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# THE MITOCHONDRIAL GENOME OF HIGHER PLANTS

*(mitochondrial DNA, cytoplasmic male sterility, S cytoplasm)*

C. S. LEVINGS, III* AND D. R. PRING**

*Department of Genetics
North Carolina State University
Raleigh, North Carolina 27650

**USDA, Florida-Antilles Area
Department of Plant Pathology
University of Florida
Gainesville, Florida 32611

## SUMMARY

*Mitochondrial (mt) DNAs of higher plants have molecular weights ranging from 70-165 x $10^6$, which is by far the largest found in nature. Their native configuration is probably a covalently closed circular molecule. In some species, the entire mt genome is coded on a single molecule but in others several classes of mtDNA molecules have been indicated.*

*The informational content of the mt genome of higher plants is poorly determined. The large size predicts that additional information is coded by these mt genomes. Evidence is presented which suggests that cytoplasmic male sterility (cms) is one of the additional traits.*

*Sequence differences have been detected among mt DNAs isolated from plants from different cytoplasmic backgrounds. Although the sequence distinctions are of unknown consequence, it is important because it establishes that diversity exists among mt genomes within a species.*

*Associated with mitochondria from the S cytoplasm of maize are two unique plasmid-like DNAs. Seemingly, these unique DNAs are responsible for the S type of cms as well as the unstable behavior of this cytoplasm.*

## SIZE OF THE MITOCHONDRIAL GENOME

The mitochondrial (mt) DNA of animals has been intensely studied (BORST 1977). The total informational content of the mtDNA is coded for by a single duplex circular molecule of the size 5-6 μm (9-12 x $10^6$ molecular weight). The fungi and protozoa have mtDNAs of intermediate size; they have molecular weights which range from 18 - 49 x $10^6$. Although most have circular configurations, the mtDNA of *Paramecium* and *Tetrahymena* exist as

linear molecules. The largest of the mtDNAs occur in higher plants where the reported sizes are 70 x $10^6$ or more.

In reviewing the literature, it is immediately evident that striking differences have been reported in the size of the mtDNA of higher plants. Some of these differences may be accounted for by distinctions among species. The mtDNAs of pea, lettuce, and spinach, have been isolated as 30 μm circles with molecular weights of approximately 70 x $10^6$ (KOLODNER & TEWARI 1972a 1972b). Electron microscopy and renaturation kinetic studies of pea mtDNA estimated the molecular weight to be 70 and 74 x $10^6$, respectively. Furthermore, these studies indicated no evidence of inter- or intra-molecular heterogeneity. More recently, a French group has studied the mtDNA from several higher plants and suggested that the mtDNA is composed of several different molecular classes (QUETIER & VEDEL 1977). The mtDNAs of Virginia creeper, cucumber, wheat, and potato were found to have molecular weights of 165, 120, 140, and 90 x $10^6$, respectively, when determined by sizing the fragments resulting from digestion with the restriction enzyme, *Eco*RI. In these investigations, mtDNA was isolated as covalently closed circular molecules from the lower band of a dye - CsCl gradient. When the mtDNAs of Virginia creeper and potato were examined by electron microscopy, molecular weights of 60 - 70 x $10^6$ were determined. Hence, the two procedures produced a marked discrepancy in the size of the mtDNAs. Based on these findings, they postulated that the mtDNA contained a heterogeneous population of molecules and that the molecules seemingly had the same contour lengths but differed in their sequence arrangements.

Electron microscopy examinations of maize mtDNAs have similarly indicated molecular heterogeneity (SHAH *et al.* 1976, 1978). These studies revealed at least three discrete classes of circular molecules which had contour lengths of 16, 22, and 30 μm and corresponding molecular weights of 36, 47, and 66 x $10^6$ (Fig. 1). These values sum to a total molecular weight of 149 x $10^6$ for maize mtDNA. This result is in reasonable agreement with the molecular weight of 131 x $10^6$ ascertained from the *Hin*dIII restriction enzyme pattern (PRING & LEVINGS 1978). The somewhat larger value obtained by electron microscopy can be explained if multiplicities of DNA bands on agarose gels are considered. In addition, a few molecules of approximate 43 μm size were observed. Since these molecules occur at low frequencies and are of appropriate size to be either dimers of the 22 μm class or chloroplast DNA circles present as trace contaminates (KOLODNER & TEWARI 1975), we have discounted them from our considerations of genome size. In this connection, we have also observed minicircles, molecules of less than 5 μm. The importance of minicircles is not clear, but they have also been reported in chloroplast DNA preparations (KOLODNER & TEWARI 1975). At any rate, it is abundantly clear that several classes of circular DNA molecules can be isolated from maize mitochondrial preparations. Recent electron microscopy studies of soybean mtDNA have also revealed the presence of several different size classes of circular molecules. Thus these results have extended the molecular heterogeneity phenomenon to soybeans (SYNENKI *et al.* 1978).

