

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/cm³, 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

organelle DNA has been postulated for Euglena chloroplast DNA by J. R. Mielenz and C. L. Hershberger (Biochem. Biophys. Res. Commun. 58:769, 1974), who have identified five species of covalently closed circular chloroplast DNA that differ in buoyant density.

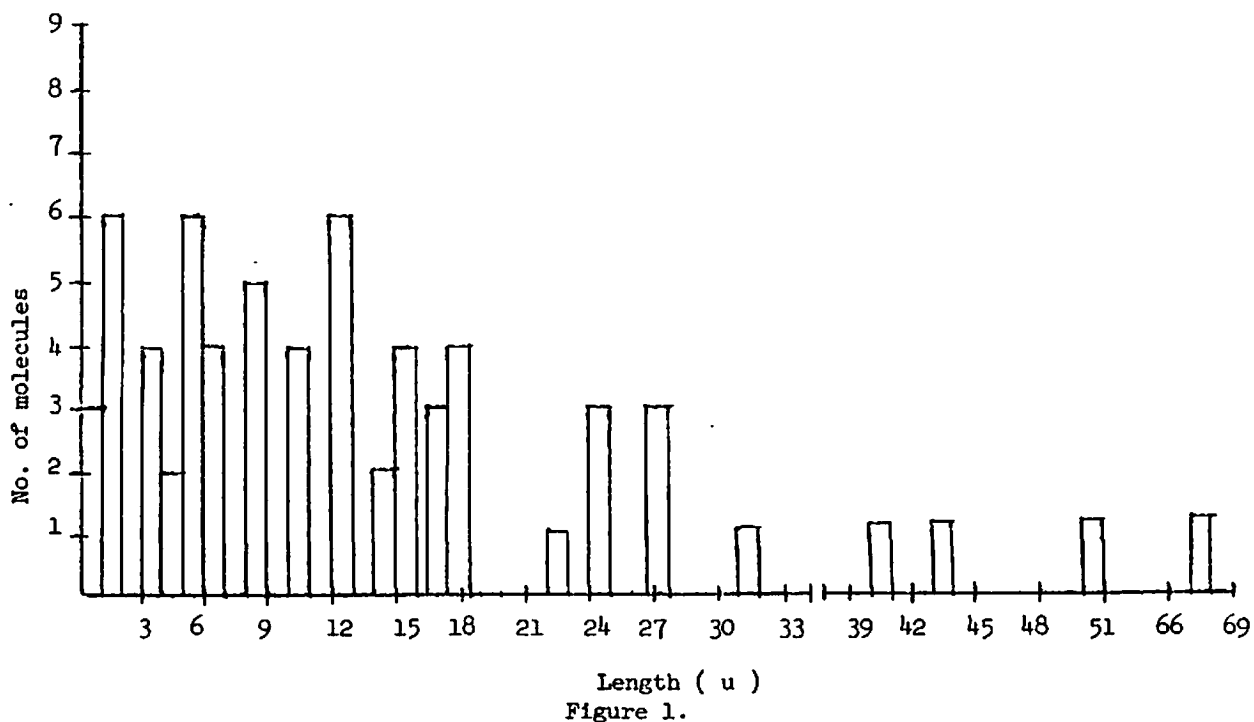


Figure 1. Frequency distribution of circular mitochondrial DNA.

We have also observed a significant number of mini-circles in our mtDNA preparations with the average molecular lengths of 0.6, 1.7 and 3.6 μ . A fraction of these mini-circles has been found to be resistant to digestion by Eco RI restriction endonuclease. Mini-circles have also been demonstrated in mitochondria of other higher plants. The significance of these mini-circles is presently unknown.

Finally, we have identified circular molecules with attached double-stranded tails (rolling circles). The length of the tail varied from 6.4% to 880% as compared to the length of the attached circle, which varied from 1.7 to 17.9 μ . This suggests that rolling circles are a mechanism for mtDNA replication in corn and also a probable means to provide for amplification of mtDNA.

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Preliminary genetic analysis of the maize catalase inhibitor

We have previously reported on quantitative variation of catalase inhibitor levels in several inbred lines (MGCNL, Vol. 49). We have presently screened over forty inbreds, and inhibitor levels in all can be categorized into one of three distinct groups having either high, intermediate, or low inhibitor activity. The F₁ hybrids between lines in the low and intermediate categories suggest that the low levels may be dominant to intermediate levels:

| | <u>Inhibitor Specific Activity</u> |
|-----------|------------------------------------|
| Line 386 | 21.7 U/mg |
| Line 399 | 9.4 U/mg |
| 386 x 399 | 11.6 U/mg |