The results from our study suggest that the multiple aleurone layering character is controlled by a few genes, possibly two with partial dominance where both dominant genes are necessary.

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Relation of hydroxamic acid concentration (DIMBOA) to resistance to the corn leaf aphid

In 1959, the cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was first reported in maize and has since been directly implicated in resistance to several pathogens and insects. DIMBOA occurs naturally in the glucosidic form and is converted to the toxic aglucone through mycelial penetration or insect injury.

The objective of this study was to correlate DIMBOA concentration with resistance to the corn leaf aphid, Rhopalosiphum maidis (Fitch), through bioassay and field experiments. The bioassay experiment was performed to test toxic effects of DIMBOA on the corn leaf aphid. An artificial diet consisting mainly of a mixture of amino acids and vitamins was prepared for use in the bioassay. To the diet were added various amounts of DIMBOA (0.1, 0.25, and 0.5 mg/g diet) to give concentrations similar to those found in host plant tissue. Control diets contained no DIMBOA. Approximately 15 first instar apterous aphid nymphs were fed the diets through a Parafilm membrane. Mortality counts were recorded every 48 hours for 12 days.

In field trials twelve inbred lines of corn were evaluated for corn leaf aphid resistance under natural infestation. Aphid damage was evaluated at the mid-silking stage using a visual rating scale and an index system. Index values, indicating severity of aphid infestation, were compared to concentrations of DIMBOA found in each line using a colorimetric procedure based upon the reaction of DIMBOA with $FeCl_3$.

Results from the aphid bioassay demonstrated significant effects of DIMBOA on aphid mortality. DIMBOA concentrations of 0.1, 0.25, and 0.5 mg/g diet produced 5.1, 12.8, and 20.5 percent mortality, respectively, using Abbott's formula. Aphid index values from the field data ranged from 15.0 to 65.0, representing mild and severe damage, respectively. DIMBOA concentrations in plants at the fifth to sixth leaf stage ranged from 0.03 to 1.48 mg/g fresh weight. A significant correlation ($r = -0.72$, df. = 34) was obtained between these two traits, indicating that inbred lines containing a high concentration of DIMBOA generally have improved resistance to the corn leaf aphid.

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Localization of factors controlling the Texas type of cytoplasmic male sterility

The location of the factors responsible for the Texas type (cms-T) of cytoplasmic male sterility is unknown. Recent studies (e.g., Science 173:67, 1971; Phytopathology 63:1357, 1973) have shown differences in the response of mitochondria from maize with normal and "Texas" cytoplasms when challenged by toxins produced by race T of Helminthosporium maydis Nisikato and Miyake (southern corn leaf blight) and Phyllosticta maydis Arny and Nelson (yellow leaf blight). Since these studies suggest the involvement of mitochondria, mitochondrial DNA (mtDNA) must be considered as a possible site of factors responsible for traits associated with cms-T.

The traditional means of genetic analysis for nuclear genes are generally not available for studying cytoplasmically inherited traits of higher plants. Another approach lies in characterizing the organelle DNA's and attempting to relate them to the cytoplasmically inherited traits. When a homogenous DNA is digested by a site-specific restriction endonuclease, a characteristic set of fragments is generated. If the DNA is of low complexity, fractionation of the restriction fragments by gel electrophoresis results in a characteristic fragment pattern. This pattern can serve as a fingerprint of the original DNA molecule in a manner analogous to the tryptic fingerprints of protein.

We have isolated mtDNA from maize with normal (fertile) and "Texas" male-sterile cytoplasm. When the mtDNA's from the two cytoplasms were subjected to restriction enzyme fragment analysis the patterns were readily distinguishable. This distinction in fragment patterns has been demonstrated consistent by examining mtDNA from normal and "Texas" cytoplasms in different genetic (nuclear) backgrounds. These results establish an association between mtDNA and the "Texas" type male-sterile cytoplasm.

