# DNA FROM MAIZE WITH AND WITHOUT B CHROMOSOMES: A COMPARATIVE STUDY

MARY-DELL CHILTON AND BRIAN J. McCARTHY*

*Departments of Microbiology and Genetics, University of Washington, Seattle, Washington 98195*

Manuscript received March 19, 1973
Transmitted by DAVID SCHLESSINGER

ABSTRACT

DNA preparations from 5B and 0B maize seedlings are indistinguishable in their buoyant density distribution in CsCl gradients. Their renaturation kinetics are identical at several stringency criteria. DNA competition studies fail to detect any component in 5B DNA redundant sequences which is lacking in 0B DNA. Homologous and heterologous duplexes formed between 5B and 0B DNA have virtually identical melting profiles. The DNA of B chromosomes is concluded to be very closely related to that of A chromosomes.

IN addition to the twenty standard or A chromosomes present in diploid maize nuclei, various numbers of supernumary heterochromatic B chromosomes occur in many strains (RANDOLPH 1941). The appearance of the plant is not visibly affected by the presence of as many as ten B chromosomes, although higher numbers result in decreased fertility and vigor, increased cell size and abnormal seed development (RANDOLPH 1941). The anomolous male transmission of B chromosomes has been ascribed to mitotic nondisjunction in the second microspore division (ROMAN 1947).

A dramatic difference was reported in the base composition of seed DNA from 4B and 0B strains (VAN SHAIK and PITOUT 1966), but this was not confirmed by later investigations of the buoyant density and base composition of leaf DNA (RINEHART and SANSING 1966; PITOUT and VAN SCHAIK 1968), which showed no measurable differences in either parameter. By contrast, the DNA of two separate B-chromosome-containing populations of grasshoppers, *Myrmeleotettix maculatus*, has been found to contain a substantial fraction of low GC satellite not present in non-B-containing populations (GIBSON and HEWITT 1970). This satellite apparently differs among populations of grasshoppers and is not homologous to main band DNA (GIBSON and HEWITT 1972).

The failure of B chromosome DNA in maize to appear obviously foreign by base compositional criteria leaves open the question of its genetic origin. The present investigation was undertaken in the hope that a detailed comparison of DNA bouyant density distribution, DNA renaturation kinetics and DNA/DNA duplexes formed between 5B and 0B maize DNA's might reveal small differences

* Present address: Department of Biochemistry, University of California, School of Medicine, San Francisco, California 94122.

which earlier investigations had failed to detect. Such differences might be exploited to separate or enrich for B chromosome DNA, which could then be characterized by hybridization to various potentially related plant DNA's.

## MATERIALS AND METHODS

*Seed stocks:* Plants used in these experiments were grown from 5B and 0B maize seeds generously provided by DR. J. KERMICLE, Department of Genetics, University of Wisconsin. Seeds were of inbred W22 substrains with and without B chromosomes of Black Mexican sugary origin. B chromosomes were introduced into W22 by backcrossing repeatedly. DR. KERMICLE determined the number of B chromosomes in the parent plant which was self-pollinated to produce our seed stock. In addition, MR. JAMES NISHITANI, Department of Botany, University of Washington, confirmed the average number of B chromosomes (and their absence in the non-B-containing plants) by cytological examination of squashes of microsporocytes at the pachytene stage. This additional check on the seed stocks was performed because of the negative results of attempts to detect DNA differences, described below.

*Growth of plants and labeling of DNA:* Seeds were surface-sterilized by soaking for two minutes in 1% sodium hypochlorite solution. They were transferred to sterile petri dishes containing dampened filter paper and 2 ml of 10% Captan suspension, a garden fungicide. The Captan suspension was sterilized by exposure to germicidal UV light with shaking.

Seeds which germinated were transferred to sterile beakers. Those to be labeled (batches of 18 plants) were given 500 $\mu$C $H^3$ thymidine or 5 mC $P^{32}$ (neutralized with sterile tris buffer) in a minimal volume of water for 6–9 days. Unlabeled plants were grown on dampened sterile perlite.

