(5) Hydrolyze in 1NHCl at 60°C for 20 minutes. Rinse thoroughly with distilled water.
(6) Place in leuco-basic fuchsin for 30-45 minutes.
(7) Wash in distilled water for 1 hour.
(8) If the root tip has not previously been cut off, excise and place in a 5% cellulose - 5% pectinase solution at pH 4.2 for 2 hours.
(9) Cut the deeply stained tip onto a clean slide and macerate in a drop of propionic carmine. A flattened end of an ivory stick is suitable for maceration and spreading.
(10) Heat gently.
(11) Add cover slip and flatten with the rounded end of a glass or steel rod. Invert onto bibulous paper and apply pressure with thumbs.
(12) Seal. Temporary smears may be stored several months in a freezer. Temporary smears may be made permanent by conventional techniques.

R. M. Brown

4. The mitotic karyotype of maize.

Using the smear technique outlined in the previous note, a number of metaphases suitable for photographing have been studied. Two notable features have emerged as the karyotype has been prepared.
(a) A definite gradation in chromosome size from large, nearly metacentric chromosomes to small, submetacentric chromosomes; and consistent arm ratios of the three classes of chromosomes - large, metacentric; medium, submetacentric and short, submetacentric.
(b) A secondary constriction with satellites is easily seen in most preparations in two chromosomes.

At present we are interpreting the karyotype on the basis of the measurements (Rhoades, Jour. of Heredity 41: 58-67, 1950) presented for pachytene chromosomes. Arm ratios quite similar to those reported for pachytene chromosomes are found in the mitotic metaphases. We are attempting to further qualify the mitotic karyotype with the use of trisomics.

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5. Influence of calcium concentration on pollen germination.

A suitable medium for the germination of "Seneca 60" ($\frac{su_1}{su_1}$) hybrid corn pollen was reported in MCNL 39: 169. This medium, consisting of 0.35 M sucrose, 100 ppm H_3BO_3 and 300 ppm $CaCl_2.2H_2O$, may be used simply as aqueous drops or with either 0.7% Difco Special Agar-Noble or 1% to 2% methyl cellulose. Calcium ion has been shown to be required for corn

pollen germination and a series of experiments were designed to investigate changing calcium concentrations on per cent germination.

Fresh "Seneca 60" pollen produced in the greenhouse was placed on drops of media in well-slides and allowed to germinate for 30 minutes. Densities were kept well above those which might reduce germination because of a "population effect" (see following note).

Sucrose and boron concentrations were held constant at the levels given above. The data were recorded and stored on film.

The addition of 1% methyl cellulose to the aqueous medium had no influence on per cent germination. Agar was not tried. Only a trace of germination was recorded at 50 ppm calcium and below. Optimum concentrations were in the range 300-400 ppm. Above 400 ppm the per cent germination slowly fell off but was still above 50% of optimum at 900 ppm. 100 ppm and 200 ppm gave 20-40% and 60-80% of optimum values, respectively.

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6. The "population effect" in corn pollen germination.

Brewbaker and Kwack (1963) described a pollen population effect whenever pollen grains are germinated in vitro. Small populations germinate poorly or not at all under conditions which support good germination of larger populations. Although no population effect with corn pollen could be shown on the medium to which Noble agar had been added (MNL 39: 170), the effect was evident with small populations on the aqueous medium containing only 0.35 M sucrose, 100 ppm H_3BO_3 and 200 ppm $CaCl_2,2H_2O$, i.e. sub-optimal concentrations of calcium.

Greenhouse-produced "Seneca 60" corn pollen was placed in wells of slides containing 20 µl of the medium and allowed to germinate for 30 minutes. Numbers of grains and per cent germination were counted under a dissecting microscope. Germination in all experiments was compared with that of "high" populations.

Below 10 grains per 20 µl there was only a trace of germination. At 100-150 grains per 20 µl, per cent germination approached a maximum. These results are similar to those reported by Brewbaker and Kwack for the pollen of Saintpaulia, Haworthia and others.

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