3. Effect of su_2 on sh_2.

When $\underline{Sh}_2$ $\underline{sh}_2$ is segregating in ears homozygous for $\underline{su}_2$ $\underline{su}_2$, the highly collapsed phenotype typical of $\underline{sh}_2$ $\underline{sh}_2$ is not obtained. The doubly recessive $\underline{su}_2$ $\underline{su}_2$ $\underline{sh}_2$ $\underline{sh}_2$ kernels are similar to $\underline{su}^{am}$ $\underline{su}^{am}$ $\underline{du}$ $\underline{du}$ kernels in phenotype. This reaction is similar to the effect of $\underline{ae}$ on $\underline{su}_1$.

-- Herbert H. Kramer

4. A new "salmon silk."

A salmon silk character which shows good expression in the absence of red pericarp has appeared in progeny from U. V. treated pollen. If allelic to $\underline{sm}$, linkage studies on chromosome 6 should be facilitated.

-- Herbert H. Kramer

PURDUE UNIVERSITY
Lafayette, Indiana
Department of Botany and Plant Pathology

1. The Wx/wx locus.

A. Segregation in backcross progenies

The frequency of $\pm^{wx}$ pollen grains in plants of a backcross progeny (90 x C) x C has been investigated with greater numbers than previously. At the same time the F_1 was grown as a standard. The mean frequency of $\pm^{wx}$ pollen grains in the F_1 plants sampled was 74×10^{-5} with the individual plant estimates being 73; 68; 80; 89; 66; 69; 80; 82; 89; 77; 74; 58; 67; 86; 59. These represent single estimates from each of 15 plants. In 1958 the mean frequency of $\pm^{wx}$ pollen grains from the same cross was 88×10^{-5}.

Sixty plants from the BC progeny were sampled. Of these 34 plants had a $\pm^{wx}$ frequency of less than 2×10^{-5} (or 0 after correction for parental $\pm^{wx}$ frequency). For the remaining 26 plants the mean frequency of $\pm^{wx}$ was 89×10^{-5} with the individual plant estimates being 98; 103; 75; 65; 107; 138 (125, 151); 57; 93; 87; 65 (51, 78); 89, 63; 74; 108; 87; 75; 63; 103; 115; 92; 68; 99; 114; 138 (143, 132); 70; 78. These figures represent single estimates for each plant with the exception of three which are an average of the estimates enclosed in parentheses. Some of the frequencies estimated for BC plants appear obviously to be outside the range of estimates for the F_1 plants and may represent an effect of genetic background on this recombinational process.

Another backcross progeny (C x H21) x C has also been sampled. Here there were 37 plants of which 18 had a $\pm^{wx}$ frequency of less than 2×10^{-5} while 19 plants had high frequencies.

The relative proportions of zero frequency (after correction for parental frequency) plants to high frequency plants in the two backcross progenies are not in disagreement with the ratio of 1 zero frequency plant: 1 high frequency plant expected if heterozygosity at the waxy locus were a prerequisite for the production of a high frequency of $\pm^{wx}$ pollen grains.

B. Investigation of intragenic recombination by conventional techniques.

With the realization that an F_1 plant of a cross between two independently occurring waxy mutants could have a frequency of $+^{wx}$ pollen grains averaging 88 x 10^{-5}, it becomes possible to study recombination in such a high frequency F_1 by seed classification.

The highest frequency F_1 is 90 x C. The C stock (for Cornell), which was obtained from the Maize Coop in 1951, is marked on only one side as c sh wx^C. However, a stock from E. H. Coe which is sh bz wx v_1 behaves in crosses as does wx^C with respect to frequency of $+^{wx}$ pollen in F_1 crosses with other mutants. The allele from Coe's stock and wx^C are probably the same, but for the record it will be referred to as wx^{Coe}.

The F_1 between 90 and sh bz wx^{Coe} v_1 gives a mean frequency of 90 $+^{wx}$ x 10^{-5} pollen grains. An estimated population of 1.4 x 10^6 pollen grains from 26 plants was scanned. These 26 (+ other) F_1 plants were pollinated by bz wx^{Coe} v_1 pollen, and pollen from them was put on bz wx^{Coe} v_1 plants. The results are given in Table 1. It is clear that there is a significant difference in the frequency of $+^{wx}$ kernels between the reciprocal crosses. While the $+^{wx}$ frequency where the F_1 was the female is in the range which might be expected from the pollen studies (.072% from a relatively small sample of kernels as compared to .090% from the pollen), there is a greatly reduced frequency when the pollination is made in the other direction.

