STADLER SYMP. Vol. 13 (1981) University of Missouri, Columbia 93

CHARACTERISTICS OF *cms*-S REVEVERSION TO MALE FERTILITY IN MAIZE

(cytoplasmic male sterility, male fertility restoration S-type cytoplasm, mitochondrial DNA)

JOHN R. LAUGHNAN, SUSAN GABAY-LAUGHNAN and JOHN E. CARLSON

Department of Genetics and Development
University of Illinois
Urbana, IL 61801

SUMMARY

The association of cytoplasmic reversion of cms-S male-sterile strains to male fertility with disappearance of the S1 and S2 mtDNA plasmids as discrete molecules has been established for all 23 cytoplasmic revertant strains that have been studied so far. This correspondence between mutational step and molecular event provides the first unequivocal evidence that the genetic determination of cms-S male-sterility; male-fertility expression is located in mtDNA.

When cytoplasmic reversion of cms-S strains to the male-fertile condition occurs, at least some, perhaps all, of the S1 and S2 plasmid sequences are transposed and integrated into the main high molecular weight mitochondrial DNA.

These findings are consistent with the model proposed earlier for cytoplasmic reversion that connected it with "fixation" at the cytoplasmic level of an episomal fertility element carried by reversion-prone cms-S strains.

Whether a corresponding transposition and integration of S1 and/or S2 mtDNA sequences into nuclear chromosomes is involved in the nuclear reversions, as called for by the model, is uncertain. If such a correlation were established it would provide the first example in higher plants of inter-organellar transposition of a naturally occurring genetic element.

The S1 and S2 mtDNA plasmids, like the IS elements of bacterial transposons, have terminal inverted repeat sequences that probably equip them for integration into high molecular weight genomes. The possibilities for use of S1 and S2, and other mtDNA plasmids that have been identified in maize, as vehicles in interorganellar gene transfer are discussed briefly.

The frequencies and types of cms-S revertants vary widely among established inbred lines of maize. The results of a conversion experiment presented here confirm that there is a strong influence of nuclear genotype on both cms-S reversion characteristics.

Most inbred lines of maize carrying cms-S exhibit equimolar amounts of the S1 and S2 mtDNA plasmids. Inbred line M825 cms-S versions, however, show a striking reduction in S2 as compared with S1, whereas the reverse situation obtains in cms-S versions of inbred line 38-11. Evidence is presented indicating that these differences, interpreted as differential plasmid replication (or degradation) are under nuclear control.

Analysis of the mitochondrial translation products of six cms-S cytoplasmic revertants indicates that in each case the reversion event was correlated with the disappearance of eight high molecular weight polypeptides normally synthesized by mitochondria isolated from the source cms-S strains. This correlation in behavior, upon cytoplasmic reversion, between S1 and S2 mtDNA plasmids and the high molecular weight polypeptides invites the interpretation that the latter are encoded by S1 and/or S2, for which there is no evidence at this time.

INTRODUCTION

Male sterility in higher plants such as maize involves the failure to produce functional pollen, defined as pollen that is able to accomplish fertilization. Since normal pollen production involves a number of successive developmental events starting with floral initiation and culminating with fertilization, it is not surprising that the male-sterile phenotype may result from a variety of underlying genetic alterations. In most cases that have been investigated the inheritance of the male-sterile phenotype is quite well understood, but only recently, from studies of several male-sterile strains, has there been an approach to an understanding of the molecular basis for this trait. One such strain in maize exhibits cytoplasmic male sterility (*cms*) of the S type and is referred to as *cms-S*. Our goal here is to review the current genetic and molecular evidence from studies on *cms-S* strains, and to a lesser extent on other *cms* strains, in order to obtain a better understanding of the events that lead to manifestations of the *cms-S* phenotype.

BACKGROUND

The occasional appearance of male-sterile individuals in natural populations in higher plant species appears to be a general phenomenon (EDWARDSON 1970). These male-sterile plants could result from recently occurring mutations but in most cases they represent the expression of a mutant gene that has been propagated through numbers of preceding generations.