The specific activity of $H^3$ DNA isolated was 7500–8800 cpm/$\mu$g; that of $P^{32}$ DNA was 1700 cpm/$\mu$g. Batches of 18–20 plants typically yielded 50–150 $\mu$g of DNA when harvested 14 days after sowing. In preparative CsCl gradients containing 20 $\mu$g of labeled DNA, the radioactivity was found to track with the $OD_{260}$ profile, indicating that the radioactive precursor was incorporated into plant DNA, not that of contaminating bacteria (BENDICH 1972).

*DNA isolation:* Batches of 15–30 plants were cut up (discarding seeds) and frozen at −80°C in a Revco freezer. A 4–6" mortar with pestle was precooled to −80°C and used to reduce the frozen plant material to fine powder (about 5 minutes of grinding). The powder was shaken into a beaker containing 10 ml redistilled phenol (equilibrated with water and adjusted to pH 7.8 with NaOH solution) and 10 ml of 0.15 M NaCl, 0.1 M EDTA, pH 8, 1% sodium dodecyl sulfate. This mixture was allowed to warm to room temperature with stirring and was stirred for 10 minutes longer. The aqueous phase was separated by centrifugation and DNA was wound out after addition of 2 volumes of ethanol. The DNA was redissolved in 3 ml of 0.1 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) and dialyzed against 0.1 × SSC to remove phenol. The solution was adjusted to 1 × SSC, treated with RNase as described by MARMUR (1961), phenol extracted, and the DNA was wound out once more. After one further phenol extraction and winding out, the DNA solution (1 ml) was dialyzed exhaustively against 0.1 × SSC to remove phenol and the solution was stored in a deep freeze.

*CsCl density gradient centrifugation:* The DNA solution was diluted to 4.00 ml with 0.1 × SSC, and to it was added 5.1 g. CsCl (Optical Grade Powder, Harshaw Chemical Company). The solution was placed in a cellulose nitrate centrifuge tube which fits the Spinco 40 rotor. Heavy mineral oil was introduced through the screw hole until the tube was filled. The sealed tube was centrifuged in a 40 rotor for 60–72 hours at 20°C. Fractions were collected from the bottom of the gradient after piercing with a syringe needle.

*DNA filters:* Filter-bound DNA was prepared by a modification of a previously described procedure (MCCONAUGHY and MCCARTHY 1968). Millipore filter apparatus was used to prepare a 35-mm diameter filter. A 100-$\mu$g sample of denatured DNA (5B maize) was adjusted to 4 × SSC and applied to the filter by the usual procedure, except that suction was used. The filter was treated with DENHARDT's (1966) preincubation mixture. The filter was not saturated with DNA, but even under subsaturating conditions, the DNA distribution across the filter appears to

be uniform, and small circles punched from the big filter gave reproducible hybridization results. The estimate of DNA bound per little filter is based on the assumption that all the applied DNA binds under these conditions.

Filter hybridization reactions were performed as previously described (McCONAUGHY and McCARTHY 1968).

*DNA renaturation and melting curve determination:* DNA renaturation reactions were performed in various mixtures of formamide and 4 $\times$ SSC at 37°C. By varying the concentration of formamide, the renaturation conditions can be made to mimic the stringency of any desired higher temperature aqueous reaction conditions (McCONAUGHY, LAIRD and McCARTHY 1969). When a very small volume of reaction mixture was prepared, e.g. in the experiment described in Figure 5 below, the formamide was weighed out to increase the accuracy of composition of the reaction mixture. When small volumes of solutions were to be incubated for long times, aliquots were sealed in disposable 10 or 20 $\mu$l pipets before denaturation and incubation.