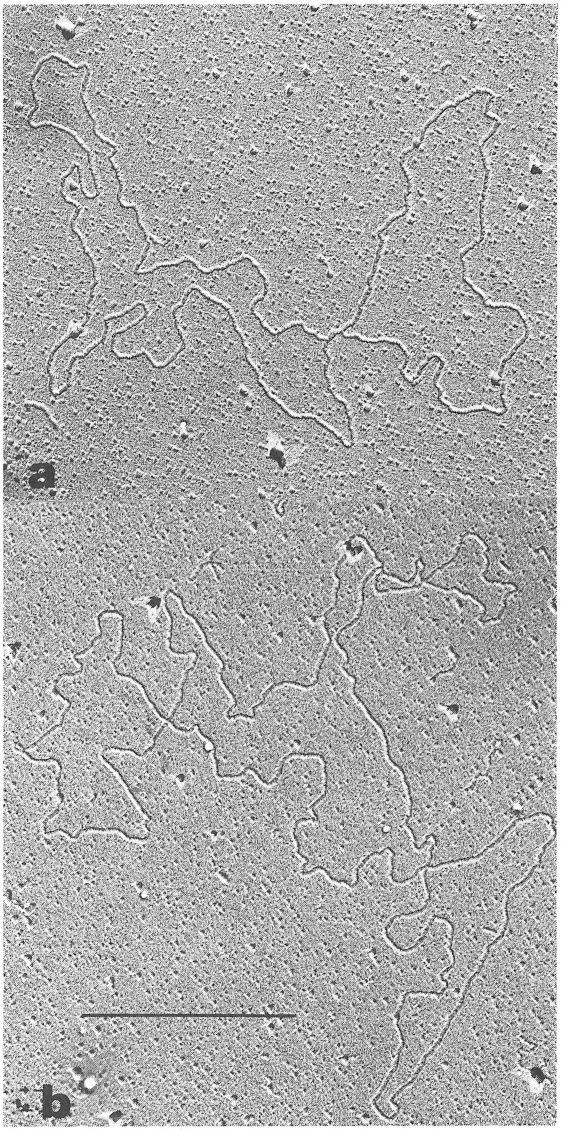


FIGURE 1. Typical relaxed circular mtDNA molecules isolated from maize: a) Circular molecule of 16 μm; b) Circular molecule of 21 μm. Bar equals 1 μm.

Why does intermolecular heterogeneity exist among circular mtDNA molecules of higher plants? Unfortunately, the explanation is not clear, but a number of possibilities can be consi-

dered. The informational content of the mitochondrial genome may be encoded on more than one molecule. This would be analogous to the situation in the nucleus where the information is coded on multiple chromosomes. Although multiple mitochondrial chromosomes have not been previously reported in higher plants, this possibility is compatible with our findings. This would be contrary to the case with chloroplast DNAs where the whole genome is apparently coded on a single molecule. Heterogeneous populations of mtDNA molecules are not without precedence in the lower forms. Circular molecules of varying lengths have been found in *Neurospora crassa* (AGSTERIBBE *et al.* 1972). Linear mtDNA molecules of *Tetrahymena pyriformis* have been reported which range in length from 17 - 26 μm without any distinct classes (ARNBERG *et al.* 1977).

A speculative alternative is the possibility that mitochondrial populations are not homogeneous. In this event, different mitochondrial types might very well contain their own unique DNAs of varying lengths. Presently, there is no supportive evidence for this hypothesis.

Different sizes of circular molecules could be generated by recombinational events among mtDNA molecules. Recombination is recognized among bacterial plasmids (CLOWES 1972), and recently, recombination among mtDNA molecules has been demonstrated in interhybrid somatic cells of animals (HORAK *et al.* 1974). In addition, molecular heterogeneity could also result from incomplete or superfluous replication of the parental molecule. The presence of a dimeric class has been suggested earlier.

Another explanation is that companion DNAs are found associated with the main mtDNA molecule. This would be analogous to the situation with bacterial chromosomes and their associated plasmids. In fact, this possibility is indicated by the unique plasmid-like DNA found in the S cytoplasmic types (PRING *et al.* 1977). This special case will be considered in greater detail elsewhere.

Lastly, contamination by alien DNAs from other organelles or sources is an important concern. Although this possibility cannot be completely discounted, it seems remote. This is suggested in previous investigations where we have demonstrated consistent differences among mtDNAs by restriction enzyme fragment analysis irrespective of genetic backgrounds, (LEVINGS & PRING 1976; PRING & LEVINGS 1978).

Although the occurrence of circular molecules has been demonstrated in the mtDNA of corn and soybeans, the percentage of circular molecules has been extremely small. Furthermore, supercoiled molecules have proven exceptionally difficult to isolate. Difficulties in isolating mtDNA in supercoiled configuration have also been encountered in eukaryotes other than animals, for example, yeast, *Neurospora* and *Euglena* (HOLLENBERG *et al.* 1970; SCHAFER *et al.* 1971; NASS *et al.* 1974). Perhaps the difficulties are simply isolation problems; however, the possibility cannot be ruled out that mtDNA in higher plants exists predominantly in the linear form and that circularization occurs only in a

small fraction of the mtDNA molecules at any one time by means of covalent linkage or cohesive ends. In this connection, the unique DNA species associated with S cytoplasm in maize have been shown to exist in a linear form and to have terminal inverted repeats (PRING *et al.* 1977; LEVINGS *et al.* 1978).

It is evident from this review that the organization of the mitochondrial genome is not clear. First, the native conformation of the mtDNA molecules has been reported to be covalently closed circles, but linear molecules have also been observed. The mt genome size reportedly ranges from 70 x $10^6$ in pea to as high as 160 x $10^6$ in Virginia creeper. Finally, there is evidence suggesting that the entire mitochondrial genome is coded on a single molecule; however, there are contrasting studies indicating the presence of several classes of mtDNA molecules. Some of these distinctions are probably due to variations among species. Additional studies will be necessary to resolve the discrepancies.

## INFORMATIONAL CONTENT OF THE MITOCHONDRIAL GENOME

Although there are seemingly wide variations in the size of the mtDNAs among higher plants, it is certain that the mtDNAs of plants are substantially larger than those found among animals, fungi and protozoa. In animals and yeast, the informational content is largely known. Certain information coded by the mtDNAs of these organisms must also be coded by plant mtDNAs.

Our knowledge of known gene products of higher plant mitochondrial DNA is limited. To date, no ribosomal RNA (rRNA) or other cellular RNAs, and only a few specific proteins, are unambiguously known to be encoded by higher plant mtDNA. Higher plant mitochondria, including maize, are known to contain unique rRNAs, which differ from cytoplasmic or chloroplast rRNAs (LEAVER & HARMEY 1973; PRING 1974; PRING & THORNBURY 1975; CUNNINGHAM *et al.* 1976; CUNNINGHAM & GRAY 1977). It has also been shown that higher plant mitochondria also contain a unique 5S RNA, probably associated with the heavy rRNA (LEAVER & HARMÉY 1976; CUNNINGHAM *et al.* 1976). It is tacitly assumed that these mitochondrial RNAs are mtDNA gene products.

Discrete products of *in vitro* protein synthesis by isolated mitochondria have been described (LEAVER 1976), but no functional identification has been made. Interestingly, the number and size range of these proteins were similar to polypeptides assumed to be gene products of fungal and mammalian mtDNA.

