7. The E_4 esterase.

The E_4 esterase in maize migrates toward the positive electrode in starch gel electrophoresis at pH 8.5. There are five different alleles of the gene responsible for the production of the E_4 esterase. Four of the alleles are distinguishable by the relative rates of migration of the enzymes which they produce in electrophoresis. The fifth form is a null gene which produces no active E_4 esterase. In the roots of seven day seedlings, each of the four active alleles produces a series of enzyme bands. In each case, the slowest moving band of the series is the most intense, with each faster moving band having a lower intensity than the band below it. Each of the four alleles is distinguished by the position of the slowest moving band of the series. Diagram 1 shows the relative position of each enzyme series in starch gel electrophoresis.

When samples of root extracts from seedlings with different genotypes are run side by side on the same piece of filter paper, it is observed that the bands produced by the different alleles correspond. That is, the slowest moving band produced by allele E_4^F migrates to the same position as the second band produced by allele E_4^E; the slowest band produced by allele E_4^E migrates to a position identical to the second band of the E_4^D series, etc. This correspondence holds for all four series.

In heterozygotes between any two of the four active alleles, the enzyme produced by each allele migrates to the same position as that found in the homozygote. For example, a E_4^C/E_4^D heterozygote produces an enzyme series in which the bands migrating to the C and D positions are intense, with the faster moving bands being less concentrated. A E_4^C/E_4^E heterozygote produces a series in which the bands migrating to the C and E positions are intense with the other bands being less concentrated. There is no evidence of hybridization by dimer formation in the E_4 series.

The question arises as to the nature of the differences between the enzyme bands which cause them to migrate to different positions in starch gel electrophoresis. There are several possibilities which could explain the differential migration rate. One possibility is that the differences in migration rate could be due to significant differences in molecular weight, as would occur if the different bands were to represent different degrees of enzyme polymerization. The differences in migration rate could also be due to differences in charge between enzyme forms in the different bands. This charge difference could be due either to differences in charged side groups associated with the enzyme molecule or to differences in net charge of the amino acids in the polypeptide. Two lines of evidence support the theory that the differences in migration rate are due to differences in charge rather than to significant differences in molecular weight.

The first line of evidence comes from experiments in which extracts from roots of seven day seedlings with genotype $\underline{E_4}^D/\underline{E_4}^D$ were run in electrophoresis using starch gels of different concentration. One set of starch gels contained 10.5 grams of starch per 100 milliliters of buffer while the other set contained 15 grams of starch per 100 milliliters of buffer. If the different bands were to migrate to different positions due to differences in the degree of polymerization of each band, one would expect that the larger molecules would be relatively more hindered in their movement in starch gels than would smaller molecules and thus, a change in concentration of the starch gel would affect the movement of the larger molecules more extremely than the smaller molecules. Measurements were taken of the movement of each band with respect to the origin. The results are shown in table 1. The results are expressed as ratios of the movement of the bands which migrate to the D and E positions (see diagrom 1) as compared to the movement of the band which migrates to the F position for each sample. The table shows that there is no significant difference in the rates of movement of the bands migrating to positions D and E for each sample in the two types of starch gel. Thus, the evidence indicates that there is no significant difference in the molecular weight of the enzymes of the different bands.

The second line of evidence comes from experiments in which samples were run in gels that were made with buffer that was lower in pH than the pH 8.5 buffer normally used to make starch gels. If the differences in the migration rates of the bands are due to differences in charge, then one should be able to lower the pH of the gels to a point where it is below the isoelectric point of the slower moving bands but still above the isoelectric point of the faster moving bands. At such a pH, one should be able to obtain movement of some of the bands toward the cathode while others are still moving to the anode. Samples from seedlings with genotypes $\underline{E_4}^E/\underline{E_4}^E$ and $\underline{E_4}^F/\underline{E_4}^F$ were run on gels with three different pH ranges, pH 6.5, pH 6.0 and pH 5.5. With the pH 6.5 gels, all bands from the F seedlings still migrated in the direction of the anode. However, the lowest band from the E seedlings migrated slightly to the cathodal side of the origin. At pH 6.0, all bands from the F seedlings still migrated to the anode. The lowest band from the E seedlings migrated further from the origin in the direction of the cathode. At pH 5.5, the lowest band from the F seedlings migrated slightly to the cathodal side of the origin. The lowest band from the E seedlings migrated still further in the direction of the cathode and the second band also migrated slightly to the cathodal side of the origin. Thus, by lowering the pH of the starch gels, it was possible to divide the series of bands produced by a single allele, with some migrating to the cathode, while others migrated to the anode. Therefore, the evidence indicates that the bands of a series differ in charge.

Diagram 1
Enzyme Series Produced by the Alleles of the E_4 Esterase

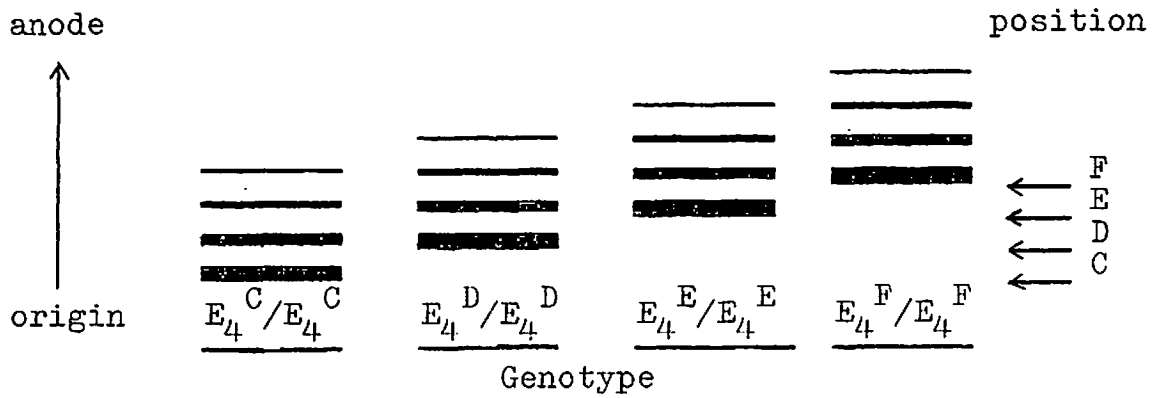

Table 1
Esterase Migration in Starch Gels of Different Concentration

Set 1 (gel conc. = 10.5 gm./100 ml.)			Set 2 (gel conc. = 15 gm./100 ml.)		
Sample	Band 1	Band 2	Sample	Band 1	Band 2
1	.76	.87	1	.78	.90
2	.77	.87	2	.75	.87
3	.76	.88	3	.74	.87
4	.75	.89	4	.75	.88
5	.77	.89	5	.75	.88
6	.77	.88	6	.77	.90
Average	.76	.88	Average	.76	.88

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.

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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