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1. Characterization of DNA from maize lines of different heterochromatic constitution.

Maize is a suitable organism with which to investigate the nature and functional significance of heterochromatin, since in this organism well characterized genetic stocks possessing different heterochromatic constitutions are available. We have used the following lines:

A. Lines with large heterochromatic knobs or chromosomal segments

K_{10} - a large heterochromatic segment on chromosome 10

K_9 - " " " knob " " 9

B chromosome line - possesses 2-4 B chromosomes/plant

F_1 - bulk seed of the hybrid C121E x C103A

B. Lines without large heterochromatic knobs

Wilbur's Flint knobless

DNA was isolated using a modified version of the Marmur technique described by Rinehart (M.N.L. 40: 1966). Etiolated epicotyls from five day seedlings were ground in liquid nitrogen and then made into a slurry with an equal weight of 0.15 M NaCl - 0.1 M EDTA pH 8.0 buffer. Sodium lauryl sulfate was added to a final concentration of 2% and the mixture was heated at 60° C for 10 minutes. Pronase (2 mg/ml final) was added followed by incubation at 45° C for 3 hours. An equal volume of buffer saturated phenol was added and after shaking for 20 minutes the emulsion was centrifuged to separate the phases. The aqueous supernatant was removed and the interphase was re-extracted with a ½ volume of buffer, recentrifuged to produce a second aqueous supernatant which was pooled with the first. The DNA was wound out after layering with two volumes of cold ethanol, washed sequentially in 70%, 80% and 95% ethanol and finally taken up in 1/10 SSC (SSC is 0.15 M NaCl + 0.015 M sodium citrate). After adjusting the ionic strength to SSC, pancreatic RNase (200 ug/ml) and T_1 RNase (20 units/ml) were added and incubated at 37° for 45 minutes. Self digested Pronase (100 ug/ml final) was added and the solution was incubated at 37° C overnight. The solution was deproteinized by shaking for 15 minutes with an equal volume of chloroform:isoamyl alcohol (24:1). The interphase was

Fig. 1 CsCl Gradients

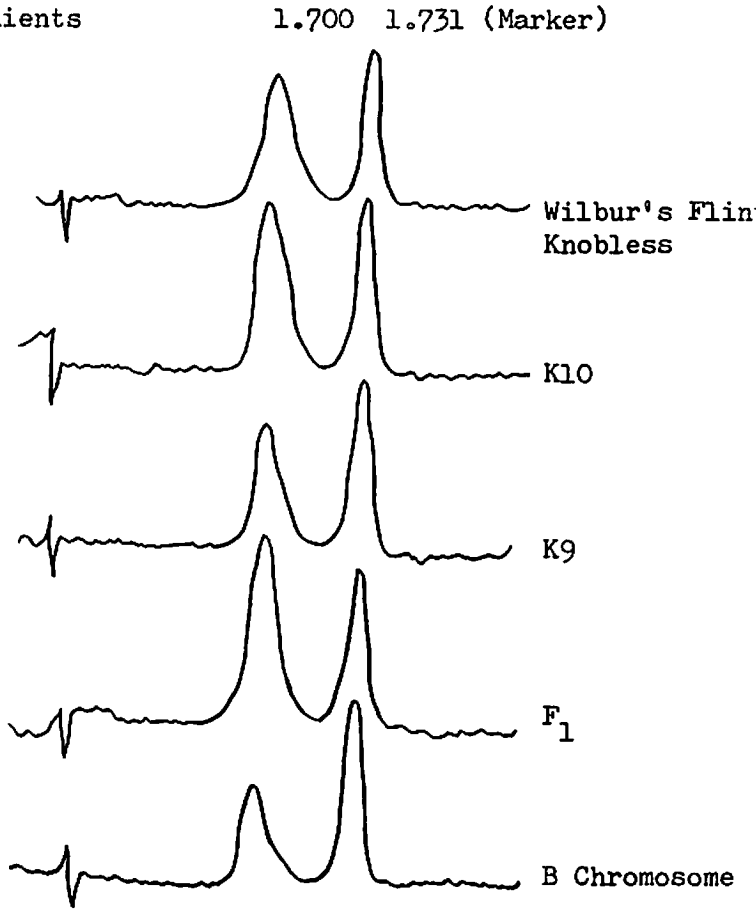
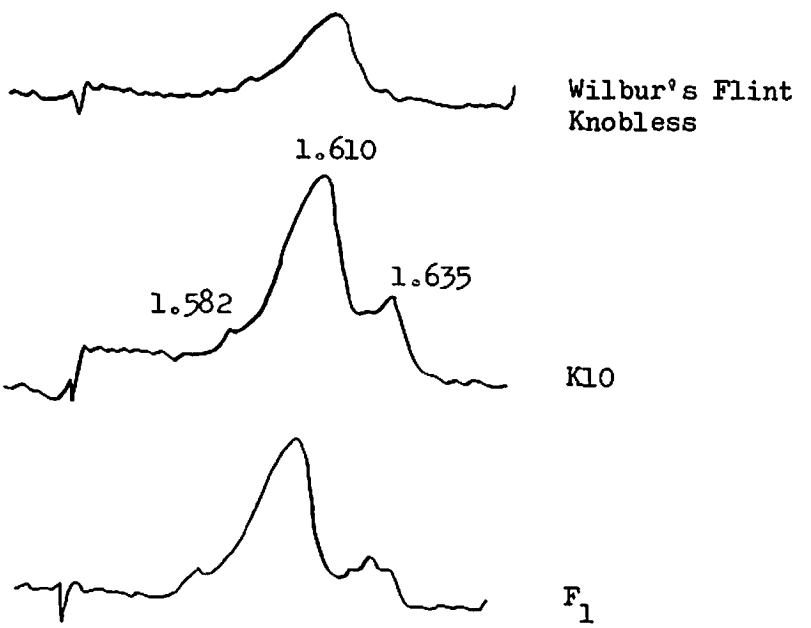


Fig. 2 Act.D-CsCl Gradients



re-extracted with a ½ volume of buffer (SSC) and aqueous supernatants were pooled. This deproteinization was repeated until no protein was seen at the interface. 3M Na Acetate (pH 6.0) was added (1/10 volume). The DNA was wound out after layering with two volumes of cold ethanol, again washed in 70%, 80%, and 95% ethanol and finally taken up in 0.01 M tris-HCl, 0.001 M EDTA pH 8.0.

Buoyant Density in CsCl

Buoyant densities were determined in a Beckman Model E ultracentrifuge. Within the limits of our measurements all 5 lines have an identical buoyant density of 1.700 (Fig. 1). In all lines there is a heavy shoulder, which is perhaps more pronounced in the B chromosome line. Rinehart (MNL 40: 1966) also found identical densities of the DNA from lines with and without B chromosomes, but measured a slightly heavier density (1.7015) than we have observed here. This difference, if significant, could be due to such factors as slightly different techniques, different marker DNA's or to different maize lines.

Fractionation using Actinomycin D

Act D is known to bind to guanine bases in DNA thus causing a reduction in the buoyant density. We would like to report here the preliminary results of analytical CsCl centrifugation of DNA in the presence of Act D at a molarity approximately equivalent to the molar phosphate of the DNA. Striking differences have been repeatedly observed between the DNA's of the three lines thus far tested (Fig. 2). All three DNA's appear to contain both light and heavy satellites (with respect to the main band) but differ widely in the relative content, particularly of the heavy satellites (those sequences binding less Act D). At present we are purifying these various satellite fractions by preparative centrifugation and then hope to be able to quantitate the relative amounts present in different lines by filter hybridization.

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