A significant question is what additional information could be coded by the large mtDNAs of higher plants. One answer is that no additional information is coded on the larger mtDNAs of plants, but instead the extra DNA serves only a "spacer" function. In fact, this belief has received some support in yeast, where nearly 50% of the mtDNA is composed of very AT-rich stretches (PURNELL & BERNARDI 1974). If the amount of mtDNA present in animals is adequate for all the informational content needed by mitochondrial genomes, in general, then more than 85% of the mtDNA of plants would have to function as "spacers." This is

not an attractive alternative. The possibility that additional information is carried by large plant mtDNAs is suggested by the fact that several traits which are unique to higher plants are inherited in an extrachromosomal fashion. Cytoplasmic male sterility (cms), disease susceptibility, and reduced kernel size are a few examples of this type. This does not include traits involved in the photosynthetic mechanisms, because they are, or appear to be, coded by the chloroplast genome.

Several approaches are available for investigating the informational content of mtDNAs; unfortunately, many of these are not practical in higher plants. We have chosen to study this question by characterizing and contrasting the mtDNAs from populations which vary with respect to cytoplasmically inherited traits. The rationale behind this approach is that cytoplasmic variants may be associated with organelle DNAs of substantially altered sequence arrangements.

Restriction enzymes are especially useful for characterizing the smaller DNAs. These enzymes are endonucleases which cleave double-stranded DNAs at sequence-specific sites. When DNAs of small complexity are cleaved by a restriction enzyme and fractionated by gel electrophoresis, a characteristic pattern, a fingerprint, of the original DNA is produced. This technique is termed restriction-enzyme fragment analysis. The restriction pattern of a particular DNA is a function of the number and position of the cleavage sites generated by the restriction endonuclease. Therefore sequence distinctions among related DNAs can be resolved if they involve changes in the number or position of cleavage sites. Importantly, changes in the position of a cleavage site can be too small for discrimination by this technique.

In higher plants, cytoplasmic male sterility is one of the more ubiquitous of the extrachromosomally inherited traits. EDWARDSON (1970) has reported that cms occurs in 80 species, 25 genera and six families. Several years ago we began studying the organelle DNAs from fertile (normal) and male-sterile cytoplasms of maize by restriction enzyme fragment analysis. Although these male steriles were known to be inherited in a strict maternal fashion (DUVICK 1965), the location of the factors responsible for this trait was unknown.

Restriction-endonuclease fragment analyses of maize organelle DNAs have been extensively utilized in our laboratories as a probe to ascertain the nature and variability of these genomes (LEVINGS & PRING 1976, 1977; PRING & LEVINGS 1978). It was apparent from these studies that there is substantial variability among mitochondrial DNAs of male-sterile cytoplasms (e.g. Fig. 2) and little variability among chloroplast DNAs. These results then favor the mitochondrion as a carrier of determinants conditioning cytoplasmic male sterility.

Investigations of mtDNA from normal cytoplasms (LEVINGS & PRING 1977) revealed that some "background" mtDNA heterogeneity existed among isolates (inbreds) of *Z. mays*. Five of nine cytoplasms, for instance, displayed unique *Hin*dIII restriction sites, while *Sal*I produced three distinct groups from nine inbreds or

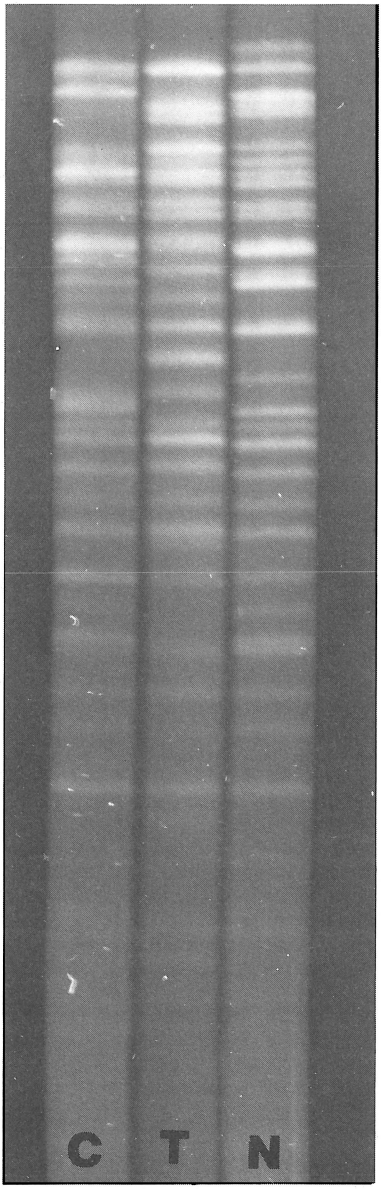


FIGURE 2. Agarose gel electrophoresis pattern (upper three quarters) of maize mtDNA after digestion with the restriction endonuclease *Sal*1. Letters on the gels designate the cytoplasmic male-sterile type except N which is a normal (fertile) cytoplasmic type. All are in the same nuclear background, B37 x NC236.

single crosses. The extent of these differences, from one to five band shifts among the approximately 50 bands produced by *Hin*dIII, is suggestive of more than simple point mutation among normal cytoplasms. Interestingly, *Bam*Hl failed to distinguish any of nine cytoplasms tested, which would suggest caution in interpreting negative data based on the use of one or a few endonucleases. When similar studies of the mtDNAs from soybeans were carried out, small differences, one or two bands, were detected in the banding patterns among some of the soybean varieties (SISSON *et al.* 1978). Clearly, both corn and soybean mtDNAs contain modest levels of sequence diversity.

The significance of the sequence diversity found among the mtDNAs of normal (wild type) maize and soybean is obscure. Presently, the variation cannot be related to a particular phenotypic expression; in fact, it is not certain that the diversity involves sequences which are transcribed. Nonetheless, the diversity may eventually prove valuable as a genetic tool for understanding the informational content and organization of the mitochondrial genome. Furthermore, when the question of cytoplasmic vulnerability is considered, it is instructive to know that sequence diversity exists among the mitochondrial genomes of two major crop plants.