In maize, as in numbers of other species of plants, two kinds of inherited male sterility are known, genic (nuclear) and

cytoplasmic (extranuclear). In the first case the male-sterile phenotype is determined solely by nuclear genotype, usually a homozygous recessive condition, and inheritance is in strict accordance with Mendelian principles; about twenty such gene loci, designated *ms*, have been identified in maize. In the second case, the basis for male sterility is extranuclear and the trait is transmitted in non-Mendelian fashion according to a pattern of uniparental (maternal) inheritance. As indicated above, these are referred to as *cms* strains. Although crossing experiments indicate that the primary determination of the male-sterile trait in *cms* strains is extranuclear, it is established in many cases that certain nuclear genes, called restorers of fertility (*Rf*), can overrule the male-sterile effect of the cytoplasm. Thus, *cms* plants are phenotypically male-sterile unless they carry an *Rf* nuclear gene that can lead to the production of functional pollen in spite of the presence of male-sterile cytoplasm. In general, restorer genes produce no permanent (heritable) change in the cytoplasm, and may therefore be regarded as suppressors of the *cms* phenotype. An interesting departure from this rule has been described in *Vicia faba* (BOND, FYFE & TOYNBEE-CLARKE 1966) in which a nuclear restorer gene effects a restoration to fertility that appears to persist in succeeding generations.

The first reported case of cytoplasmic male sterility (BATESON & GAIRDNER 1921) involved two strains of flax that gave male-sterile F_2 offspring when crossed in one direction, but not in the other. Since then, *cms* has been found in many higher plant groups. EDWARDSON (1970) reports that *cms* has been identified in 153 species, 51 genera and 22 families of plants. The first case of *cms* in maize was described by RHOADES (1931, 1933). This strain was lost but many discoveries of *cms* in existing strains were later made by maize geneticists and breeders. Studies based on fertility restoration indicate, however, that these many *cms* strains represent only several major categories. In maize, two types of *cms* were early recognized (see DUVICK 1965; EDWARDSON 1970); one of these is *cms-T* (Texas) and the other is *cms-S* (USDA). A third major category, *cms-C* (Charrua), was identified by BECKETT (1971). If other major *cms* groups exist in maize, strains that carry them must occur in extremely low frequency.

The three types of *cms* described above were originally identified on the basis of field studies that indicated differing restoration responses. Thus, male-sterile strains T, P, Q, HA and RS represent independent discoveries of *cms* strains, but all are assignable to the *cms-T* group because they give similar male-fertile or male-sterile responses in the presence of a series of inbred-line nuclear genotypes (BECKETT 1971), indicating that some inbred lines do, and others do not, carry the nuclear restorer genes for the *cms-T* group. On similar criteria, male-sterile strains C, RB and EL, as examples, were placed in the *cms-C* group, and male-sterile strains S, VG, RD, ML, I and J, as examples, were assigned to the *cms-S* group.

There have been many independent discoveries of *cms* in maize strains from isolated sources (DUVICK 1965; BECKETT 1971)

but so far as we are aware there is no established instance of a mutation from normal (male-fertile condition) to *cms* that occurred while a strain was under experimental culture. In the course of propagations of inbred strains, maize geneticists and plant breeders have had virtually unlimited opportunities to observe such mutations and, because of their potential value in hybrid seed production, strong incentive to identify and characterize them; that none has been found suggests that mutations from male-fertile to *cms* in maize are extremely infrequent. Indeed, it is possible that the mutation to *cms-S* has occurred only once in the history of maize, that all of the *cms-S* subgroups descend from that original event, and that minor differences observed between subgroup strains represent evolutionary perturbations. The same might apply to *cms-C* and *cms-T*.