Duplex formation was followed by the hydroxylapatite assay as previously described (GROUSE, CHILTON and McCARTHY 1972). Two kinds of hydroxylapatite were used in the course of this investigation, Biorad HTP and Clarksen, the latter of which gave better column flow rate but several degrees higher Tm values in melting curves (cf. Figures 4 and 5).

## RESULTS

*Buoyant density studies:* Examination of the buoyant density of 5B and 0B DNA (three different preparations of each) in analytical CsCl gradients using *Myxococcus xanthus* DNA as density marker ($\rho = 1.728$) failed to reveal any reproducible differences in density distribution, confirming the findings of RINEHART and SANSING (1966). The density distributions were further analyzed by preparative CsCl gradient analysis of mixtures of 25 $\mu$g unlabeled 0B with 1 $\mu$g $H^3$ 5B (Figure 1A) and 25 $\mu$g unlabeled 5B with 1 $\mu$g $H^3$ 0B DNA (Figure 1B). In the former, the radioactivity tracks perfectly with the $OD_{260}$ profile; in the latter, the slight shift of radioactivity toward higher density is not significant because the radioactivity in this preparation appeared at a slightly greater density than its own $OD_{260}$ in a control gradient (not shown). Such slight disparities in plant DNA preparations may be due to variable incorporation of label into chloroplast or mitochondrial DNA.

*DNA filter competition studies:* Several attempts were made to detect filter-bound DNA duplexes formed with 5B DNA which could not be competed by 0B DNA. Under filter-bound DNA reaction conditions, only the redundant portion of the genome is able to react; isolated unique sequences of labeled mouse DNA do not give measurable reaction with mouse DNA filters over a period of six days. Thus this experiment asks specifically whether any unusual component is contributed by 5B DNA to the redundant sequences of the plant.

A number of competition curves were determined, corresponding to the two kinds of filter-bound DNA reacting with either kind of labeled DNA in solution and competed with each kind of unlabeled competitor. No reproducible differences were found. Figure 2 shows a pair of double-label competition experiments, in which a mixture of $P^{32}$-5B DNA and $H^3$-0B DNA was allowed to react with 5B filter-bound DNA in the presence of increasing concentrations of each kind of unlabeled competitor DNA. The competition curves are superimposable, showing that there are no redundant sequences in 5B DNA which cannot be competed by 0B DNA.