The mtDNA from the three (T,C,S) cms groups a) varies markedly from that of normal cytoplasms, and b) displays marked variation between groups (PRING & LEVINGS 1978). These conclusions have been based on restrictions with *Eco*RI, *Hin*dIII, *Sal*I, *Bam*Hl, *Sma*I, and *Xho*I. Patterns produced by these enzymes range from about 50 fragments with *Hin*dIII to about 30 with *Sma*I. Variations of the cms mtDNA patterns from those of normal cytoplasms range up to 17 new or missing bands, with *Hin*dIII, for example. Most of the fragment patterns show a similarity to normal cytoplasms of 65-80%; that is, 20 out of 30 or 40 out of 50 bands are common to all maize cytoplasms.

The complexity of the restriction patterns precludes accurate molecular weight determinations. The *Hin*dIII patterns, for instance, are characterized by numerous bands of apparent double or triple stoichiometry, and by bands of very low intensity. Attempts to separate and quantitate these bands have been largely unsuccessful. The highest molecular weight *Hin*dIII fragment, about 7 x $10^6$ molecular weight, is very close to the linear portion of a log molecular weight - distance migrated plot, making molecular sizing of clearly resolved fragments reliable. *Sma*I fragments are well separated and easily resolved; however the largest fragments are probably over 20 x $10^6$ molecular weight, well beyond a useful range for accurate molecular weight determination by current electrophoresis procedures. Estimates of molecular weight of *Hin*dIII or *Sma*I patterns then each have disadvantages. With these reservations, we have calculated minimum molecular weights for *Hin*dIII digests as 131 x $10^6$, and a minimum estimate of 150 x $10^6$ for *Sma*I digests. French workers (QUETIER & VEDEL 1977) found similar values (120-165 x $10^6$) for Virginia creeper, wheat, and cucumber mtDNA when digested with *Eco*RI. We have recently examined sorghum mtDNA and obtained similar complex patterns and molecular weights of between 100 and

200 x $10^6$, depending on which endonuclease is employed (PRING, CONDE, & WARMKE in preparation). It is thus apparent that the higher plant mitochondrial genome is probably far larger than any fungal or mammalian mtDNA previously examined.

The ease with which each of six endonucleases clearly distinguishes N, T, C, and S mtDNA from each other strongly indicates that the differences are not simple point mutations, but rather that these mtDNAs differ in significant sequence organization. It is intriguing to speculate that deletion may be operative, but our data, although suggestive, are not unambiguous in this area. Several enzymes, for instance, result in fragment patterns with minimum molecular weights of N mtDNA in excess of T mtDNA. Furthermore, the T mtDNA-*Hin*dIII pattern has 11 missing bands out of about 50, when compared to N mtDNA, but only six new bands are generated. The remainder of the missing DNA may be obscured in the closely spaced *Hin*dIII fragments, but deletion could be a viable alternative.

The repeated and invariant nature of the C, S, and T mtDNA restriction patterns in several nuclear backgrounds, some backcrossed over 20 generations, attests to the stable and conserved nature of maize mtDNA. Texas cytoplasm was transferred to F44, for instance, beginning in the early 1950's (J. R. EDWARDSON, personal communication). We assume that some of our T cms lines were developed much later, yet we have not observed any variation in the mtDNA fingerprint. The collective data also provide strong evidence of lack of paternal transmission, or more concisely, lack of expression of the paternal mtDNA genome in progeny.

While these studies on mtDNA of cms sources in maize are suggestive of a role in the inheritance and expression of cms, the chloroplast genome must be considered. This is especially true since chloroplast-coded fraction one protein subunits in tobacco vary with cytoplasm and have been shown to be associated with the female parent in crosses which result in cms (CHEN *et al.* 1975). These data distinguish a unique chloroplast genome. Investigations with N, C, S, and T maize cytoplasm chloroplast DNA (ctDNA) have shown a one-band displacement of S cms ctDNA when digested with *Hin*dIII (PRING & LEVINGS 1978). *Eco*RI and *Sal*I failed to differentiate any of the ctDNAs. More recently (unpublished) we have been able to show that *Hae*III, which produces over 55 fragments with ctDNA, results in one extra band from T cms ctDNA. Thus the seemingly minor variation among ctDNAs, and the substantial mtDNA variation, would provide compelling evidence implicating the mitochondrion. Similar results were obtained with mt- and ctDNA from an interspecific *Triticum aestivum-Triticum timopheevii* cross, which results in male sterility (QUETIER & VEDEL 1977). However, only *Eco*RI was used in these studies.

A more perplexing case has been observed in sorghum (PRING, CONDE, & WARMKE in preparation). Mt- and ctDNA from the male-sterile line was readily distinguishable from their counterparts in a maintainer line. The data suggest strict maternal inheritance, but offer no clue to date as to what role(s) each organelle may play in cms.

Other kinds of evidence point toward the mitochondrion as the probable site of determinants conditioning the expression of cms in maize. WARMKE AND LEE (1977) observed mitochondrial degeneration in the tapetum and middle layer of T anthers at the tetrad stage of microsporogenesis; no changes in plastids were detected until late in anther development. No alterations of mitochondria or plastids were noted in N anthers. BARRATT AND PETERSON (1977) recently identified T cms-specific protein differences from submitochondrial particles and from a partially purified ATPase complex, suggestive of unique gene products in constituents of the mitochondrion known to contain mtDNA-coded proteins (SCHATZ & MASON 1974).

Substantial evidence now points toward mitochondria as the target organelle involved in the maternally-inherited susceptibility of T cms lines to southern corn leaf blight. Toxins produced by the causal organism (HOOKER *et al.* 1970), race T of *Bipolaris maydis* (Nisikado) SHOEMAKER, preferentially affect T cms lines but not lines in N cytoplasm (MILLER & KOEPPE 1971; PETERSON *et al.* 1975). An association of toxin activity with several aspects of T cms behavior provide clues to a coupling of the susceptibility-sterility phenomena. Lines restored to fertility by the use of *Rf*, *Rf2* genes, for instance, display a modified reaction to the fungus, and the response of mitochondria from these lines to the toxin is similarly altered (WATRUD *et al.* 1975, BARRATT & FLAVELL 1975). Such changes are suggestive of nuclear gene products influencing or altering the T mitochondrion. GENGENBACH AND GREEN (1975) and GENGENBACH *et al.* (1977) utilized callus tissue culture to select cultures which were resistant to the toxin. Mitochondria isolated from these resistant calli were unaffected by toxin. Plants differentiated from the calli, after suitable exposure of the cultures to toxin, were resistant to the fungus, and mitochondria were similarly resistant to the toxin. Most of these plants were fertile. The "male sterile" resistant plants exhibited abnormal morphological characteristics, and the sterility is probably not cytoplasmic in origin (B. G. GENGENBACH personal communication). Thus selection for toxin resistance in these callus tissue cultures was essentially selection for mitochondrial resistance, and selected resistant cells gave rise to fertile plants.

