

All the crosses except the ones that involve 4519 had the expected reduction in sugary kernels, namely to about 15 percent when crossed as a male, female or selfed. When 4519 was used as a female or selfed the percentage of sugary kernels was 25 or more - no reduction. Used as a male, the percentage of sugary kernels was reduced to 15 as observed before.

Since 4519 derivatives in paired pollinations give 25 percent sugary when used as females, but 15 percent sugary when used as males, it suggests the presence of a new allele at this locus. This allele seems to be different from those known up to now since the action of this gametophyte factor is confined to the male gametophyte.

Another explanation of these results would be the existence of a fertility factor  $F$  closely linked to  $Ga$ , 4519 being  $F Ga$  and all the other popcorns  $f Ga$ . This  $F$  factor in dominant condition nullifies the action of the gametophyte factor, by removing the selectivity of the silks for  $Ga$  pollen. Then both  $ga$  and  $Ga$  pollen have the same chance of effecting fertilization. The inclusion of this fertility factor would give 25 percent sugary kernels when 4519 is used as a female. All the other popcorns would be carrying  $f$  and as a consequence  $Ga/ga$  silks will screen  $ga$  pollen and this would account for the 15 percent sugary kernels observed when 4519 was the male.

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### 3. Association of recombination and mutation to colorless and near-colorless aleurone in plants heterozygous $R^r R^{st}$ .

It has been observed that in  $R^r R^{st}$  plants mutations occur to colorless and to near-colorless aleurone, and that some of the mutants carry both the red plant color characteristic of  $R^r$  and the paramutagenic action characteristic of  $R^{st}$  (Ashman, Genetics 45:19). This finding suggests that such mutants result from intragenic recombination at the  $R$  locus. To obtain information bearing on this possibility a second test was made utilizing genetic markers on either side of the  $R$  locus. The following cross was made in the latter test:

$$\begin{array}{c} + \quad R^r \quad + \\ \hline g \quad R^{st} \quad M^{st} \end{array} \quad X \quad \begin{array}{c} g \quad r^g \quad + \\ \hline g \quad r^g \quad + \end{array}$$

Golden ( $g$ ) is 14 units proximal to  $R$ , and  $M^{st}$ , a modifier of the stippled phenotype, is 6 units distal to  $R$ . Ears from the above cross were scored for colorless and near-colorless kernels. The kernels selected were planted and the resulting plants were scored for golden and for plant color; the ears produced on the plants were pollinated

with pollen from plants heterozygous  $\underline{R}^F \underline{r}^G$ . The ears were harvested and examined for any non-mutants, which would be segregating either of the parental phenotypes,  $\underline{R}^F$  or  $\underline{R}^{St}$ ; however, no such ears were found. The kernels on the ears were examined and the mutants were tentatively classified for aleurone phenotype, i.e. colorless or near-colorless; the classification was verified in a later generation when the mutants were isolated in homozygous stock cultures. The distal marker,  $\underline{M}^{St}$ , was tested for by pollinating several  $\underline{r}(m)/\underline{r}^G$  plants of each mutant with  $\underline{R}^{St}$  pollen. The frequency of colored areas in the aleurone is much greater in  $\underline{r} \underline{M}^{St}/\underline{r} \underline{M}^{St}/\underline{R}^{St} \underline{M}^{St}$  kernels than in  $\underline{r} +/\underline{r} +/\underline{R}^{St} \underline{M}^{St}$  kernels; therefore, those mutants carrying  $\underline{M}^{St}$  could be identified.

A total of 262 ears was scored for seed color mutants, and the non-mutant  $\underline{R}^F$  and  $\underline{R}^{St}$  kernels totaled 50,515 and 49,446 respectively. Thirty-nine seed color mutants were obtained, and they have been characterized for aleurone phenotype, plant color, and proximal and distal genetic markers. These data are presented in Table 1.

Table 1 - Classification of the seed color mutants isolated from the cross  $+ \underline{R}^F +/\underline{g} \underline{R}^{St} \underline{M}^{St} \times \underline{g} \underline{r}^G +$  for aleurone pigment and plant color, and for the distribution of the proximal and distal genetic markers.