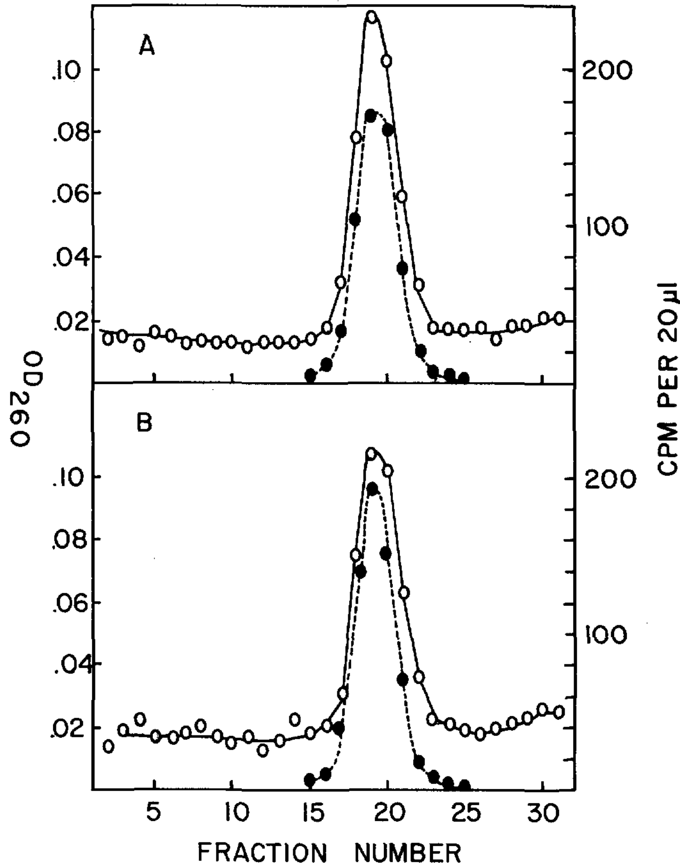


FIGURE 1.—Comparison of distribution of 5B and 0B maize DNA in CsCl equilibrium density gradients. A mixture of 25 µg unlabeled and 1 µg labeled DNA was centrifuged to equilibrium in CsCl as described in the text. In separate gradients of the labeled DNA's alone (not shown), radioactivity was measured throughout the gradient and all TCA insoluble radioactivity was found in a single symmetrical band which tracked with the $OD_{260}$ of the sample.

A: $H^3$ 5B plus unlabeled 0B DNA

B: $H^3$ 0B plus unlabeled 5B DNA

○ = $OD_{260}$ of the fraction to which 0.1 ml $H_2O$ was added

● = cpm per 20 µl of the diluted fraction, as measured by TCA precipitation.

*DNA renaturation kinetics:* The renaturation kinetics of 0B and 5B DNA as measured by the hydroxylapatite assay were indistinguishable over the range studied for three different DNA renaturation conditions in formamide, corresponding to low (60°C, 1 × SSC), intermediate (75°C, 1 × SSC) and high (84°C, 1 × SSC) stringency. Renaturation kinetics displayed as BRITTEN and KOHNE's (1968) $C_0t$ plot are shown in Figure 3.

*Melting of homologous and heterologous duplexes:* Thermal dissociation profiles were measured for duplexes made from the four possible combinations of labeled and unlabeled DNA from 5B and 0B plants. When duplexes were formed at intermediate stringency by renaturation to $C_0t = 1$ (66% duplex), the Tm

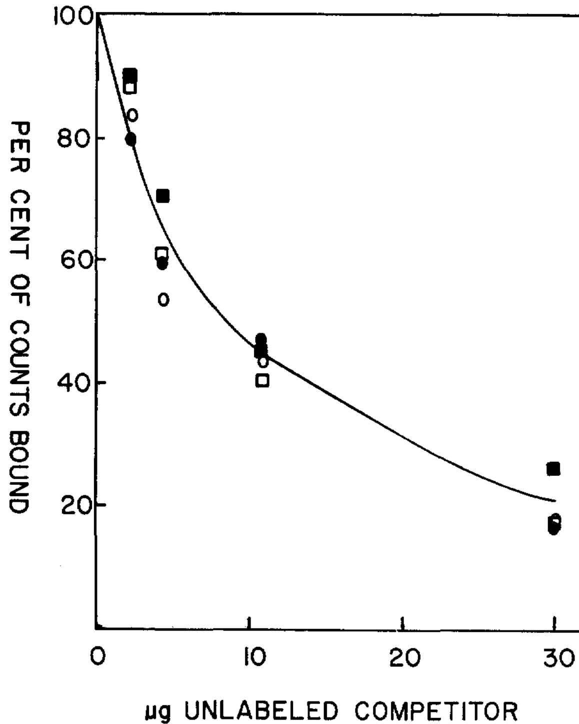


FIGURE 2.—Competition curves for homologous and heterologous filter duplexes *vs.* 5B and 0B DNA. Filters containing 3 $\mu$g unlabeled DNA and 5B maize were incubated with 0.4 $\mu$g labeled DNA which was a 1:1 mixture of $H^3$ 0B DNA and $P^{32}$ 5B DNA. Various concentrations of competing 5B or 0B unlabeled DNA were included in the incubation mixture, 0.21 ml total volume, 28.6% formamide, 2 × SSC. Filters were incubated at 45°C for 48 hours. These reaction conditions are calculated to be similar in stringency to 60°C, 2 × SSC. At zero competitor, 9.2% of the $P^{32}$ DNA bound while 17.4% of the $H^3$ DNA bound. The preferential binding of 0B DNA in this experiment was not reproducible; when $H^3$ 0B and $H^3$ 5B DNA were compared, the opposite difference was found.

