be due to recombination. The findings bear out this conjecture only in part. The reduced rate in Rst r' (16.2 × 10⁻⁴, Table 3) relative to the average of high-frequency combinations (21.7 × 10⁻⁴, Tables 2 and 3), for example, could relate to the absence in Rst r' of recombination-associated, self-colored mutations. But there remain unexplained on this basis differences such as between the 16.2 × 10⁻⁴ of Rst r' and 6.3 × 10⁻⁴ reported for Rst r (ASHMAN 1965). Additional evidence of a source of variation other than recombination comes from a test of Rst stability when hemizygous. Plants having a Rst-bearing, standard chromosome 10 and the 10b chromosome of translocation B-10a in place of a normal homologue yielded 14 germinal Rsc changes among a population of 3,550 gametes tested through matings to rgrP8. This situation, in which absence in 10b of the distal two-thirds of chromosome 10's long arm precludes interhomologue exchange in the R region, nevertheless gave a higher Rsc frequency (39 × 10⁻⁴) than Rst Rst and high mutation frequency heterozygotes.

McWHIRTER and BRINK proposed a model of R-stippled function whereby an inhibitor (I") acts in conjunction with a pigment determinant of the R class to
Figure 1.—Phenotypes of the parental $R$-stippled and $R$-Navajo alleles (upper two rows), their heterozygote (center row) and its crossover derivatives (lower two rows). From top to bottom the endosperm genotypes are $R^stR^stR^st$, $R^{nj}R^{nj}R^{nj}$, $R^stR^stR^{nj}$, $R^{cj}R^{nc}R^{cc}$ and $R^{nj}:stR^{nj}:st$. Collectively, the two derivatives possess the same $R$ components as the parental alleles, but carry them in different coupling relations.
produce collectively the unstable phenotype. The finding that the pigmenting
determinant (here termed $Sc$ to denote the type of $R^p$ allele) is separable by
recombination in appropriate combinations from a closely distal inhibitory, or
instability factor confirms this view. The average frequency of $R^{sc}$ cases from
$R^{st}R^{st}$, $R^{st}R^{st}(1)$ and $R^{st}R^{st}; R^{st}$ heterozygotes attributable to a recombinational
origin can be estimated from the data of Table 3 as follows. Those mutations
having the distal marker of the $R^{st}$ homologue are taken as $.943$ and $.98$, for $M^{st}$
and $Lc$, respectively, of $R^{sc}$ mutations occurring independently of recombination.
After removing in this way recombinants equal to the number normally expected
in the $R-M^{st}$ or $R-Lc$ region, there remains an excess of $6.4 \times 10^{-4}$ recombinant
$R^{sc}$ mutations, about one-third of the total $R^{sc}$ cases from these heterozygotes.
Based on the mutational and recombinational evidence, the $R^{st}$ complex is denoted
$Sc-I^R$, where the $(\cdot)$ signifies lack of functional independence of $Sc$ from $I^R$.

The recombination-associated loss of $I^R$ in $R^{st}$ homozygotes holds particular
significance for the interpretation of $R^{st}$ structure. To have crossover dependence
in this circumstance, the exchanges should be unequal, implying presence of a
tandemly duplicated chromosome region. Because it is the instability property
rather than the basic seed-pigmenting determinant which is lost in this manner,
$I^R$ but not necessarily $Sc$ should be associated with a differentiated region of the
duplication.

Upon separation from $Sc$ by crossing over, what property does $I^R$ confer on the
$R$ components in the homologue to which it is transferred? There was obtained
from $R^{st}R^{st}$ no colored seed of phenotypes other than stippled and self-colored.
Adding a second $I^R$ to the $Sc-I^R$ complex therefore seems to have had no readily
discernible effect on stippling. Opportunity for detecting $I^R$ action in a different
chromosomal relationship is improved in heterozygotes of $R^{st}$ with stable alleles.
The 15 unstable $R$-Navajo cases, $R^{n1}; R^{st}$, isolated from $R^{st}R^{n1}$ are considered to be
of just this sort. $I^R$ was transferred from $R^{st}$ ($Sc-I^R$) to $R^{n1}$ in forming $R^{n1}; R^{st}$
($Nj-I^R$) and $R^{sc}$. It will be recalled that each of the 15 $R^{n1}; R^{st}$ isolates was recom-
binant, having that combination of markers complementary to the excess recom-
binant $R^{sc}$ class. The frequency of $R^{n1}; R^{st}$, $4.8 \times 10^{-4}$, probably minimal due to the
technical difficulty in identifying isolated kernels of this phenotype, nevertheless
falls only slightly below the recombinant $R^{sc}$ rate of $6.4 \times 10^{-4}$.