Plant color	Constitution of proximal and distal markers			
	Non-recombinants		Recombinants	
	+ +	$\underline{g} \underline{M}^{St}$	+ $\underline{M}^{St}$	$\underline{g} +$
	Near-colorless aleurone			
Green	0	2	8	1
Red	0	0	13	0
	Colorless aleurone			
Green	0	1	0	0
Red	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{11}{32}$	$\frac{1}{2}$

The data show that 32 of the 39 mutants received the proximal marker from the  $\underline{R}^F$  parental chromosome and the distal marker from the  $\underline{R}^{St}$  parental chromosome. Also, these 32 mutants were not of a single class but included both those with colorless and near-colorless aleurone, and those with red and green plant color. The seven other mutants were comprised of one that carried both markers from the  $\underline{R}^F$  parental chromosome, four that carried both markers from the  $\underline{R}^{St}$  parental chromosome, and two that carried the proximal marker from the  $\underline{R}^{St}$  parental chromosome and the distal marker from the  $\underline{R}^F$  parental chromosome.

It is evident from the data that the majority of mutants, regardless of aleurone phenotype or plant color, are associated with a single crossover in the  $R$  locus region. A certain proportion, perhaps all, of the seven exceptional mutants could be explained as instances of a mutation producing crossover occurring coincidentally with recombination between the  $R$  locus and either the proximal or distal genetic marker. Such coincident crossovers, barring interference, would be expected to give  $0.14 \times 39$  or five mutants carrying  $\underline{g}$  (six were obtained), and  $0.06 \times 39$  or two mutants not carrying  $\underline{M}^{st}$  (three were obtained). The observed number of mutants in these two classes is, therefore, no greater than would be expected from coincident crossing over.

The two mutants carrying  $\underline{g}$  and not  $\underline{M}^{st}$  require the seemingly unlikely occurrence of three coincident crossovers in a 20 unit chromosome segment. The expected frequency of such multiple crossover mutants, again barring interference, can be calculated as  $(.14 \times .06) \times 39$  or 0.3 mutants. Triple crossing over would appear to be an inadequate explanation for the occurrence of these two mutants. However, this may be another instance of the "negative interference" phenomenon observed at other loci in maize and in other organisms.

The above data support a conclusion that most, and possibly all, mutations to colorless and near-colorless aleurone in  $\underline{R}^r \underline{R}^{st}$  heterozygotes are associated with recombination, the mutants being composed of that portion of the  $\underline{R}^r$  chromosome proximal to the  $R$  locus, and that portion of the  $\underline{R}^{st}$  chromosome distal to the  $R$  locus. In referring the results of these and previous tests to the fine structure of the parental  $\underline{R}^r$  and  $\underline{R}^{st}$  alleles, a reasonable hypothesis could assume that the phenotype characteristic of  $\underline{R}^{st}$  is dependent on the presence of two closely linked components, and that these two components are separable by conventional genetic recombination. Stadler and Emmerling have presented evidence that the  $\underline{R}^r$  allele is composed of two such closely linked components, a plant color component ( $P$ ) and a seed color component ( $S$ ). A two component structure for  $\underline{R}^{st}$  would assume that the stippled phenotype is lost when a crossover occurs that separates the two units, and in the heterozygous combination tested above,  $\underline{R}^r \underline{R}^{st}$ , the crossover strand carrying the colorless or near-colorless mutant may or may not also carry the plant color component ( $P$ ) from  $\underline{R}^r$ . A more detailed hypothetical structure of the  $\underline{R}^{st}$  gene is deferred, pending a more complete characterization of the 39 mutants isolated in the above test. At least one of the mutants with near-colorless aleurone and green plant color has shown evidence of back mutations to self-colored aleurone, and several mutants originally isolated with green plant color have, in later generations, given evidence of mutating to red plant color. Also, the important characteristic of paramutagenic action, both qualitative and quantitative, is yet to be determined.

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