■ $P^{32}$ 5B *vs.* 5B
● $P^{32}$ 5B *vs.* 0B
□ $H^3$ 0B *vs.* 5B
○ $H^3$ 0B *vs.* 0B

was 88°C for all kinds of duplexes, compared to 95°C for native maize DNA (Figure 4). Duplexes formed at the same stringency but renatured to $C_0t = 8900$ (97% duplex) show a very slight difference: when labeled 5B DNA is renatured with a 200-fold excess of unlabeled 0B DNA, the Tm of the heteroduplexes is 85.5°C, compared to 86°C for homologous (5B/5B) and 86.5°C for homo- and heteroduplexes made with labeled 0B DNA (Figure 5). Native 5B standard DNA melted at 91°C on this brand of hydroxylapatite; thus 4°C must be added to these Tm values in order to compare with the first set. The duplexes formed at $C_0t$ 8900 thus have corrected Tm's of 89.5°–90.5°, 1.5 to 2.5 degrees higher than those at $C_0t = 1$, a surprisingly small change considering the 50% increase in duplexes

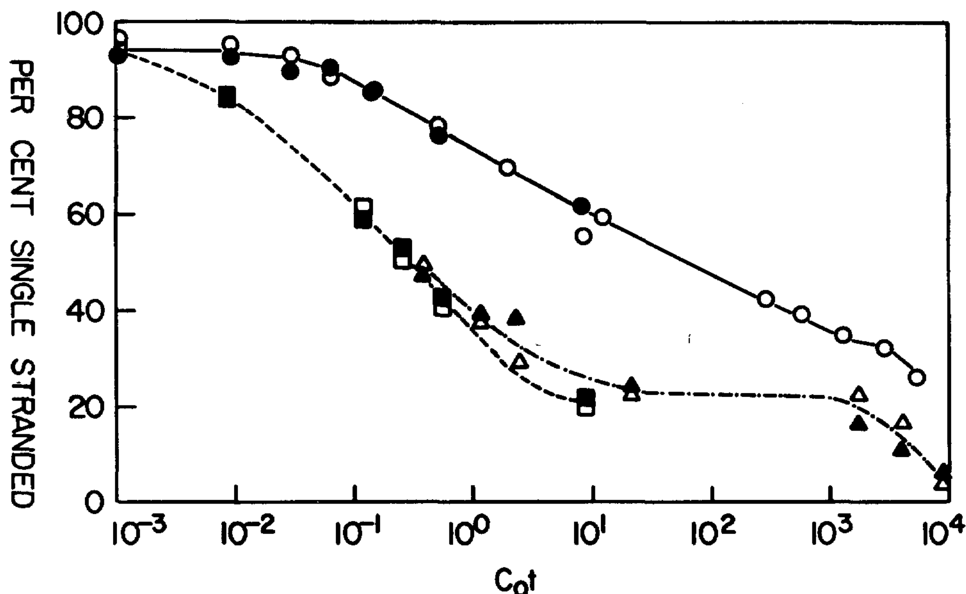


FIGURE 3.—Renaturation kinetics of 5B and 0B maize DNA. Renaturation kinetics were followed by the hydroxylapatite assay. Labeled DNA was degraded to low molecular weight by 33 minute depurination (GROUSE, CHILTON and MCCARTHY 1972). Reaction mixtures were all incubated at 37°C following denaturation of the DNA sample at 100°C. The three reaction mixtures were calculated to mimic in stringency 60°, 75° and 84°C in 1 × SSC, respectively.

