Evidence supporting the theory that the charge differences are due to differences in charged side groups comes from experiments with glyceraldehyde. When extracts of seedlings of each of the four genotypes ($\underline{E_4}^C/\underline{E_4}^C$, $\underline{E_4}^D/\underline{E_4}^D$, $\underline{E_4}^E/\underline{E_4}^E$, $\underline{E_4}^F/\underline{E_4}^F$) are incubated for 24 hours in the presence of glyceraldehyde and then run in electrophoresis, it is found in each case that the series of enzyme bands is converted to a single more acidic band. In all four cases, the converted bands move to the same position in the starch gel. Thus, glyceraldehyde eliminates the charge differences between the various enzyme bands while maintaining their esterase activity. These results favor the theory that the charge differences are located in side groups attached to the enzyme molecules, since it seems unlikely that glyceraldehyde would cause breakage of the peptide linkages between amino acids in the enzyme molecules, resulting in the loss of charged amino acids. It is interesting to note that glyceraldehyde also has an effect on another, non-allelic esterase in maize, the $\underline{E_1}$ esterase. In this series, charge differences between enzymes in the series are also eliminated by glyceraldehyde treatment. In this case, it has been demonstrated (Schwartz, Genetics 52: 1295-1302, 1965) that glyceraldehyde exerts its effect on the esterase molecule by causing a change in the net charge of the molecule rather than by causing dissociation of the molecule into smaller subunits.

John W. Harris

8. Maize DNA composition: analysis of plants with and without B-chromosomes.

DNA was extracted from etiolated maize seedlings by the following modified Marmur technique. Plants were ground in liquid nitrogen to a fine powder in a mortar. The powder was added to an equal weight of NaCl-EDTA solution (0.15 M NaCl + 0.1 M EDTA, pH 8.0). Sodium lauryl sulfate (25% in H_2O) was added to a final concentration of 2% and the suspension was lysed at 60°C for 10 minutes. After the solution cooled to room temperature, 5 M NaCl was added with rapid stirring to a final concentration of 1.4 M NaCl. The suspension was centrifuged at 3000 x g for 5 minutes. The supernatant was filtered through silk, layered with ice-cold ETOH and the DNA was wound out on a glass rod and dissolved in dilute saline citrate (DSC) (0.015 M NaCl + 0.0015 M sodium citrate). The solution was brought to standard saline citrate concentration (SSC) (0.15 M NaCl + 0.015 M sodium citrate) using concentrated saline citrate (CSC) (1.5 M NaCl + 0.15 M sodium citrate). The DNA solution was deproteinized three times by shaking 15 minutes with an equal volume of chloroform-isoamyl alcohol (24:1, v/v, layering the aqueous phase with ice-cold ETOH, and winding out the DNA. In all cases, the DNA was dissolved in DSC and brought to SSC with CSC (all steps must be carried out using a minimum volume of saline citrate). RNase (5mg/ml in H_2O: heated for 10

minutes at 70°C) was added to a concentration of 50 µgm/ml and incubated for 30 minutes at 37°C. Deproteinization was repeated until free of protein. After the last deproteinization the DNA was dissolved in DSC and 1/10th volume acetate-EDTA (3.0 M sodium acetate + 0.001 M EDTA, pH 7.0) was added while stirring. The DNA was wound onto the stirring rod upon dropwise addition of 0.54 volume of isopropyl alcohol. After washing the DNA progressively in 70, 80, and 95% ETOH to remove the acetate, it was dissolved in SSC and stored over chloroform. This technique was obtained from Dr. Gene Williams, Botany Dept., I. U. The amount of DNA was calculated by absorption at 260 mµ assuming 1.0 O.D. unit equals 45 µgm of DNA. In runs with the marker DNA present (Figures 1 & 2) 2 µgm of the sample DNA and 1.5 µgm of the marker DNA were dissolved in 0.80 ml of SSC and added to 1.0300 gm of CsCl (optical grade obtained from The Ealing Corp., Cambridge, Mass., Cat. #90-495). This gives the sample a density of approximately 1.71 g/cm^3. In the run shown in Figure 3, 4.2 µgm of DNA were used. All samples were centrifuged in a Beckman Model E Analytical Ultracentrifuge at 44,770 rpm at 25°C. After 20-22 hours photographs were taken using UV optics and the developed photograph was scanned on a Beckman Analytrol Densitometer. The marker DNA was Aerobacter aerogenes which has a buoyant density of 1.715 gm/cm^3 ** and a G-C content of 56%. Heat denaturation studies of maize were carried out on a Beckman DU equipped with a high temperature cell.

Fig. 1

Fig. 2

1.715
A. aerogenes

Fig. 3

1.7015
maize

Figure 1 shows the densitometer tracing of the photograph produced by centrifugation of DNA extracted from Black Mexican Inbred Line with no B-chromosomes present. Figure 2 is the tracing from a run using another line with an average of 4.8 B-chromosomes per diploid genome. Both of these runs used Aerobacter as the marker DNA. It can be seen that both samples of maize DNA band at the same place in relation to the marker DNA. The density of the maize DNA calculated from the marker density is 1.7015. This

** (determined in relation to E coli at 1.710 gm/cm^3)

corresponds to 42% G-C content (Ifft et. al., 1961, J. Phys. Chem. 65: 1138-1145).

Figure 3 is the tracing of a run when excess DNA from plants with an average of 4.8 B-chromosomes per diploid genome was used in order to note any minor amounts of DNA of a different density. As can be seen from this curve, no significant minor peaks are present. All centrifuge runs reported banded at the same point in relation to the reference markers in the centrifuge.

This study indicates that the B-chromosome DNA has an overall G-C content of 42%, the same as the DNA from the A-chromosomes. Heat denaturation studies of plants with B-chromosomes give results consistent with this base-ratio. Dr. Norman Sansing at The University of Georgia has analyzed the DNA from a single cross hybrid of maize using CsCl centrifugation, heat denaturation, and enzymatic hydrolysis and subsequent column chromatography. He determined a G-C content of 42% for this stock.

Note: van Schaik and Pitout in this MNL have reported that they find differences in base-ratio for three different stocks studied. Their base-ratio determination for the inbred agrees with those reported here, but their determinations for the other lines do not. Differences in extraction and analysis procedures exist and at this time no definite conclusions can be stated.

Karl Rinehart

9. Loss of dominant markers in single chromosomes.

In the 1964 field planting and in the 1965 greenhouse crop, crosses of $\underline{A}_1\ \underline{Sh}_2/\underline{A}_1\ \underline{Sh}_2$ male parents on $\underline{a}_1\ \underline{sh}_2$ silks gave a few a sh seeds. Many and perhaps all of these had colored embryos. A large number of crosses were made in 1965 involving the same $\underline{A}_1\ \underline{Sh}_2$ stock as male parent with female parents homozygous for recessive genes on several different chromosomes (su, pr, r, wx, c, $\underline{a}_1$). The resulting ears showed a low frequency of the mutant present in the female parent. Although these kernels must be tested further, it is evident in several cases that contamination is not the explanation. For example, the a sh kernels on ears resulting from a cross of a sh dt females with A Sh/A Sh, Dt/Dt males were also Dt. In crosses of r wx females with R/R Wx/Wx males, colorless kernels were found which were Wx in phenotype. A few colorless waxy kernels also were found and these are probably contaminants. Kernels with small sectors of mutant tissue have also been observed but the frequency of fractional deficiencies is much less than that of whole kernel losses in the "high loss" ears. Preliminary observations indicate the highest rate of loss occurred for markers on chromosome 3.