Table 1. Backcrosses of (wx^{90} x wx^{Coe}) with wx^{Coe}.

Cross	No. Ears	No. Kernels	No. $+^{wx}$ k	% $+^{wx}$ k
$\frac{+\ wx^{90}\ +}{bz\ wx^{Coe}\ v_1}$ x bz wx^{Coe} v_1	68	23591	17	.072
bz wx^{Coe} v_1 x $\frac{+\ wx^{90}\ +}{bz\ wx^{Coe}\ v_1}$	309	61004	12	.019

Two possibilities suggest themselves as explanations of this reciprocal difference. The first is that some of the pollen grains which are scored as $+^{wx}$ are not carrying fully functional $+^{wx}$ alleles but intermediate alleles. In the case of such alleles, gene dosage could be the critical factor in determining whether or not we detect the kernel carrying the recombinant. Some support is given to this idea by the observation that using our standard stain pollen grains from a mutant which gives 5% amylose in the endosperm stain as normals. In any case, it will be relatively simple to determine by chemical analyses of the stocks arising from the $+^{wx}$ kernels if intermediacy constitutes the basis for the reciprocal differences observed.

A second possibility is that the bz wx^{Coe} v_1 stock is carrying a gametophyte factor. Then, it may be assumed that the event or events resulting in the formation of the $+^{wx}$ locus in the F_1 plant usually place this locus in a chromosome not carrying the gametophyte factor. Such gametes will be at a disadvantage in effecting fertilization on the bz wx^{Coe} v_1 when competing with others carrying the gametophyte factor and this would reduce the frequency of $+^{wx}$ kernels when the F_1 is used as the male but not as the female parent. We know, however, that in the cross bz wx^{Coe} v_1 x $\frac{+\ wx^{90}\ +}{bz\ wx^{Coe}\ v_1}$ there are no deficiencies of bz kernels. We are testing now for v_1 so that the possibility of a gametophyte factor can be either excluded or explored further.

This is the second year in which a reciprocal difference has been found. In 1958, we used the F_1, 90 x C, as both male and female parent in crosses with various wx stocks. When the cross was made (90 x C) x wx we found 4 +wx kernels in a total of 4054 (.1%) kernels. For the reciprocal, wx x (90 x C), there were 2 +wx of 7724 total (.03%). But the numbers are small, and without markers contamination could not be excluded. Thus, it was not thought to be noteworthy until this year's data showed a similar effect.

Since outside markers bz and v_1 were present in this year's test, it is of interest to note their assortment in the +wx kernels. There were a total of 29 +wx kernels of which 27 germinated so that they could be scored for both markers. Of the 27, 12 were bz +v, 7 bz v, 6 +bz v, and 2 +bz +v. The kernels in this last group may represent contaminants. The outside markers are so far from the wx locus that great reliance should not be put on the data. It seems, however, reasonable to conclude that not all +wx kernels arise from an orthodox recombinational event in one direction.

C. Technique

In the past year, it has been found that the greatest possible differentiation between wx and +wx pollen grains is found when preparations are made using anthers from the less mature floret (which is distal to the tassel branch) at a time when the proximal or more mature floret is ready to extrude its anthers. Making pollen preparations in this manner will not change the estimate of +wx frequency, but the preparations are easier to score.

It has not been mentioned, but to work successfully with wx^a or crosses involving wx^a it is necessary to reduce the strength of the iodine from the 45 mg./25 ml of the standard stain to 31 mg./25 ml. and to allow somewhat more time for destaining to take place before scoring.

D. Preliminary tests of nitrogen base analogues as mutagens on maize.

One of the problems in the Wx/wx locus work is the lack of a number of mutants induced in a common background. As part of the attack on this problem, we tested for mutagenicity an assortment of purine and pyrimidine analogues as well as a few other compounds which have been reported as having mutagenic activity. The advantage of such compounds (where they are effective mutagens) is their propensity to produce point mutations. Specifically with maize we would hope that some of these would be pre-meiotic in time of origin.

The base analogues tested were 2,6,8-trichloropurine; 2,6-di(diethyl-amino) purine; 2-thioxanthine; 5-bromodeoxyuridine; 2-thioadenine; 2-oxypurine; isoguanine; 2,6-diaminopurine; 6-methyl-2-oxypurine; 6-azathymine, 6-oxy-2,8-thiopurine; 2-amino-6-thiopurine; and 5-bromouracil. Acridine and proflavine were also tested. I am indebted to Dr. Seymour Benzer for supplies of the above chemicals.

