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1. The difference in Wx frequency between male and female gametes from $\frac{wx^{Coe}}{wx^{90}}$.

In 1963 a conventional genetic analysis of the heterozygote $\frac{Bz\ +90\ V}{bz\ C+\ v}$, $\frac{ae}{ae}$ showed an interesting difference in $\frac{Wx}{}$ frequency in the male and female gametes. When the heterozygotes were used as males onto the $\frac{bz\ wx^{Coe}\ v}{}$, $\frac{ae}{}$ tester, 76 apparent $\frac{Wx}{}$, $\frac{ae}{}$ recombinants were found in 133,358 kernels. This is a frequency of 57×10^{-5}. The weighted average of $\frac{Wx}{}$ frequency in the pollen of the heterozygotes was 75×10^{-5}. When heterozygous plants of the same genotype were used as female parents, 19 apparent $\frac{Wx}{}$, $\frac{ae}{}$ recombinants were found in 94,158 kernels or a frequency of 20×10^{-5}. The probability that the observed distribution would be found if $\frac{Wx}{}$ gametes were equally likely for both male and female populations is .0001 (from expansion of the binomial distribution and summation).

The verification of the presumed recombinants was hindered by poor germination. Test pollinations (by $\frac{bz\ wx^{Coe}\ v}{}$, $\frac{ae}{}$) were obtained on only 36 plants. Of these 31 came from $\frac{Wx}{}$, $\frac{ae}{}$ recombinants, 2 from $\frac{Wx\ Ae}{}$ contaminants, and 3 from $\frac{wx\ ae}{}$ gametes. These latter could arise by heterofertilization events or misclassification.

The same type of test was repeated in 1965. Plants of the constitution $\frac{Bz\ +90\ V}{bz\ C+\ v}$, $\frac{ae}{ae}$ were used as male and female parents in crosses with the tester stock $\frac{bz\ wx^{Coe}\ v}{}$, $\frac{ae}{}$. When the heterozygotes were used as males, 18 presumed $\frac{Wx}{ae}$ were found in 35,497 kernels. This is a frequency of 51×10^{-5}. The weighted average of $\frac{Wx}{}$ in the pollen of the plants used as male parents is 72×10^{-5}. When the heterozygotes were used as female parents, 17 presumed $\frac{Wx\ ae}{}$ were found in 85,679 kernels or a frequency of 20×10^{-5}.

The agreement between the results of 1963 and 1965 indicates that the difference in $\frac{Wx}{}$ frequency between male and female gametes for $\frac{wx^{Coe}}{wx^{90}}$ heterozygotes is real and reproducible.

In tests with $\frac{Bz\ Wx\ V}{bz\ wx\ v}$ plants that are as closely related as possible to the $\frac{Bz\ wx^{90}\ V}{bz\ wx^{Coe}\ v}$ heterozygote, no differences were found for the $\frac{bz\ wx}{}$ interval (♂♂ 20.0% and ♀♀ 19.1%) or the $\frac{wx\ v}{}$ interval (♂♂ 5.6% and ♀♀ 5.4%).

Oliver Nelson

2. Reconstitution of the R^{st} allele.

Near-colorless aleurone mutants from $\frac{R^r}{R^{st}}$ are associated with crossing over between outside markers and possess all

or part of the paramutagenic action characteristic of the $\underline{R}^{st}$ parental allele. These facts suggest that the stippled phenotype may depend on two or more components that are separable by crossing over. Tests have been made for $\underline{R}^{st}$ reconstitution in various heterozygous combinations of mutants derived from $\underline{R}^{st}$, and an apparently successful test involved the following alleles:

$\underline{R}^{scl}{}_{113}$: Self color mutant from $\underline{R}^{lst}$; nonparamutagenic.

$\underline{R}^{scl}{}_{132}$: Self color mutant from $\underline{R}^{lst}$; as paramutagenic as $\underline{R}^{st}$.

$\underline{r}^{g}(I)^{2}$: Near-colorless aleurone, green plant mutant isolated from $\underline{R}^{r}\underline{R}^{st}$; unstable seed color giving mutations to self color; stable plant color. Mutants of this type are not associated with recombination when isolated from $\underline{R}^{r}\underline{R}^{st}$ plants, also occur in $\underline{R}^{st}\underline{R}^{st}$ plants, and are as paramutagenic as $\underline{R}^{st}$.