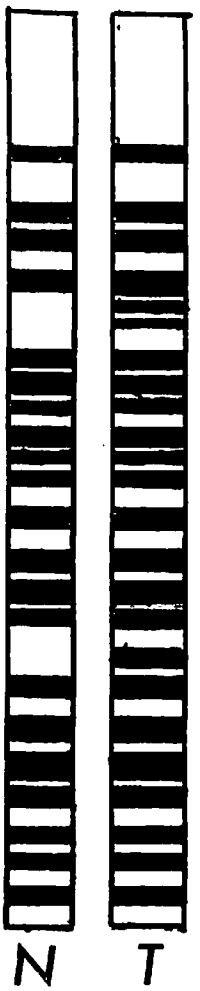
Mitochondria were isolated from etiolated maize coleoptiles and treated with DNase I prior to lysis with 1% Sarkosyl. The DNA from such preparations was centrifuged in CsCl-ethidium bromide gradients and mtDNA was obtained by collecting upper (nicked) or lower (supercoiled) DNA bands. The DNA's were extracted with isopropyl alcohol and dialyzed against restriction buffers. Purified mtDNA's were then digested with R endo·Eco R I or R endo·Hind III, and the resultant DNA fragments were separated by electrophoresis in 0.7 or 1.0% agarose gels. Restriction fragments were visualized by fluorescence after staining with ethidium bromide.

The patterns obtained from the mtDNA digestion with endo R·Hind III revealed about 50 bands, while endo R·Eco R I produced about 40 bands. The agarose gel electrophoretic fragment patterns of endo R·Eco R I (Fig. 1) and of endo R·Hind III (Fig. 2) digestions clearly show that the normal and cms-T fingerprints are different. Although the cleavage patterns from the two restriction endonucleases are not similar, both nucleases generated fragment distinctions between the mtDNA's of normal and "Texas" cytoplasms. We have examined mtDNA from normal cytoplasm of several inbreds or crosses and found all the fragment patterns to be similar when cleaved by the same endonuclease. Similarly mtDNA from several inbreds or crosses in cms-T resulted in indistinguishable patterns when restricted by the same endonuclease. Although the two types of cytoplasm yield readily distinguishable fragment patterns, there is considerable homology in the DNA's as evidenced by the large number of common bands.

Although these experiments were designed to localize the factors responsible for cytoplasmically inherited traits, they also provide unique evidence of uniparental inheritance of the mitochondrial genomes. In our study, we had hybrids in which the male parent contained normal cytoplasm and the female, "Texas." Since the mtDNA's of the two cytoplasms are distinguishable, the parental DNA's are marked. The Hind III digestion pattern of normal mtDNA contains several fragments (Fig. 2) which are not present in the cms T pattern. These fragments effectively mark the male parent, and we have repeatedly been unable to observe these specific fragments in the progeny. The pattern of the mtDNA of the cross was always that of the female parent. The same result was found for cms-T inbred lines, which must be maintained by repeated crossing with male fertile (normal cytoplasm) lines.

The purity of our mtDNA is an important concern. We have examined mtDNA by buoyant density determinations in neutral CsCl. Maize mtDNA is resolved as a single band with a density of 1.706 g/cm^3, which is typical of other higher plant mtDNA's. This density is different from that of maize nuclear or chloroplast DNA's, 1.702 and 1.700 g/cm^3 respectively. Nonetheless, we cannot unequivocally state that our preparations do not contain an alien DNA. The possibility of a DNA-containing virus or virus-like agent in our maize lines is difficult to eliminate.

These results have important implications. To our knowledge this is the first report of restriction endonuclease fragment analysis of any higher plant mtDNA,



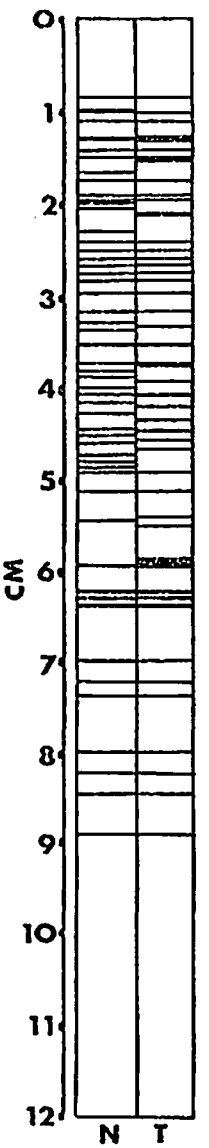


Figure 1 (left). Partial agarose gel electrophoretic patterns of endo R·Eco R I-digested maize mitochondrial DNA's from NC 7 x T204 with normal (N) and Texas (T) cytoplasms. Only the first 5.5 cm of the 12 cm gel is shown; most of the pattern differences occurred in this portion of the gel.