In the case of *cms-T* there are two known gene loci for restoration, designed *Rf* and *Rf2*; they are dominant and both are required for fertility restoration. *Rf* is in chromosome 3 (DUVICK, SNYDER & ANDERSON 1961) and *Rf2* is placed in chromosome 9 (SNYDER & DUVICK 1969). Restoration of *cms-T* is sporophytic since all the pollen produced by an *Rf rf Rf2 rf2* plant is functional even though only one-fourth of the pollen grains from this plant carry both restoring alleles *Rf* and *Rf2*. A restorer gene for *cms-2*, designated *Rf3* is dominant but, in contrast with *cms-T*, it acts at the gametophytic level (BUCHERT 1961) so that the *cms-S Rf3 rf3* plant produces one-half normal and one-half aborted pollen grains, the former carrying *Rf3*, the latter *rf3*. The originally identified *Rf3*, carried by a number of established inbred lines of maize, is located in chromosome 2 (LAUGHNAN & GABAY 1975) but, as indicated below, there is now evidence suggesting that transposable *cms-S* restorer elements may occupy other sites in addition to the standard one in chromosome 2. Restoration of *cms-C* strains is sporophytic and dominant in character, but the location of the *cms-C* restorer(s) is not established.

THE *cms-S* REVERSION PHENOMENON

While induced reversions for male-sterile to male-fertile in *cms-T* strains have been obtained from progeny of plants treated with gamma rays or with EMS (CASSINI et al., 1977), and from plants regenerated from callus grown in tissue cultures with and without *Helminthosporium maydis* race T pathotoxin (GENGENBACH, GREEN & DONOVAN 1977; BRETTELL, THOMAS & INGRAM 1980), so far no spontaneous reversions of either *cms-T* or *cms-S* have been described. On the other hand some *cms-S* strains revert rather frequently to the male-fertile state. The first reported case of this sort (JONES 1956) involving a reversion in inbred line WF9 background was interpreted as a heritable cytoplasmic change but it was not studied intensively. Additional cases of cytoplasmic reversion of *cms-S* in different genetic backgrounds were later reported (SINGH & LAUGHNAN 1972; LAUGHNAN & GABAY 1973). These spontaneous reversions are initially observed in the tassel of the mature plant and they may be expressed either as entirely fertile tassels or as fertile sectors on otherwise sterile tassels. The fertile sectors range

in size from only a portion of one tassel branch up to almost entirely fertile tassel with a single verticle file of sterile tassel branches. On the basis of relatively simple testcross procedures involving the use of pollen from such revertants in crosses into *cms-S* male-sterile tester plants, it was determined that such spontaneous reversions to fertility fall into two classes, those in which the genetic change is extranuclear (cytoplasmic), and those in which the change is in the nucleus. Revertant strains in the first category exhibit uniparental inheritance of the male-fertile trait, which is consistent with a change at the cytoplasmic level from S to normal (male-fertile). Those in the second category exhibit Mendelian inheritance of the male-fertile trait, as though they had, through reversion, acquired a nuclear restorer gene.

To explain these results we postulated (LAUGHNAN & GABAY 1973) the existence of a male-fertility element in the cytoplasm and suggested that it might be identical with the nuclear restorer(s). When the analysis of a sample of 10 independently occurring nuclear revertants revealed that restoring elements in these strains were located in different sites in different chromosomes, we proposed (LAUGHNAN & GABAY 1975; 1978) that S male-sterile plants carry an episomal fertility element F that is capable of being "fixed" either in the cytoplasm (cytoplastic reversion) or in the nucleus (nuclear reversion) leading, in either case, to the manifestation of male fertility.

Cytoplasmic revertants identified by the testcross procedure described above are stable in subsequent generations; among thousands of such propagates we have never observed second-cycle forward mutations to male sterility and must conclude that the cytoplasmic revertant strains are as stable as their corresponding maintainer stocks. The same can not be said for the nuclear revertant strains, however, not because there is evidence that they are unstable but because the homozyhous lethality or semilethality of most such revertants prevents us from making the determination.

