2. Fluorescent compounds in Bf-1.

Isolation and identification of the anthranilic acid-containing blue fluorescent substances in anthers of <u>Bf-l</u> are being carried out (see MGCNL 32:28, 1958). Extraction of strongly fluorescent fatty substances has been found to improve paper chromatography and chemical fractionation of the blue fluorescent components. The main fluorescent compounds are easily oxidized during purification. One of the substances has been obtained in crystalline form.

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1. Recombination values for 11 alleles at the Wx locus.

Presented here are the recombination values (Wx pollen grains x 10-5) for 54 of the possible 55 F₁'s between 11 wx alleles of independent origin. The results of intercrosses between the alleles C, a, H21, 90, and B have been reported previously. Of the new alleles 1, 2, 1, 6, and 8 were kindly supplied by Dr. R. A. Brink who detected the mutations in an experiment designed to test mutation rate in a PVV stock. After being received at Purdue the mutant alleles were separated from the allele (probably C) in the tester stock used to detect the mutational event. The R allele was furnished by Dr. D. W. Richardson who found the mutation in a stock of popcorn.

The recombinational values for the crosses between the new alleles and between the new alleles and the old alleles are given with considerable reservations. In the first place, the stocks are very heterogeneous with respect to background. We now know that in crosses between two different wx alleles, differences in genetic background can have a pronounced effect upon recombination values. In the second place, data for these new crosses have come from only two plants for each cross. For these reasons, it is felt that the recombination values given may be poor estimates of the frequency of recombination that would be shown by 2 alleles in a common genetic background.

It is felt, however, that a reliable datum for each cross is whether or not any recombination is observed (frequencies below 2 x 10⁻⁵ are considered as not showing recombination since some of the parental stocks may have a frequency this high). Table 1 presents the data in both forms for each cross. The order of the alleles is arbitrary as will be shown.

Inspection of the table will show that using lack of recombination as a criterion, one can separate the alleles into three distinct sets. They are (1) \underline{C} which recombines with every other allele; (2) the set delimited by \underline{R} and including $\underline{H21}$, \underline{L} , and $\underline{2}$, none of which recombine with \underline{R} ; and ($\underline{3}$) the set delimited by \underline{B} and including \underline{a} , $\underline{1}$, $\underline{90}$, $\underline{6}$, and $\underline{8}$, none of which recombine with \underline{B} . Any member of a set will recombine with all alleles in the other 2 sets. Within a set, 2 alleles may or may not show recombination.

Table 1. Qualitative and quantitative recombination data for 54 F₁ progenies between 11 wx alleles of independent origin. The quantitative data are given as frequencies x 10-5. For the qualitative data, a (0) indicates no recombination observed, a (+) indicates recombination.

reco	mbinat C	ion. a	ı	В	90	6	8	4	H21	R	2
c		+	+	+	+ .	+	+	+	+	+	+
a	5	_	0	0	0	0	?	+	+	+	+
1	19	0		0	0	0	0	+	+	+	+
В	26	0	0		0	0	Ō.	+.	+	+	+
90	80	0	0	0	(0	0	+	+	+	+
6	59	0	<u>l</u> t	0	0		0	+	+	+	+
8	47	?	6	0	4	0		+	+	+	+
14	49	50	106	<u>4</u> 8	43	514	112		0	0	+
H21			49	36	32	71	39	0		0	+
R			·	32	47	149	40	0	0		0
	-					20	125	6.	1 13	0	
2											

On the basis of recombination or lack of recombination, the alleles can be arranged in a linear array within each set (according to the criterion of Benzer, 1959, P.N.A.S.) although there are too few alleles in any set to make this particularly meaningful. The sets, however, can be arranged in any order with respect to each other and still meet Benzer's specifications.

On the same basis, H21 and L are genetically indistinguishable from each other. Either B or 90 are genetically indistinguishable from a (depending on whether or not a should show recombination with 8) but are distinguishable biochemically since a allows limited synthesis of amylose. All other alleles can be shown to be different on this basis which is a rigorous one.

The results emphasize the necessity for having all alleles in a common genetic background if recombination frequencies are to be meaningful. We're meeting this requirement as rapidly as possible.

Oliver Nelson

2. A test for intermediacy of Wx recombinants.

It had been suspected that some Wx alleles arising in crosses between wx90 and wxC or wxCoe were not fully functional. In fact, in the large-scale conventional test of recombination between wx90 and wxCoe where Wx kernels were detected by staining, a few Wx kernels were tentatively identified as intermediates because of their weak-staining propensities.

In 1961, 59 viable <u>Wx</u> recombinants from the 1960 test of <u>Bz</u> <u>wx</u> 90 <u>V</u> / <u>bz</u> <u>wx</u> Coe <u>v</u> were crossed by and onto <u>bz</u> <u>wx</u> Coe <u>v</u> in order to verify their genotypes. The kernels resulting from these crosses were then available to test for amylose percentage.

In the past several months, 20 Wx alleles arising by recombination have been tested for their ability to support amylose synthesis. recombinants carrying one or both of the recessive outside markers from the cross Bz wx90 V / bz wxCoe v were used in order to be certain that no Wx contaminants were tested. Starch was isolated from the stocks to be tested by the method of Shuman (Report #4 to the Corn Industries Research Foundation, Inc.). Such starch isolates had less than 1% protein as measured by Lowry's assay using the Folin phenol reagent. Protein (gluten) would be the most likely contaminant of any starch sample. The amylose percentage of the starch isolates was measured by the "Blue Value" method given by Ulmann and Augustat (Z. Anal. Chem. 162:337-3141, 1958). The "blue value" read in the assay was converted to amylose percentage by reference to an amylose calibration curve constructed from mixtures in varying proportions of 3X recrystallized amylose and amylopectin. Such a standard curve was run with every group of assays.

The results of the assays are given in Table 2. Note that the kernels being tested were either $\frac{Wx}{Wx} \frac{Wx}{wx}$ ($\frac{Wx}{wx}$ recombinant x the bz $\frac{wx}{x}$ tester, 15h92) or $\frac{Wx}{wx} \frac{Wx}{wx}$ ($\frac{Dz}{x}$ $\frac{wx}{x}$ tester x recombinant). The duplicate values given for each cross are assays on different days from the same starch isolate.

Reference to Table 2 shows no definite indication that any of the recombinants can be considered significantly less effective than the check in supporting amylose synthesis. The three recombinants where the reciprocal crosses were analyzed were originally thought to be intermediates because of their weak-staining properties. Even with these recombinants, effectiveness in supporting amylose synthesis cannot be considered less than normal.

It is interesting to observe that our results confirm the observation of Sprague, Brimhall, and Hixon and Sager that the Wx dosage effect on amylose percentage is not linear. However, we derive quite a different dose effect curve. Considering all isolates from Wx/Wx/wx kernels, the mean amylose percentage is 19.8. For all isolates from Wx/wx/wx kernels, the mean amylose percentage is 13.1. Both these values are well below those given by Sprague et al. and Sager.