3. A negative transformation experiment.

Simple modifications of the Marmur technique (J. Mol. Biol. 3: 208-218, 1961) yield very promising DNA preparations from young seedlings.

Germination: 500g of kernels are soaked in aerated water for 8 hours, then placed in a pipette basket wrapped in black plastic and sprayed with a mist for 4 days, loosening frequently by shaking, then harvested and weighed (1-2 kg).

Extraction: Seedlings, including kernels, are chilled in iced distilled water, then blended for 2 minutes, in portions, with minimal volumes of cold saline-EDTA (0.15 M NaCl plus 0.1 M EDTA, total 250-300 ml) and portions of saturated sodium lauryl sulfate (20 ml). The mash is strained crudely through two layers of cheesecloth, divided into two cold one-liter stoppered graduates, brought to 1M sodium perchlorate by addition of 1 part cold 5M sodium perchlorate to 4 parts mash, then mixed and emulsified with a half-volume of iced chloroform-isoamyl alcohol (24 parts chloroform to 1 part isoamyl), and shaken for 20 minutes, with chilling. The emulsion is centrifuged in the cold (10 min., 5,000 rpm) and the upper (aqueous) layer is collected in a large beaker. Two volumes of iced ethanol are added to precipitate the nucleic acids in bulk, and precipitation and settling are allowed to proceed in an ice bath for 30-60 minutes. Most of the liquid is decanted off, and the precipitate is collected by centrifugation (5 min., 3,000 rpm) and promptly dissolved in cold saline-citrate (0.15 M NaCl plus 0.015M sodium citrate, 40 ml or more in portions, preferably dissolving rapidly and completely in small volumes of 1/10 strength followed by addition of 10x strength to bring to proper concentration). Sodium perchlorate is then added (5M, 1 part to 4), and the solution is shaken with an equal volume of chloroform-isoamyl for 15 minutes, centrifuged, and the aqueous layer removed and overlaid carefully with two volumes of ethanol. The threads at the interface are then collected by winding on a wooden swab stick. The collected threads, largely DNA, are redissolved in saline-citrate and can be deproteinized repeatedly by proceeding through the perchlorate-chloroform step several times. Saline-citrate seems to be a satisfactory solvent for injection into corn seedlings.

Transformation trial: Source seedlings were B A Y Pl Yg C Sh Wx R^r. Recipients were b A Y Pl yg c sh wx R^g, permitting observation in the immediate plants for B, Yg, and R^r, and in seed and seedling progenies for Y, Yg, C, Sh, and Wx. Crude preparations with and

without spermine (0.05%), a stabilizing agent, were injected by puncturing repeatedly into the area of the growing point, and half of the treated seedlings and controls were x-rayed promptly (1000 r) in hopes of opening membranes, inhibiting nucleases, and providing sites for incorporation. Seedlings, were injected at 1 to 2 weeks, 3 weeks, and 4 weeks after planting, including some repeated at all three stages. Although variable leaf-streaking simulating Yg was seen in a few plants, no B sectors or purple anthers were observed in over 250 treated survivors. In over 26,000 seeds obtained from intercrosses among the plants, five exceptional seeds were found (4 C Sh Wx, 1 C sh? Wx), in both treated and control material; these are presumably contaminations but are to be tested. Half of the 26,000 seeds were planted in the sand beach and scanned for Yg; no exceptions were found.

E. H. Coe, Jr.
K. R. Sarkar

NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina

1. Chromosome knobs of North Carolina inbred lines.

Cytological examination was made of the inbred lines of two varieties which have been and are being studied for quantitative analysis. The objectives are: 1) To find inbreds from both varieties with identical knob constitutions to provide material for further studies involving K10 effects on genetic variances and recombination and, 2) to provide a characterization of the differences between the two varieties with respect to frequency of various knobs which, in turn, may provide a useful background in planning experiments to study the nature of quantitative genetic differences between varieties.

Two sets of inbred lines have originated in 1953 from 300 random selfed ears of varieties Jarvis Golden Prolific and Indian Chief, and each ear was used to establish an inbred line. In order to minimize selection during the following inbreeding period, every line in every generation was raised from a single selfed ear of the first plant in the row in the preceding generation. Currently available are 64 lines of Jarvis and 125 lines of Indian Chief.