■ 0B, 44.5% formamide, 4 × SSC.
□ 5B, 44.5% formamide, 4 × SSC.
▲ 0B, 64% formamide, 4 × SSC.
△ 5B, 64% formamide, 4 × SSC.
● 0B, 78% formamide, 4 × SSC.
○ 5B, 78% formamide, 4 × SSC.

at the longer incubation time. In a duplicate experiment (not shown), four $C_0t$ = 1 samples were prepared and radioactivity and $OD_{260}$ melting profiles were measured. In all cases, the radioactivity and $OD_{260}$ gave identical results. This substantiates that the label of the DNA samples is largely in nuclear maize DNA and yields results representative of the total genome, not plasmid DNA.

## DISCUSSION

The results of several types of analysis of native and homologous and heterologous renatured duplexes of 5B and 0B maize DNA fail to reveal convincing differences ascribable to the presence of foreign DNA sequences in B chromosomes. In order to assess the significance of this negative finding, it is crucial to know how much DNA the 5B chromosomes contribute to total 5B maize DNA. From the measurements of AYONOADU and REES (1971) we calculate that 21.5% of 5B DNA should derive from its B chromosomes. This should be a sufficient amount of foreign DNA to detect if it had differed grossly in any of the parameters investi-

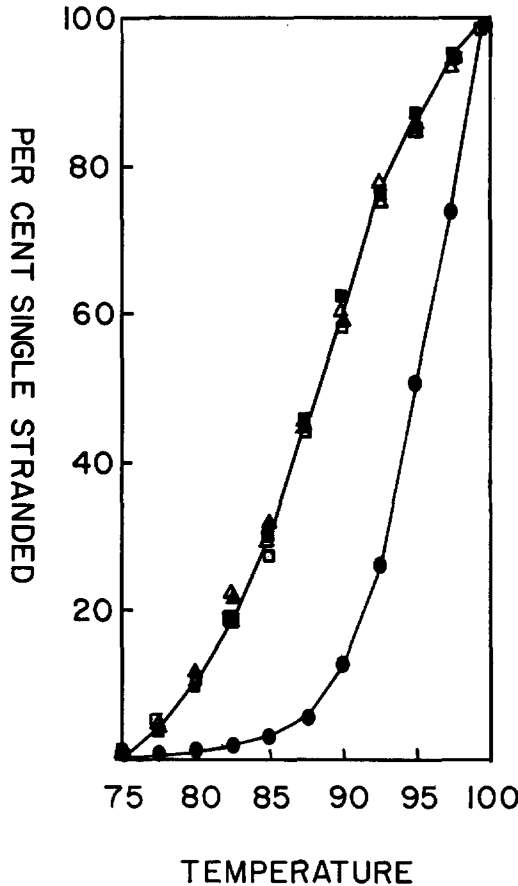


FIGURE 4.—Thermal dissociation profiles of homologous and heterologous duplexes formed at low $C_0t$. Mixtures of 40 $\mu g$ unlabeled plus 0.2 $\mu g$ labeled 33-minute depurinated DNA in 2 ml of 64% formamide, 4 × SSC were denatured and incubated at 37°C for 4 hrs 38 min. ($C_0t = 1$). The samples were about 66% duplex by hydroxylapatite assay. Samples were allowed to adsorb to hydroxylapatite columns at 60°C in 0.12 M phosphate buffer (BRITTEN and KOHNE 1968) and were eluted with the same buffer at increasing temperatures. Radioactivity was measured in the effluent by TCA precipitation.

■ $H^3$ 5B + unlabeled 5B
▲ $H^3$ 5B + unlabeled 0B
□ $H^3$ 0B + unlabeled 5B
△ $H^3$ 0B + unlabeled 0B
● native 5B (standard)

gated: buoyant density in CsCl, general renaturation kinetics, or melting behavior of renatured duplexes formed at low and high $C_0t$.