## THE S CYTOPLASM OF MAIZE

The S cytoplasm is one of several cytoplasms which confers the cms trait to maize. It is distinguished from the other recognized cms, T and C, in that there is a difference in the mode and nuclear genes required for fertility restoration. *Cms-S* is restored to pollen fertility by a single locus, *Rf3*, which has been mapped on chromosome 2 (LAUGHNAN & GABAY 1975b). Pollen restoration is unusual in that it is gametophytic in nature (BUCHERT 1961). This means that the genotype of the pollen grain determines its phenotype with respect to pollen fertility. The fertile pollen grains have the genotype *Rf3*, while the aborted grains are *rf3*. Recently, additional distinctions have been discovered which are particularly relevant. These findings have come from investigations of the mtDNA of the S cytoplasm and

from studies of mutations involving the S cytoplasm and its restorer. Although the S cytoplasm may be a special case, these results seemingly provide insight into our understanding of the organizational and informational content of the mitochondrial genome.

MtDNA from the S cytoplasm has been isolated and partially characterized (PRING *et al.* 1977, PRING & LEVINGS, 1978). When the mtDNA from S cytoplasm plants was fractionated by gel electrophoresis, two unique plasmid-like DNAs were identified which were in addition to the usual high molecular weight mtDNAs (Fig. 3). These unique DNAs had molecular weights of 3.45 (S-F) and 4.10 (S-S) x $10^6$. Electron microscopic examination has revealed that these molecules exist in a linear form. However, it may be that the linear molecules have resulted from the breakage of native circular molecules.

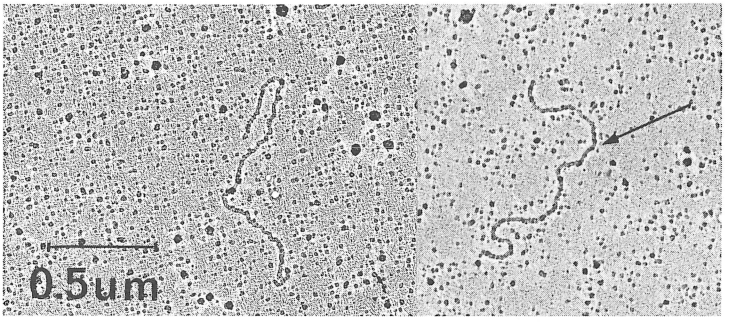


FIGURE 3. Unique DNA molecules associated with mitochondria from the S cytoplasm. Mounting was by the formamide technique. Linear molecules from the S-S and S-F (arrow) bands.

Significantly, the small unique DNAs associated with the S cytoplasm have not been detected in mtDNA preparations from normal (fertile) T, or C cytoplasms. This result has been repeatedly confirmed among many sources of these cytoplasms. Furthermore, the unique DNAs are not seen in mtDNA preparations from teosinte and tripsacum, which are close relatives of maize, nor from male-sterile sorghum. On the other hand, these unique DNAs have been verified in every S cytoplasm studied, irrespective of source or nuclear background. Nine different sources of the S cytoplasm were included in these studies. A causal relationship between the unique DNAs and the S type of male sterility is suggested by these associations.

The unique DNAs associated with the S cytoplasm have only been successfully isolated from mitochondrial preparations. Repeated efforts have failed to isolate these unique DNAs from chloroplast or nuclear preparations. Strict maternal transmission of the unique DNAs associated with S cytoplasm have been demonstrated (unpublished). Earlier, maternal transmission of mtDNAs of maize was verified by restriction enzyme fragment analyses

(LEVINGS & PRING 1976).

Unusual sequence arrangements have been discovered in the unique DNAs (S-S and S-F) by electron microscopy investigations (LEVINGS *et al.* 1978). After these DNAs were denatured and then briefly self-annealed, stem-loop configurations were observed in formamide spreads by electron microscopy. With both the S-S and S-F DNAs, the stem-loop structures consist of a short double-stranded stem and a large single-stranded loop. The formation of stem-loop structures was interpreted as being due to the presence of terminal inverted repeats on the molecules. When terminal inverted repeats pair, intrastrand stem-loop structures form with first-order kinetics (BOKER *et al.* 1977). The inverted repeats were estimated to be 195 and 168 nucleotides long in the S-S and S-F molecules, respectively. These lengths were determined by electron microscopic measurements of the double-stranded stems of stem-loop structures. The inverted repeats constitute 3.1 and 3.2 percent of the S-S and S-F DNAs, respectively. Several other studies have verified our interpretation of the stem-loop structures.

The significance of terminal inverted repeats on the unique DNAs from the S cytoplasm is not understood. Interestingly, inverted repeats are often prominently involved with insertional events in lower organisms. It is tempting to consider that the unique DNAs described here are in some manner associated with the unstable nature of the S cytoplasm.

For several years Laughnan and his associates have been investigating the stability of the S cytoplasm of maize (see LAUGHNAN & GABAY 1975b). In particular, they have sought cases where the S cytoplasm changed from male-sterile to the male-fertile condition. Their efforts have been successful in that they have found several hundred mutations where male-steriles have reverted to fertiles. Two kinds of changes were observed, cytoplasmic mutations from the male sterile to the male fertile and nuclear mutations giving rise to new fertility-restoring genes. Details concerning the breeding procedures used in these studies can be found in LAUGHNAN'S papers (1973; 1975a; 1975b; SINGH & LAUGHNAN 1972). Before proceeding, it is important to point out the difference in stability between the S and the T and C cytoplasm. Substantiated cases of T or C reverting from the cytoplasmic male-sterile to male-fertile condition have not been reported. In fact, DUVICK (1965) has noted that these cytoplasms are notoriously stable.