$\underline{r}^{g}(I)^{3}$: Near-colorless aleurone, green plant mutant isolated from $\underline{R}^{r}\underline{R}^{st}$; stable seed color; unstable plant color giving mutations to red plant. Mutants of this type are associated with recombination when isolated from $\underline{R}^{r}\underline{R}^{st}$ plants, and are as paramutagenic as $\underline{R}^{st}$.

The two near-colorless mutants were made heterozygous with each of the two self color mutants, and plants of the four heterozygous combinations were pollinated with $\underline{r}^{g}$, $\underline{wx}$ pollen. Stippled kernels were selected from these ears and grown out for verification. The results are shown in Table 1. Tests to definitely exclude the possibility of the stippled kernels having resulted from pollen contamination are not yet complete, but evidence to date makes this very unlikely.

One of the three $\underline{R}^{st}$ mutants isolated from $\underline{R}^{scl}{}_{113}/\underline{r}^{g}(I)^{3}$ was atypical in phenotype, the colored spots being smaller than those characteristic of the standard $\underline{R}^{st}$ allele. The two $\underline{R}^{sc}$ alleles were not tested for back mutations to $\underline{R}^{st}$ in homozygous plants, but McWhirter (MGNL 35:142) tested 98 $\underline{R}^{sc}$ mutants for back mutations to $\underline{R}^{st}$ and none were recovered in over one million gametes.

Positive verification of the reconstitution of $\underline{R}^{st}$ in certain of the heterozygous combinations would indicate that: (1) the stippled phenotype is dependent on two or more genetic components, (2) the components of $\underline{R}^{st}$ can be separated and reassembled by crossing over, (3) the component(s) of $\underline{R}^{st}$ carried by the near-colorless crossover mutant was complementary to the one(s) carried by the $\underline{R}^{sc}$ mutants, (4) the component(s) of $\underline{R}^{st}$ carried by the

near-colorless noncrossover mutant, if any, was not complementary to the one(s) carried by the $\underline{R}^{sc}$ mutants, (5) mutations of $\underline{R}^{st}$ to $\underline{R}^{sc}$ and to near-colorless alleles not associated with crossing over involve alterations of a common $\underline{R}^{st}$ component, (6) paramutagenic and nonparamutagenic $\underline{R}^{sc}$ mutants carry the same unaltered components of $\underline{R}^{st}$, and (7) secondary changes may occur in the separation and reassembling of $\underline{R}^{st}$ components as evidenced by the altered phenotype of one of the reconstitued $\underline{R}^{st}$ alleles.

Table 1

Occurrence of reconstituted $\underline{R}^{st}$ in four heterozygous combinations of $\underline{R}^{sc}$ and near-colorless aleurone, green plant mutants, and in two near-colorless, green homozygotes.

Combination of alleles	Total No. of kernels scored	No. of stippled kernels Selected	Verified as $\underline{R}^{st}$	Non-mutant	Not verified
$\underline{R}^{scl_{113}}/\underline{r}^{g}(I)^3$	24,459	5	3	1	1
$\underline{R}^{scl_{132}}/\underline{r}^{g}(I)^3$	14,877	1	1	0	0
$\underline{R}^{scl_{113}}/\underline{r}^{g}(I)^2$	28,033	0	-	-	-
$\underline{R}^{scl_{132}}/\underline{r}^{g}(I)^2$	19,952	0	-	-	-
$\underline{r}^{g}(I)^3/\underline{r}^{g}(I)^3$	22,260	0	-	-	-
$\underline{r}^{g}(I)^2/\underline{r}^{g}(I)^2$	32,155	0	-	-	-

R. B. Ashman

3. Seed color mutations from $\underline{R}^{r}\underline{R}^{sc}$ heterozygotes.

Three general classes of mutations to or toward colorless aleurone in $\underline{R}^{r}\underline{R}^{st}$ plants have been identified: near-colorless aleurone, green plant; near-colorless aleurone, red plant; and colorless aleurone, red plant. The near-colorless, green mutants do not form a homogeneous group, varying in seed and plant color stability and in their association with recombination between outside markers. Tests have shown that near-colorless mutants possess either all or part of the paramutagenic action of $\underline{R}^{st}$, and that colorless mutants are nonparamutagenic. The apparent association between the near-colorless phenotype and paramutagenic action was examined further in the following test.