Because $I^R$ acts through pigmenting components such as $Sc$ or $Nj$, its nature is
observed only indirectly. Distinctively it interferes with action of $R$ pigmenting
components in the cis but not in the trans linkage phase (compare, for example,
$Nj$ expression in the center and bottom rows of kernels in Figure 1). This effect
of position is similar to that of transposable elements associated with certain other
instances of mutable alleles in maize. As McWhirter and Brink point out,
stippled’s instability can be accounted for by ascribing to $I^R$ the known properties
of such elements. A specific test for $I^R$ transposability, however, proved negative
(McWhirter 1961). Two of the present $R^{st}$ cases are of interest in this connec-
tion. The gametes in which the two mutations were isolated carried a factor that
intensified $R^{st}$ spotting much as $M^{st}$, but which segregated independently of $R$.
Initially it was considered that these might represent instances of transposed $I^R$. 
The two cases derive, however, from the group of 66 $R^{ac}$ mutations reported in Tables 2 and 3 where $M^{st}$ was employed as marker. Hence the possibility that such independently segregating modifiers originate from $M^{st}$ transposition, as Ashman (1960) has suggested, cannot be rigorously excluded. Based on the mechanism of transposition of Modulator in the variegated pericarp system (Greenblatt and Brink 1962), the possibility of $M^{st}$ transposition remains, even though it could be shown that the two $R^{ac}$ chromosomes continued to carry an $M^{st}$ in the closely linked, standard position.

At $6.4 \times 10^{-4}$, the crossover reversion of $R^{st}$ to $R^{ac}$ is frequent compared with mutable alleles known to be under controlling element suppression. In larger populations than reported here, Neuffer (1965) observed no separation of the suppressors of the $Dt$ and $Ac-Ds$ systems from $A_s$. And recently, Nelson (1968) has placed the controller of two $Ac-Ds$ induced alleles and one $Spm$ case at different sites within the $W_s$ gene.

The association in stippled of somatic instability with a chromosome duplication suggests analogy with variegated-type position effects which often accompany chromosome rearrangements in Drosophila. The irregular action of $Sc$ in $R^{st}$, that is, could reflect a lack of functional independence between the chromosome 10 regions brought together in formation of the duplication. A germinal change of $R^{st}$ to $R^{ac}$ by recombination would be equivalent to the restoration of normal function which accompanies the return of a gene expressing position-effect variegation to a structurally standard chromosome (Dubinin and Sidorov 1935, with Drosophila; Catcheside 1939, with Oenothera).

Does position-effect variegation also account satisfactorily for stippled's mitotic instability? The variegation pattern in stippled, like certain of the Drosophila systems (Becker 1959; Baker 1967), is distinctly clonal. Baker (1963) presents several lines of evidence consistent with the view that variegation in Drosophila is a phenomenon of gene expression rather than somatic mutation. The heritability of $R^{ac}$ selections which represent changes in $R^{st}$ male gametophytes, in contrast, clearly implicates mutation as a mechanism for $R^{st}$ spotting. Somatic mutation is understandable, of course, on a transposable-element basis. Still other mechanisms for release of the inhibition can be readily envisioned, however. Unequal exchange between $R^{st}$ sister chromatids, for example, could exclude $I^{re}$ in a manner similar to the unequal meiotic exchange between $R^{st}$ homologues demonstrated for $R^{st}R^{st}$ homozygotes. The restriction of the mitotic instability of $R^{st}$ principally to those stages of the life cycle following meiosis assumes significance on this interpretation.

A fuller description of $R^{st}$ structure should also incorporate the allele's interhomologue influence on $R$ action, i.e., its paramutagenic action. Included in the questions yet to be answered is the precise relationship between paramutagenicity and the intrachromosomal determination of the $R^{st}$ phenotype. That the relation is intimate is indicated by McWhirter and Brink's finding that slightly more than half of $R^{ac}$ mutations from $R^{st}R^{st}$ are coincidently changed in paramutagenic action. The nearly one-half which remained unchanged, on the other hand, argues against an obligate, functional association of the two properties. The two
might vary concomitantly simply as a consequence of close linkage, both being separated from Sc by particular crossover events. Accordingly, the present mutations seem to provide favorable material for correlating the recombinational loss of instability with change in paramutagenic action.

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SUMMARY

The finely spotting seed-color factor, R-stippled (Rst), mutates to the stable, uniformly pigmented form termed self-colored (Rsc) at meiosis, during development of the gametophytes and the embryo, and, rarely, during sporophyte development. The frequency of meiotic mutations is higher in Rst homozygotes and in heterozygotes with Rsi, r'(I), and Rsi;st than in heterozygous combination with the particular r' and r alleles studied. Approximately one-third of the mutations from high-frequency combinations originate in association with recombination of flanking markers, separating the basic pigmenting-determinant Sc from an instability property, Ir, slightly distal. The change to self-color in Rst homozygotes also is recombination-associated, indicating that Ir is situated in a differential segment of a tandem duplication. Association with a chromosome rearrangement and the position-effect interaction of I with Sc bring the meiotic instability of Rst into close analogy with position-effect variegation in Drosophila. Unlike variegation in Drosophila, however, the mitotic instability of Rst is mutational. Crossover Rsc mutations were not obtained from Rst in r' (W22 source) heterozygotes, probably due to chromosome structural polymorphism in the immediate R region. In RstRsi plants, the instability of Rst was transferred to Rsi with a frequency equal to, and with a linked-marker combination complementary to, the crossover Rsc category. Stippled's mitotic instability parallels the behavior of unstable genes under the control of transposable elements, but conclusive evidence of Ir transposability was not obtained.

LITERATURE CITED