The vast majority of LAUGHNAN'S reversions from the male-sterile to male-fertile conditions arose by a cytoplasmic change. Over 300 independently occurring cases of cytoplasmic reversions have been identified and confirmed by his test procedures. These newly arisen fertile cytoplasms have persisted through subsequent generations of propagation. Perhaps of importance, most of the cytoplasmic revertants happened in one inbred line which seems especially prone to the event. The cytoplasmic revertants arose either as fertile chimeras or completely fertile tassels.

A number of male-fertile reversions have been identified

whose testcross analyses showed that the change did not occur at the cytoplasmic level. In appropriate testcrosses, these new male fertiles exhibited a behavior expected of nuclear restorers of the S cytoplasm. To date, ten new restorer strains have been isolated and partially characterized. These ten appeared as fertile chimeras or fully fertile tassels.

The phenotypic expression of the ten new restorers has been characterized in relation to *Rf3*, the natural occurring restorer of *cms-S*. The manner of restoration for the ten new restorers is gametophytic as it is with the standard S restorer, *Rf3*. They contrast with the standard *Rf3* locus in several respects. For instance, the new restorers have reduced transmission through the female gametophyte, a reduction in kernel size, and a lethality of the restorer homozygotes. One of the new restorers, designated IV, appears to be without adverse effects, which is especially interesting because it originated in a maintainer plant.

Mapping studies of the ten newly arisen restorer genes have provided surprising results (LAUGHNAN & GABAY 1975b). Roman numeral designations have been given to the new restorer genes. *Rf3*, the standard restorer locus, has been mapped on chromosome 2, probably in the long arm. New restorers IX and X are on chromosome 1; IV and VII are on chromosome 3; and I and VIII are on chromsome 8. The remaining four restorers, II, III, V, VI, have not yet been mapped, but they are known not to be allelic with *Rf3*. Therefore, it seems that each new restorer is located at a unique chromosomal site.

Based upon these findings, the investigators have proposed the existence of a male-fertility element which has the characteristics of an episome (LAUGHNAN & GABAY 1975a, 1975b). In bacterial systems, episomes can be transposed from one site to another, or be lost entirely. The transposing phenomenon is suggested by the newly arisen restorer genes in that male-fertility elements seemingly have been integrated at various chromosomal sites. In this regard, they have speculated about the causes of the deleterious side effects manifested by kernels carrying the newly arisen restorer genes. They propose that the aberrant behavior of new restorers may result from differences in integration sites (position effect) in the chromosomes or in qualitative distinctions among the fertility elements. Support for the latter possibility is indicated by the fact that restorer IV, the only restorer without adverse side effects, is the only one which arose in a fertile maintainer cytoplasm. Similarly, the cytoplasmic revertants from male-sterile to male-fertile could be due to the transposition of a fertility element.

In spite of the fact that male-fertile revertants have involved changes at either the cytoplasmic or nuclear level, the investigators have argued that both events have a common origin. This argument is supported by the fact that both kinds of male-fertile reversions have arisen in the same strains and both were initially expressed as either wholly male-fertile tassels or as fertile-sterile tassel chimeras. Accordingly, they propose that the male-fertile element is fixed in the cytoplasm when a

cytoplasmic change from a male-sterile to male-fertile condition occurs. Conversely, if the element is fixed in the nucleus, it behaves as a restorer strain.

The positive association between the S type of male sterility and the unique DNAs, S-S and S-F, constitutes additional supportive evidence that cms trait is involved with the mitochondrial genome. It is tempting at this juncture to entertain the hypothesis that S-S and S-F DNAs are indeed the "fertility elements" which LAUGHNAN'S group has described to explain the unstable nature of the S cytoplasm. Unfortunately, rigorous data unequivocally linking these S-S and S-F DNAs to the phenomenon are not available. Interestingly, in maize several nuclear systems, Ac-Ds (MCCLINTOCK 1956), Dt (RHOADES 1941) and others (FINCHAM & SASTRY 1974) have been described in which genetic elements are postulated to move and bring about changes in phenotypic expression. The S-S and S-F molecules may be only one example of several transposable elements present in maize.

The organizational relationship between the unique DNAs, S-S and S-F, and remainder of the mtDNA is not clear. In a sense, this relationship seems analogous to that of the bacterial chromosome and its plasmids. However, since molecular heterogeneity of mtDNA exists in other cytoplasms besides S, this may represent still another version of the heterogeneity phenomenon. The evidence indicating the occurrence of transpositional events has important implications on the evolution of the organelle and nuclear genomes of higher plants. It suggests that the various genetic systems are not closed and that genetic elements may be transposed among them.

## ACKNOWLEDGEMENTS

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## LITERATURE CITED

AGSTERIBBE, E., A. M. KROON and E. F. J. VAN BRUGGEN 1972 Circular DNA from mitochondria of *Neurospora crassa*. Biochim. Biophys. Acta 269: 299-303.

ARNBERG, A. C., R. W. GOLDBACH, E. F. J. VAN BRUGGEN and P. BORST 1977 The structure of *Tetrahymena pyriformis* mitochondrial DNA. II The complex structure of strain GL mitochondrial DNA. 477: 51-69.

BARRATT, D. H. P. and R. B. FLAVELL 1975 Alterations in mitochondria associated with cytoplasmic and nuclear genes concerned with male sterility in maize. Theor. Appl. Genetics 45: 315-321.

BARRATT, D. H. P. and P. A. PETERSON 1977 Mitochondrial banding pattern difference in electrofocused polyacrylamide gels between male-sterile and non-sterile cytoplasm of maize. Maydica 22: 1-8.

BOKER, T. R., L. SOLL and L. T. CHOW 1977 Underwound loops in

self-renatured DNA can be diagnostic of inverted duplications and translocated sequences. J. Mol. Biol. 113: 579-589.

BORST, P. 1977 Structure and function of mitochondrial DNA. Pp. 237-244. International Cell Biology 1976-1977. (Brinkley, B. R. and K. R. Porter, Eds.). Rockefeller Univ. Press.