All the compounds were tested by injecting a solution of the compound into wells bored into the stem of a plant at three-day intervals extending from a time judged to be premeiotic until the first pollen was shed. Multiple injections were made, therefore, into each plant. Because most of the compounds have limited solubilities in water, saturated aqueous solutions were generally used. Even with the wells bored into the plants only .25 to .5 ml could be injected at one time. On each injection day after allowing time for uptake, a second injection was made. There was no effect on growth nor delay in flowering with any compound used.

The treated line was the dent inbred M14 (+bz +wx $+v_1$). Pollinations were made onto a recessive tester stock, bz wx v_1, and the ears scored for bz or wx kernels (kernels which were both bz and wx were considered to be the result of accidental self-pollination) and for bz, wx

or bz wx sectors. Between 3,000 and 6,500 gametes were sampled per treatment. The sum of apparent gametic mutations plus sectors for most compounds ranged from .03% to .09%. One compound, 2-amino-6-thiopurine, did not produce any mutations nor sectors in 3074 kernels. Two compounds, proflavine and 2-thioadenine, were apparently more effective than the other substances since the sum of mutants plus sectors was .29% for 2-thioadenine (2779 kernels) and .20% for proflavine (4645 kernels). For 5-bromouracil, only 1 pollination was obtained owing to breakage of the injected plant. One apparent mutation to wx was found in 474 kernels. All the mutant kernels are being grown to ascertain whether the embryos are homozygous or heterozygous for bz or wx as the case may be.

Pollen of untreated M14 plants was irradiated for 4 and 6 minutes under an ultraviolet lamp. The sum for the 4-minute treatment was .9%, and for the 6-minute treatment it was 1.1%.

E. Attempts to affect inter- and intragenic recombination differentially.

It is still a question as to whether intergenic and intragenic recombination have the same physical basis. If a stimulus could affect markedly one type but not the other, it would suggest a different basis for the two types. Roman has shown in yeast that ultra-violet radiation can greatly increase intragenic recombination without apparently affecting intergenic recombination. We have attempted to do the converse in maize (i.e., use agents which have been reported to increase intergenic recombination and then look for an effect on intragenic recombination). The material used here was the $F_1 \frac{+\ +\ wx^{90}\ +}{sh\ bz\ wx^{Coe}\ v_1}$ which has already been reported on in other connections. Two plants were injected as described for the base analogues with .001 M Versene (Na_2) every two days from a premeiotic stage until pollen shed. The usual pollen collections were made, and pollinations were made onto the sh bz wx^{Coe} v_1 stock. The frequency of $+^{wx}$ pollen grains in the treated plants was not different from the frequency in control plants. Nor could any increase in the frequency of recombination between sh and bz over the controls be detected in the pollinations made from the treated plants. The same negative results were garnered from 4 plants which were sprayed with .2 M $MnSO_4$ every five days from the time that they were 10 inches high until the emergence of tassels from the boot.

With the apparent inability of these agents to affect either type of recombination under the conditions of our test, it was not possible to obtain the information originally desired.

-- Oliver E. Nelson

2. The fourth chromosome gametophyte factor in some Central and South American races.

As has been pointed out previously, knowledge of the allelic constitution of a variety for the fourth chromosome gametophyte factor, ga/Ga/Ga^s, can be an aid in tracing the evolutionary history of that variety. This is so because male gametophytes carrying Ga and Ga^s exclude or nearly exclude male gametophytes carrying ga when both types are competing to effect fertilization in plants which are ga/Ga, ga/Ga^s, Ga/Ga^s, Ga/Ga, or Ga^s/Ga^s. The competitive advantage of Ga^s or Ga alleles over ga in such situations is close to 1. On the other hand, there is no advantage of ga gametophytes over Ga or Ga^s gametophytes on ga/ga plants. When either Ga or Ga^s is introduced into a stock by introgressive hybridization or mutation and becomes established, its frequency must increase rapidly until it is equal to 1. It is not possible to derive a variety which is ga/ga from a cross between two other varieties one of which is Ga or Ga^s. All United States varieties which we have tested here have been ga/ga (except for Papago Indian Corn which is Ga/Ga). This includes southern whites, northern flints, and middle western dents.