Although B chromosomes contribute a considerable fraction of DNA to the 5B nucleus, their influence on the $C_0t$ curve would be difficult to detect unless B-DNA were very unusual (e.g., all redundant or all unique). The influence of added B chromosomes on renaturation kinetics of unique sequences can be calculated, and

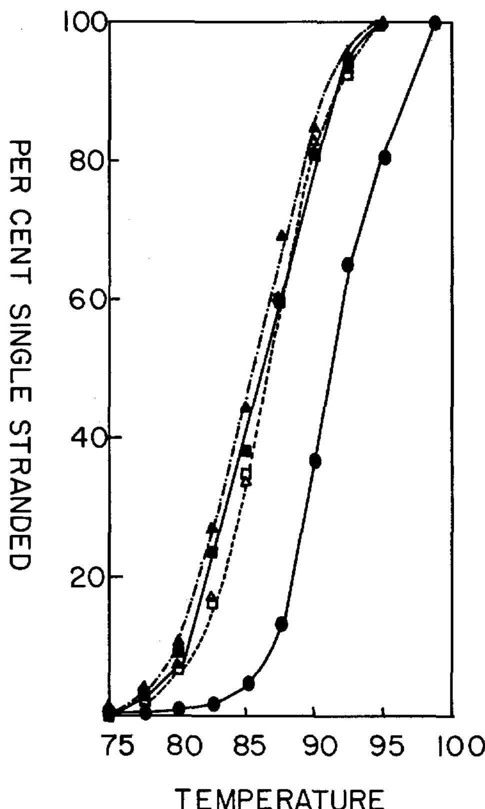


FIGURE 5.—Thermal dissociation profiles of homologous and heterologous duplexes formed at high $C_0t$. Mixtures of 100 μg unlabeled plus 0.5 μg labeled 33-minute depurinated DNA in 0.05 ml of 64% formamide, 4 × SSC, were denatured and incubated at 37°C for 17 days ($C_0t = 8900$). The samples were 97% duplex by hydroxylapatite assay. Samples were allowed to adsorb to hydroxylapatite columns at 60°C in 0.12 *M* phosphate buffer and were eluted with the same buffer at increasing temperatures. Radioactivity was measured in the effluent by TCA precipitation.

■ $H^3$ 5B + unlabeled 5B
▲ $H^3$ 5B + unlabeled 0B
□ $H^3$ 0B + unlabeled 5B
△ $H^3$ 0B + unlabeled 0B
● Native 5B DNA (standard)

for 5B DNA the changes produced would be small. Two kinds of change are expected: an increase in the observed $C_0t_{1/2}$ of the A-DNA sequences because they are diluted with B-DNA, and the appearance of a faster-renaturing component due to the multiple copies of B-DNA. The expected increase in $C_0t_{1/2}$ of A unique sequences in 5B DNA is 1.25-fold, because per mg of DNA, 5B has only 80% as much A-DNA as does 0B DNA. This small change would be difficult to measure. The presence of the faster renaturing B component in 5B DNA would produce an equally subtle alteration in the unique region of the $C_0t$ curve. Assuming that all B chromosomes are identical and different from A chromosomes, the

5B nucleus would contain five copies of B-DNA for every two copies of A-DNA, an enrichment of 2.5-fold for 22% of the DNA. If instead the 5B chromosomes are assumed identical to one pair of A chromosomes, an enrichment of 3.5-fold for about 30% of the DNA is produced. The presence of a 22–30% component of the unique sequences renaturing 2.5–3.5 times faster than the remainder would be extremely difficult to detect. The sensitivity of the measurements is limited because only a minority of the DNA renatures as unique sequences, even under very stringent conditions of renaturation. Detection of these alterations in the $C_0t$ curve should be feasible if DNA from strains with much higher numbers of B chromosomes were studied. The percentage of faster renaturing material would be increased and in addition there would be an increase in the factor by which its $C_0t_{1/2}$ changes from that of A chromosome unique sequences. Materials were not available to us to exploit this approach.