BUCHERT, J. G. 1961 The stage of the genome-plasmon interaction in the restoration of fertility to cytoplasmically pollen-sterile maize. Proc. Natl. Acad. Sci. USA 47: 1436-1440.

CHEN, K., S. D. KUNG, J. C. GRAY, and S. G. WILDMAN 1975 Polypeptide composition of fraction I protein from *Nicotiana glauca* and from cultivars of *Nicotiana tabacum*, including a male sterile line. Biochem. Genetics 13: 771-778.

CLOWES, R. C. 1972 Molecular structure of bacterial plasmids. Bacteriol. Rev. 36: 361-405.

CUNNINGHAM, R. S., L. BONEN, W. F. DOOLITTLE, and M. W. GRAY 1976 Unique species of 5S, 18S, and 26S ribosomal RNA in wheat mitochondria. FEBS Letters 69: 116-122.

CUNNINGHAM, R. S. and M. W. GRAY 1977 Isolation and characterization of $^{32}$P-labeled mitochondrial and cytosol ribosomal RNA from germinating wheat embryos. Biochem. Biophys. Acta 475: 476-491.

DUVICK, D. N. 1965 Cytoplasmic pollen sterility in corn. Adv. Genet. 13: 1-56.

EDWARDSON, J. R. 1970 Cytoplasmic male sterility. Bot. Rev. 36: 341-420.

FINCHAM, J. R. S. and G. R. K. SASTRY 1974 Controlling elements in maize. Annu. Rev. Genet. 8: 15-50.

GENGENBACH, B. G. and C. E. GREEN 1975 Selection of T-cytoplasm maize callus cultures resistant to *Helminthosporium maydis* race T pathotoxin. Crop Science 15: 645-649.

GENGENBACH, B. G., C. E. GREEN and C. M. DONOVAN 1977 Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. (USA) 74: 5113-5117.

HOLLENBERG, C. P., P. BORST and E. F. J. VAN BRUGGEN 1970 Mitochondrial DNA. V. A 25-μ closed circular duplex DNA molecule in wild-type yeast mitochondria. Structure and genetic complexity. Biochim. Biophys. Acta 209: 1-15.

HOOKER, A. L., D. R. SMITH, S. M. LIM and J. B. BECKETT 1970 Reaction of corn seedings with male-sterile cytoplasm to *Helminthosporium maydis*. Plant Dis. Reptr. 54: 708-712.

HORAK, I., H. G. COON and I. B. DAWID 1974 Interspecific recombination of mitochondrial DNA molecules in hybrid somatic cells. Proc. Natl. Acad. Sci. USA 71: 1828-1832.

KOLODNER, R. and K. K. TEWARI 1972a Physicochemical characteristics of mitochondrial DNA from pea leaves. Proc. Nat. Acad. Sci. 69: 1830-1834.

KOLODNER, R. and K. K. TEWARI 1972b Genome sizes of chloroplast and mitochondrial DNA's in higher plants. (Arceneaux, C. J., Ed.). Proc. 30th Annual Meeting Electron Microscopy Society of America. Pp. 190-191.

KOLODNER, R. and K. K. TEWARI 1975 The molecular size and conformation of the chloroplast DNA from higher plants. Biochim. Biophys. Acta 402: 372-390.

LAUGHNAN, J. R. and S. J. GABAY 1973 Mutations leading to nu-

clear restoration of fertility in S male-sterile cytoplasm in maize. Theor. Appl. Genetics. 43: 109-116.

LAUGHNAN, J. R. and S. J. GABAY 1975a An episomal basis for instability of S male sterility in maize and some implications for plant breeding. Pp. 330-349. Genetics and the Biogenesis of Cell Organelles. (Birky, C. W., Jr., P. S. Perlman and T. J. Byers, Eds.) The Ohio State University Press, Columbus.

LAUGHNAN, J. R. and S. J. GABAY 1975b Nuclear and cytoplasmic mutations to fertility in S male-sterile maize. (Walden, D. B., Ed.). International Maize Symposium: Genetics and Breeding. John Wiley and Sons, Inc. (In press).

LEAVER, C. J. 1976 Mitochondrial protein synthesis in higher plants. Pp. 779-782. Genetics and Biogenesis of Chloroplasts and Mitochondria. (Böcher, Th., W. Neupert, W. Sebald, and S. Werner, Ed.). North Holland, New York.

LEAVER, C. J. and M. A. HARMEY 1973 Plant mitochondrial nucleic acids. Biochem. Soc. Symp. 38: 175-193.

LEAVER, C. J. and M. A. HARMEY 1976 Higher plant mitochondrial ribosomes contain a 5S rRNA component. Biochem. J. 157: 275-277.

LEVINGS, C. S., III, W. W. L. HU, D. H. TIMOTHY and D. R. PRING 1978 Terminal inverted repeats in the unique DNAs associated with the S cytoplasm. Maize Genet. Coop. Newsletter (In press).

LEVINGS, C. S., III, and D. R. PRING 1976 Restriction endonuclease analysis of mitochondrial DNA from normal and Texas cytoplasmic male sterile maize. Science. 193: 158-160.

LEVINGS, C. S., III, and D. R. PRING 1977 Diversity of mitochondrial genomes among normal cytoplasms of maize. J. Hered. 68: 350-354.

MCCLINTOCK, B. 1956 Controlling elements and the gene. Cold Spring Harbor Symp. Quant. Biol. 21: 197-216.

MILLER, R. J. and D. E. KOEPPE 1971 Southern corn leaf blight: Susceptible and resistant mitochondria. Science. 173: 67-69.

NASS, M. M. K., L. SCHORI, Y. BEN-SHAUL and M. EDELMAN 1974 Size and conformation of mitochondrial DNA in *Euglena gracilis*. 374: 283-291.

QUETIER, F. and F. VEDEL 1977 Heterogeneous population of mitochondrial DNA molecules in higher plants. Nature. 268: 365-368.

PETERSON, P. A., R. B. FLAVELL and D. H. P. BARRATT 1975 Altered mitochondrial membrane activities associated with cytoplasmically-inherited disease sensitivity in maize. Theor. Appl. Gen. 45: 309-314.