Figure 2 (right). Agarose gel electrophoretic patterns of endo R·Hind III-digested maize mitochondrial DNA's from W64A with normal (N) and Texas (T) cytoplasms.

and we feel it demonstrates the application of a technique which can be used in the analysis of cytoplasmically inherited phenomena. Our results show that a maternally inherited difference in mtDNA is associated with the Texas male-sterile cytoplasm. These observations suggest that the factors conditioning cytoplasmic male sterility and the cytoplasmic inheritance of susceptibility to H. maydis and P. maydis are located on the mitochondrial genome. Although we cannot disregard chloroplasts or other cytoplasmic DNA's as potential carriers of these traits, the preferential effect of the host specific fungal toxins on mitochondria from cms T lines, together with the restriction endonuclease data, constitute strong evidence that the mitochondria are the organelle involved in the inheritance of the traits.

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Conformation and size of mitochondrial DNA of maize

The conformation and size of mitochondrial DNA (mtDNA) were studied for a corn hybrid, NC 7 x T204ms (cms-T), by electron microscopy. Crude mitochondria were obtained from 7-day-old coleoptiles and carried through an additional resuspension and centrifugation cycle. Sarkosyl lysates of this fraction were centrifuged to equilibrium in CsCl-ethidium bromide density gradients. After centrifugation the upper and lower bands were readily visualized with UV light (365 nm). The upper band contained mainly linear and nicked circular mtDNA and contaminating nuclear DNA and was not further studied. The lower band contained covalently closed circular mtDNA. The purity of mtDNA in the lower band was analyzed by CsCl density gradient centrifugation in a model E analytical ultracentrifuge. The mtDNA was found to have a buoyant density of 1.705 g/cm^3, which was in agreement with our previously reported value (Crop Sci. 14:852, 1974). Ethidium bromide in the lower band was removed by extraction with iso-amyl alcohol and the DNA was dialyzed against TES buffer (0.1 M NaCl, 0.05 M Tris, pH 8.0, 0.01 M EDTA) for 24 hours in cold. DNA-protein monolayers for electron microscopy were prepared according to the aqueous technique described by R. W. Davis et al. (Methods in Enzymology, vol. 21, part D, p. 413, 1971). The molecules were photographed at magnifications of either 4,000 or 10,000. Measurements were made at a total magnification of 80,000 or 110,000 respectively. Calibration of magnifications was done with a replica grating (E. Fullam 2160 lines/mm).

Electron microscope examination of DNA revealed the presence of circular mtDNA in corn. Figure 1 presents the frequency distribution of the circular mtDNA. It is evident that mtDNA in corn exists as a very heterogeneous population of molecules in the young coleoptile tissue. We have not yet studied the distribution of mtDNA from leaves. The high degree of intermolecular heterogeneity makes it difficult for us to accurately determine the molecular weight of maize mtDNA. The present data suggest that the total genetic information of corn mitochondrial DNA is probably distributed amongst more than one class (based on size) of mtDNA molecules differing in molecular weight. We have arbitrarily divided the mtDNA in six principal classes with average length of 5.3, 8.8, 10.7, 12.7, 15.1 and 17.4 μ. The size distribution also suggests that there are at least two, probably three, oligomeric series of circles which are integral multiples of unit size circles. There seems to be one series of 5.3, 10.7...; a second of 8.8, 17.4...; and a third of 12.7, 24.3 μ.... Further members of these series are also present but they could not be assigned to a particular series because of overlapping that is inherently involved. Our data indicate that mtDNA of corn is different from the mtDNA of other higher plants which have been studied by R. Kolodner and K. K. Tewari (Proc. Nat. Acad. Sci. USA 69:1830, 1972), who have found circles of 30 μ in the mtDNA preparations of pea, spinach, lettuce and beans with no evidence for intermolecular heterogeneity. At present, we have no evidence that intermolecular heterogeneity in corn mtDNA is a reflection of the differential amplification of mtDNA segments despite the fact that there are several species of supercoiled mtDNA present within mitochondria. Such differential amplification of