Acridine orange banding of maize chromosomes — Haploid and diploid maize seeds from the same strain were germinated at 23°C for four to seven days. A modification of Dutrillaux's technique for human chromosomes was applied to root-tips using BuDr and acridine orange.

When viewed with blue light fluorescence, the chromosomes appeared yellow/ green with red/orange bands. Chromosome 1 had three red/orange regions, one on the short arm and two on the long arm. Chromosome 4 had one on the short arm and two on the long arm. Chromosome 6 had a red/orange band at the nucleolar organizer region and two bands on the long arm, and chromosome 7 had a red/orange band on the long arm that corresponds to the regular knob region shown by acetocarmine squashes of microsporocytes at pachytene stage. Further studies of the other chromosomes are in progress.

The regions banded by the acridine orange-BuDr technique appear to correspond to the Giemsa-banded regions reported last year in the News Letter. The red/orange bands are late-replicating, indicating that there are more heterochromatic regions revealed by acridine orange than by the conventional acetocarmine technique. If acridine orange bands are the same as G bands, then the G bands are also late-replicating.

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Ultrastructure of the pollen grain ektexine of maize and its relatives — The present investigation using electron microscope methods has revealed that the pollen grain ektexines of "pure races" of maize and teosinte have very similar patterns of evenly distributed ektexine spinules at the ultrastructural level. This ektexine pattern was also found among some re-investigated fossil pollen grains (approximately 80,000 years old) from the deep-core samples from Mexico City (U. C. Banerjee, Ph.D. Thesis, Harvard Univ., 1973); these fossil pollen grains were previously described by Barghoorn and Wolfe (M.G.C.N.L. 27:17, 1953) and by Barghoorn, Wolfe and Clisby (Harvard Bot. Mus. Leaflet 16:229, 1954). Similar ektexine patterns were also recorded in fossil maize pollen from Gatun Basin, Panama, by A. S. Bartlett (Ph.D. Thesis, Harvard Univ., 1967) and by Bartlett, Barghoorn and Berger (Science 165:389, 1969) and in some archaeological pollen grain samples from Tehuacán Valley, Mexico, and from surface level at the site near

Huarmey, Peru, by Banerjee (1973). However, when introgression of teosinte occurs with maize or <u>vice versa</u>, the pollen grain ektexines of the hybrid progeny show a new "spinule-loss" type of ektexine pattern, easily recognizable ultrastructurally (Banerjee and Barghoorn, 30th Ann. Proc. Electron Micr. Soc. Amer., p. 226, 1972). This "spinule-loss" pattern was also found with the pollen grain ektexine of maize from Bat Cave, New Mexico, as reported by Banerjee (1973).

The ektexine patterns of pollen grains of <u>Tripsacum</u> spp. (both diploids and tetraploids) show a distinct ektexine spinule-clumping represented by the "negatively-reticuloid" pattern. This phenotypic ektexine pattern is dominant over the ektexine patterns of maize and teosinte (Banerjee and Barghoorn, Abstr. Amer. Assoc. Stratigr. Palyn., 1972). The introgression of <u>Tripsacum</u> with maize or teosinte can be detected by the presence of some degree of spinule-clumping if pollen grains are derived from the hybrid progeny. The oldest convincing evidence of <u>Tripsacum</u> introgression with maize was found in some archaeological maize samples recovered from the lower levels of the site near Huarmey, Peru, approximately dated 2000 to 1600 B.C. (this date is estimated by the archaeologists--personal communication with Professor Mangelsdorf, and Mangelsdorf and Cámara-Hernández, M.G.C.N.L. 41:47, 1967). The ultrastructural characters of the ektexine also show that Cuzco maize (<u>Zea mays</u> L.) from Peru, and Florida teosinte (<u>Euchlaena luxurians</u> Durieu.) from southern Guatemala, are possibly contaminated with <u>Tripsacum</u> germplasm.

The palynological investigations with Mangelsdorf's genetic stocks of maize also suggest that "teosinte" is not a hybrid of maize and <u>Tripsacum</u>, as it was considered previously (Mangelsdorf, P.C., Corn: Its Origin, Evolution and Improvement. Harvard Univ. Press, 1974).

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In situ staining of pollen grains for alcohol dehydrogenase activity — The cytochemical reduction of p-nitroblue tetrazolium chloride is a well-known assay for NAD(P)-linked dehydrogenases in the primary substrate (oxidized) to primary substrate (reduced) reaction direction. Here, the specificity of the assay lies in the enzyme's specificity for primary substrate. We have adapted the methods of Hauser and Morrison (1964; Amer. J. Bot. 51, 748) to semi-quantitate the level of ethanol dehydrogenase in shed pollen.

## Procedures:

1. Pollen is collected for a three-hour interval in a paper bag. Take whatever precautions necessary to insure healthy (stainable) pollen. In our hands, pollen