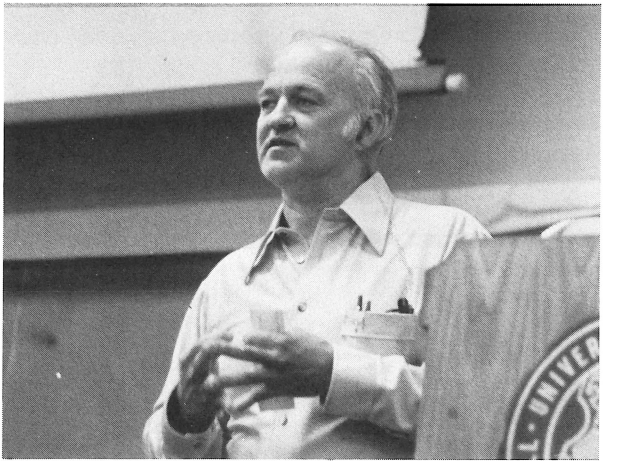
PRING, D. R. 1974 Maize mitochondria: purification and characterization of ribosomes and ribosomal ribonucleic acid. Plant Phys. 53: 677-683.

PRING, D. R. and C. S. LEVINGS, III 1978 Heterogeneity of maize cytoplasmic genomes among male-sterile cytoplasms. Genetics (In press).

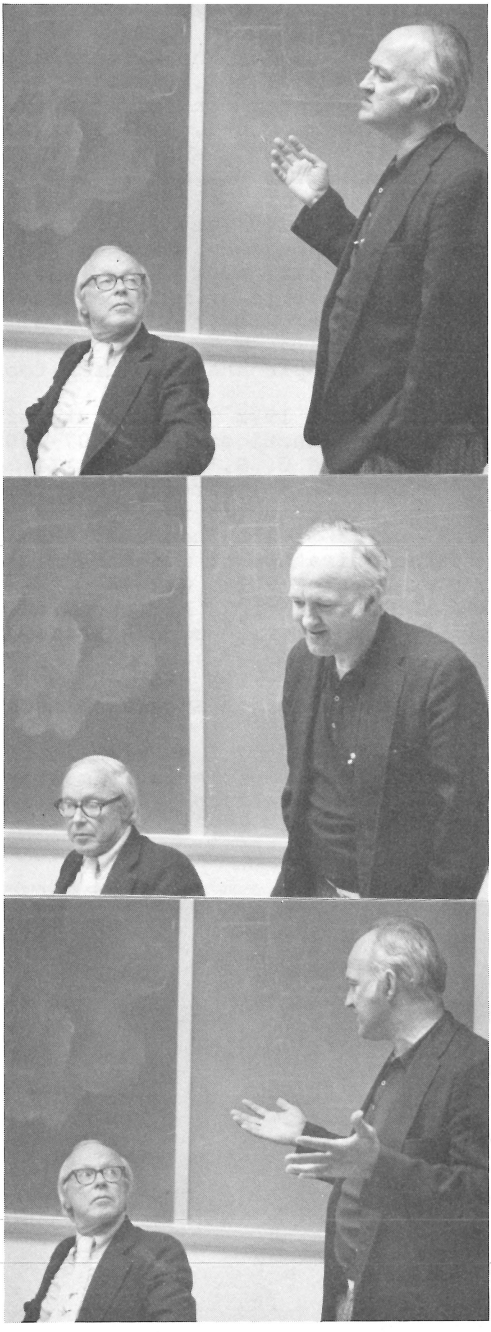
PRING, D. R., C. S. LEVINGS, III, W. W. L. HU and D. H. TIMOTHY 1977 Unique DNA associated with mitochondria in the "S" type cytoplasm of male-sterile maize. Proc. Natl. Acad. Sci. USA. 74: 2904-2908.

PRING, D. R. and D. W. THORNBURY 1975 Molecular weights of maize mitochondrial and cytoplasmic ribosomal RNAs under

denaturing conditions. Biochem. Biophys. Acta 383: 140-146.
PURNELL, A. and G. BERNARDI 1974 The mitochondrial genome of wild-type yeast cells. J. Mol. Biol. 86: 825-841.
RHOADES, M. M. 1941 The genetic control of mutability in maize. Cold Spring Harbor Symp. Quant. Biol. 9: 138-144.
SCHAFER, K. P., G. BUGGE, M. GRANDI and H. KÜNTZEL 1971 Transcription of mitochondrial DNA *in vitro* from *Neurospora crassa*. 21: 478-488.
SCHATZ, G. and T. L. MASON 1974 The biosynthesis of mitochondrial proteins. Annu. Rev. Biochem. 43: 51-87.
SHAH, D. M., C. S. LEVINGS, III, W. W. L. HU and D. H. TIMOTHY 1976 Conformation and size of mitochondrial DNA of maize. Maize Genet. Coop. Newsletter. 50: 94-95.
SHAH, D. M., C. S. LEVINGS, III, W. W. L. HU and D. H. TIMOTHY 1978 Electron microscopic analysis of mitochondrial DNAs of corn (In review).
SINGH, A. and J. R. LAUGHNAN 1972 Instability of S male-sterile cytoplasm in maize. Genetics. 71: 607-620.
SISSON, V. A., C. A. BRIM and C. S. LEVINGS, III 1978 Characterization of cytoplasmic diversity in soybeans by restriction endonuclease analysis (In review).
SYNENKI, R. M., C. S. LEVINGS, III and D. M. SHAH 1978 Physicochemical characterization of mitochondrial DNA from soybeans. Plant Physiol. 61: 460-464.
WARMKE, H. E. and S. L. J. LEE 1977 Mitochondrial degeneration in T cytoplasmic male-sterile corn anthers. J. Hered. 68: 213-222.
WARMKE, H. E. and S. L. J. LEE 1978 Pollen abortion in T cytoplasmic male-sterile corn: A suggested mechanism. Science (In press).
WATRUD, L. S., A. L. HOOKER and D. E. KOEPPE 1975 The effects of nuclear restorer genes of Texas male-sterile cytoplasm on host response to *Helminthosporium maydis* race T. Phytopathology 65: 178-182.



Dr. C. S. Levings III



Drs. Giles and Levings at a discussion session