Huarmey, Peru, by Banerjee (1973). However, when introgression of teosinte occurs with maize or vice versa, 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 Tripsacum 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 Tripsacum 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 Tripsacum 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 (Zea mays L.) from Peru, and Florida teosinte (Euchlaena luxurians Durieu.) from southern Guatemala, are possibly contaminated with Tripsacum germplasm.

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

Umesh C. Banerjee and Elso S. Barghoorn

UNIVERSITY OF CALIFORNIA

Department of Genetics, Berkeley, California

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

may be stored dessicated at 4°C for at least 2 hours without lowering stainability or in vitro germination.

2. After shaking-away most of the anthers, about 10^6 grains are dispersed onto 50 ml 0.1 M sodium phosphate buffer, pH 7.3 in a standard petri dish. After about 10 mins healthy grains will sink.

3. Freeze the pollen sample at -20°C for three hours on an iron plate. Presumably, this slow freezing procedure perforates the pollen wall and membranes, yielding 10^6 tiny dialysis bags.

4. After no more than six hours of freezing, remove the surface of the ice with running tap-distilled water and with it anthers, nonsunken pollen, and flotsam. Then defrost at room temperature on an iron plate rotating at about 30 rpm for 45-60 mins. Endogenous dehydrogenase substrates are presumed to dialyze into the buffer.

5. Remove the buffer (dialysate) with a pasteur pipet connected to an aspirator bottle, and immediately replace with dehydrogenase stain at 10-15 ml per 10^6 grains.

6. For 100 ml of alcohol dehydrogenase stain:

Stock	ml per 100
0.1 M sodium phosphate, pH 7.3	86.0
0.01 M p-nitroblue tetrazolium chloride (NBC) in 100% methanol (AR)	3.0
0.01 M NAD^+ (NBC; ethanol free)	10.0
95% ethanol (the primary substrate)	1.0

When tightly-capped, this stain is stable at 4°C for at least 24 hrs. Malic acid, sorbitol, sodium lactate and succinic acid also serve as primary substrates under these conditions.

7. Stain for 3±1 (± range that we use for absolutely unambiguous results) hrs with slow rotation. Stop the reaction by replacing stain with 100% technical-grade methanol and/or cooling the reaction to 4°C. The advantage of the latter method is that it may be done automatically.

In order to estimate the total number of grains in a sample, we suspend pollen at 4-6 x 10^3 grains per ml (by eyeball estimation) in 40% glycerine, remove aliquots for dilution and particle counts using millipore techniques, and calculate the exact grains per ml suspension. Known volumes are then dispensed into large gridded petri plates for screening, where pollen does not change position as the plate is moved; use incident light and about 16X. Screening is accompanied by motion sickness in some people; call collect with a more humane method.

Comments:

These procedures were devised using pollen segregating Adh^+/Adh^-, where the mutant Adh (from Drew Schwartz) is CRM^-. Omitting ethanol yields 100% of 10^7

yellow, translucent grains, but only if the freeze-dialysis procedure is followed. Using lactate as a primary substrate, 97-100% of the grains stain blue and opaque. With ethanol, 50% stain blue and opaque and 50% stain yellow and translucent with zero statistically significant overlap; do not overstain. One of us (MF) has screened almost 200,000,000 pollen grains using these methods; the method works. We also know that the stain is able to detect as light blue grains 5% of wild-type ADH levels.

Adh is about 1.5 mu from lw (Schwartz) and less than 1 mu from Kn (Freeling) on the long arm of chromosome one. Like wx, Adh should be of general utility. Unfortunately, simultaneous scoring for waxy and ADH may prove difficult, especially since ADH is stained in shed pollen.

The power of pollen analysis (per Oliver Nelson's prototype work with wx) as a means for recombinational frequency or topological mapping within the Adh cistron would be greatly increased if we had flanking pollen markers. Small pollen, fluorescent pollen, oblong pollen and the like would be ideal if they prove transmissible; they need not specify competitive gametophytes. If anyone has a potential marker within 20 mu of lw your cooperation is requested.

Michael Freeling and Elvin Brown*

*Elvin Brown is in the Undergraduate Honors Program in this Department.

CEREAL RESEARCH INSTITUTE
Szeged, Hungary

Effects of plant density and spacing on the grain yield of hybrids — In Szeged in 1973, the effect of different plant densities and of the cutting of the leaf surfaces on yield were examined. Two hybrids were sown with two spacings in a randomized block layout. After fertilization leaves were cut upwards from below in three different ways. Table 1 shows the extent of leaf cutting.

Table 1. Extent of leaf cutting.

	Treatments	Surface of leaves (cm^2) A90 x 153R		W64A x Oh43	
		b_1	b_2	b_1	b_2
a_1	control (without cutting)	4913.2	4251.3	5562.3	4944.7
a_2	plants with leaves cut below ear	2241.4	2119.4	2781.8	2337.1
a_3	plants with half of leaves cut above ear	820.6	753.2	1017.9	786.0

b_1 = 5 plants/m^2; b_2 = 10 plants/m^2