2. Nonrandom association between R-stippled ($R^{st}$) to self-colored ($R^{sc}$) mutation and recombination of linked markers in $R^{st}$ $R^{st}$ and $R^{st}$ $R^{nj}$ plants.

Mutation of the unstable spotting factor R-stippled to a form conditioning uniformly pigmented aleurone (self-colored, $R^{sc}$) is known to occur at several stages during the maize life cycle. The germinal mutation frequency (based on single, self-colored seed from $R^{st}$ bearing female parents) varies, however, depending upon the R locus genotype (Ashman, Genetics 45:19 and 52:835). Two fairly distinct rates were observed: approximately $7 \times 10^{-4}$ characteristic of $R^{st}$ $r^{g}$ heterozygotes, and about $20 \times 10^{-4}$ observed for certain $R^{st}$ $r^{r}$ heterozygotes and $R^{st}$ $R^{st}$ homozygotes. Subsequent tests indicate that the genotype dependent variation may be stage limited to near meiosis, suggesting crossing over as a possible basis for the two-fold excess of mutations in the high frequency combinations. Study of the possible relationship between $R^{sc}$ mutation and recombination in the g-20-R-6-$M^{st}$ region was made in genotypes giving the higher mutation frequency, therefore, even though previous tests had shown these two events to be independent in low mutation rate situations of the $R^{st}$ r type.

Of the 39 singly occurring self-colored seeds among 12,348 produced from g $R^{st}$ +/+ $R^{st}$ $M^{st}$ ♀ X g $r^{g}$ + ♂ matings, progeny of 22 proved self-colored, whereas 17 yielded the parental, stippled phenotype. Characterization of the 39 cases for g and $M^{st}$ gives the following distributions:

| Progeny class | Linked marker composition: Parental | | Recombinant | | Classification incomplete |
|---|---|---|---|---|---|
| | g + | + $M^{st}$ | g $M^{st}$ | + + | |
| $R^{sc}$ | 4 | 6 | 7 | 2 | 3 |
| $R^{st}$ | 5 | 8 | 2 | 0 | 2 |

Among the 15 failing to prove germinally $R^{sc}$, two were recombinant. This small sample accords with an average g to $M^{st}$ recombination value of approximately 25 per cent. Within the $R^{sc}$ class, on the other hand, g and $M^{st}$ assort nearly independently, with nine in the recombinant and ten in the parental group. The excess of $R^{sc}$ mutations in the recombinant class indicates that the basic seed pigmenting determinant of $R^{st}$ may be separable by crossing over from a second component responsible for the spotting action. Furthermore, since the parent was homozygous for stippled, such exchanges must be effectively unequal. Presumably the spotting component, but not necessarily that for seed pigmentation, lies within a duplicated region.

More critical information on the possible crossover origin of some $R^{sc}$ mutations derives from $R^{st}$ $R^{nj}$ heterozygotes. $R^{nj}$ (R-Navajo, kernel crown spot) has not been observed to give self-colored mutations, and also

differs from $\underline{R}^{st}$ in time of pigment formation. Three kinds of nonparental kernel phenotypes were observed among 68,883 seeds from $\underline{g}\ \underline{R}^{st}\ \underline{M}^{st}/\pm\ \underline{R}^{nj}\ \pm$ ♀ X $\underline{g}\ \underline{r}\ \pm$ ♂ matings: colorless, self-colored, and Navajo-stippled compound ($\underline{R}^{nj:st}$). The colorless type occurred infrequently and each proved to be either $\underline{R}^{nj}$ or $\underline{R}^{st}$ in progeny tests. As self-colored kernels were frequent, only those from a portion of the total population were chosen for progeny analysis. The results of testing 25 for germinal transmission and linked marker composition are given in the first two lines of the table below:

| Phenotype | | Effective kernel population | Linked marker composition | | | |
|---|---|---|---|---|---|---|
| | | | Parental | | Recombinant | |
| Kernel | Progeny | | $g\ M^{st}$ | $+\ +$ | $g\ +$ | $+\ M^{st}$ |
| $\underline{R}^{sc}$ | $\underline{R}^{st}$ | 14,035 | 11 | 0 | 0 | 1 |
| $\underline{R}^{sc}$ | $\underline{R}^{sc}$ | 14,035 | 6 | 0 | 4 | 3 |
| $\underline{R}^{nj:st}$ | $\underline{R}^{nj:st}$ | 62,815 | 0 | 0 | 0 | 15 |

The fraction of self-colored kernel selections which proved germinal, about one half, and the frequency of this class, $18.5 \times 10^{-4}$ (based on the number of $\underline{R}^{st}$ gametes), agree with the corresponding values obtained for $\underline{R}^{st}\ \underline{R}^{st}$. The distribution of linked markers also conforms to that predicted from the findings with $\underline{R}^{st}\ \underline{R}^{st}$. Four $\underline{R}^{sc}$ mutants were found to carry the $\underline{g}\ \pm$ combination whereas expectation based on independence of mutation from recombination predicts less than one. To permit interpretation of these four as separation of the two components postulated for $\underline{R}^{st}$ by single exchanges, the location of the basic seed pigmenting element should be proximal to the element suppressing its action.

The third nonparental phenotype, $\underline{R}^{nj:st}$, strongly resembles the case described by Brink (M.G.C.N.L. 34:122). The typical stippled pattern is confined to those regions of the kernel normally pigmented by $\underline{R}^{nj}$. Each of the 15 progeny groups recovered from 16 initial $\underline{R}^{nj:st}$ kernel selections yielded the compound phenotype and are found to correspond to $\underline{R}^{nj}$, rather than $\underline{R}^{st}$, in time of pigment formation. They therefore appear to combine the basic $\underline{R}^{nj}$ pigmenting property with the instability of stippled. Each proved to carry the proximal marker of the $\underline{R}^{nj}$ chromosome and the distal marker of the $\underline{R}^{st}$ homologue. That this marker combination is complementary to the principal recombinant class of $\underline{R}^{sc}$ mutants suggests that the two originate as reciprocal products of a single kind of recombinational event. Neither $\underline{R}^{sc}$ nor $\underline{R}^{st:nj}$ expresses the characteristic plant color effects of $\underline{R}^{nj}$, however, so either the two are not strictly complementary, or plant pigmentation is suppressed when brought into cis position with the inhibiting component of $\underline{R}^{st}$.

J. L. Kermicle