Although the experiments described in this paper failed to detect any definite differences between 0B and 5B DNA, some inference can be drawn concerning the relatedness of B chromosome DNA to A chromosome DNA. The renaturation kinetics (Figure 3) show that B-specific DNA contains mostly redundant sequences, since no increase in the proportion of unique sequences is observed in 5B DNA compared to 0B DNA. The redundant B-specific sequences must be able to cross-react completely with A-specific redundant sequences, as shown by the competition curves of Figure 2. However, no lower melting component is detectable in heterologous renatured redundant duplexes (Figure 4). The redundant sequences of B and A chromosomes therefore must cross react and appear indistinguishable. This finding is difficult to evaluate at present, for very few data are available concerning the relatedness of redundant DNA's of plants. Comparison of barley, rye, oats and wheat DNA (BENDICH and MCCARTHY 1970) show in general a $\Delta$ Tm of 3–7° for heteroduplexes compared to homoduplexes. We can conclude that the B chromosomes of maize bear a closer evolutionary relationship to A chromosomes than do these monocots to one another. Exactly how close the relationship is can only be evaluated by a systematic study of the Tm of redundant heteroduplexes formed from maize DNA and the DNA from a series of closely related plants.

The authors thank DR. HERSCHEL ROMAN, DR. JERRY KERMICLE and DR. CHARLES LAIRD for contributing to this work through rewarding discussions. We are very much indebted to MR. JAMES NISHITANI for determining chromosome numbers in our plants, and to DR. JERRY KERMICLE for generous contribution of seeds.

This research was supported by N.S.F. Research Grant GB-6099.

LITERATURE CITED

AYONOADU, U. W. and H. REES, 1971 The effect of B chromosomes on the nuclear phenotype in root meristems of maize. Heredity **27**: 365–383.

BENDICH, A. J., 1972 Effects of contaminating bacteria on the radiolabeling of nucleic acids from seedlings: False DNA "satellites." Biochim. Biophys. Acta **272**: 494–503.

BENDICH, A. J. and B. J. MCCARTHY, 1970 DNA comparisons among barley, oats, rye and wheat. Genetics **65**: 545–565.

BRITTEN, R. J. and D. E. KOHNE, 1968 Repeated sequences in DNA. Science **161**: 529–540.

DENHARDT, D. T., 1966 A membrane filter technique for detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**: 641–646.

GIBSON, I. and G. HEWITT, 1970 Isolation of DNA from B chromosomes in grasshoppers. Nature **225**: 67–68. ——, 1972 Interpopulation variation in the satellite DNA from grasshoppers with B chromosomes. Chromosoma **38**: 121–138.

GROUSE, L., M.-D. CHILTON and B. J. McCARTHY, 1972 Hybridization of ribonucleic acid with unique sequences of mouse deoxyribonucleic acid. Biochemistry **11**: 798–805.

MARMUR, J., 1961 A procedure for the isolation of DNA from microorganisms. J. Mol. Biol. **3**: 208–218.

McCONAUGHY, B. L., C. D. LAIRD and B. J. McCARTHY, 1969 Nucleic acid reassociation in formamide. Biochemistry **8**: 3289–3295.

McCONAUGHY, B. L. and B. J. McCARTHY, 1968 Related base sequences in DNA of simple and complex organisms. I. DNA/DNA duplex formation and the incidence of partially related base sequences in DNA. Biochem. Genetics **2**: 37–53.

PITOUT, M. J. and VAN SCHAIK, 1968 Deoxyribonucleic acid from black Mexican sweet corn. South African Journal of Science **64**: 25–29.

RANDOLPH, L. F., 1941 Genetic characteristics of the B chromosomes in maize. Genetics **26**: 608–631.

RINEHART, K. and N. SANSING, 1966 Unpublished results cited in PITOUT and VAN SCHAIK, 1968.

ROMAN, H. L., 1947 Mitotic nondisjunction in the case of interchanges involving B-type chromosomes in maize. Genetics **32**: 391–409.

VAN SCHAIK, N. and M. J. PITOUT, 1966 Base composition of DNA from maize with heterochromatic B chromosomes. South African Journal of Science **62**: 53–56.