MOLECULAR STUDIES RELATING TO *cms-S* AND ITS REVERSION

The development of techniques for the isolation and study of organellar DNA and, in particular, of restriction endonuclease technology, provided an opportunity to search for differences at this level among mutant strains that exhibit extranuclear inheritance. For our purposes such studies carried out with normal (male-fertile) and *cms* maize strains are particularly relevant and the reader is referred here to the LEVINGS & PRING paper published in Volume 10 (1978) of these Symposia for a detailed treatment of this topic. It was shown (LEVINGS & PRING 1976) that mitochondrial DNAs (mtDNAs) from normal and *cms-T* strains of maize exhibit different banding patterns after restriction endonuclease digestion followed by agarose gel electrophoresis, indicating that the mtDNAs of these strains have some differences in nucleotide sequences. It was also shown that these distinctive banding patterns are inherited mater-

nally although occasional transmission of mtDNA through the pollen would be difficult to exclude. An ensuing study (LEVINGS & PRING 1977) indicated that the banding patterns of restriction endonuclease digests of mtDNAs isolated from various normal (male-sterile) maize strains are similar but not identical. It was shown by the same procedures that each of the four maize cytoplasms, that is, normal, *cms-C*, *cms-T and cms-S*, has distinctive mtDNA characteristics, and that chloroplast DNAs from the same sources exhibit only minor differences.

These findings suggested that mtDNA may be the site of the genetic alterations in *cms* strains of maize, and that mtDNA is the carrier of the genetic determiners of male fertility at the cytoplasmic level. This viewpoint was further supported by the discovery that two low molecular weight plasmid-like double-stranded DNA molecules are found in undigested mtDNA preparations from *cms-S* maize but not in mtDNA from *cms-C*, *cms-T* or normal strains (PRING et al. 1977). These plasmids, designated S1 and S2 (formerly S-S and S-F), have molecular weights of 4.1 and 3.5 x 10^6, respectively, are linear molecules and in most *cms-S* strains occur in equimolar amounts. The S1 and S2 plasmids have similar inverted repeat sequences of about 200 base pairs at the termini of the duplex molecules (LEVINGS & PRING 1979). Restriction enzyme mapping studdies (KIM et al. 1981) reveal that S1 and S2 have about 1300 additional base pair sequences in common. The terminal reverse repeat sequences carried by the S1 and S2 mtDNA plasmids of *cms-S* strains are reminiscent of the insertion sequences (IS) described in numbers of transposable elements in prokaryotes. It was inviting therefore to consider that they equip the S1 and S2 plasmids for a similar role in integration, and that the *cms-S* S1 and S2 plasmids are the physical manifestations of the earlier postulated F fertility element.

Collaborative studies involving a number of our cytoplasmic revertant strains have revealed that the S1 and S2 plasmids do indeed play an important role in the reversion event (LEVINGS et al. 1980). Analyses of unrestricted mtDNA from seven cytoplasmic revertant strains indicate that in each case the reversion event was associated with the disappearance or virtual disappearance of the S1 and S2 plasmids. Moreover, restriction enzyme fragment studies (KIM et al. 1980; LEVINGS et al. 1980) of mtDNA from such revertants, coupled with hybridization analyses using ^{32}P-labelled S1 and S2 plasmids as probes, revealed that the reversion of *cms-S* from male-sterile to male-fertile is associated with integration of plasmid sequences into the main mtDNA genome. It also appears from these hybridization studies that independently occurring cytoplasmic reversions of *cms-S* to male fertility are associated with the integration of plasmid sequences at different sites in the high molecular weight mitochondrial genome, this conclusion is based on the interesting finding that restricted mtDNAs from cytoplasmically reverted *cms-S* strains display unique bands that hybridize with labelled S1 and S2 plasmid probes.

In addition to the seven revertant strains discussed above, 16 other cytoplasmically reverted male-fertile strains have recently been analyzed in our laboratory. In every case, the S1

and S2 plasmids present in the *cms-S* male-sterile source strains were found to be absent in the cytoplasmically reverted male-fertile strains. Thus, the association of cytoplasmic reversion with the disappearance of S1 and S2 as unique plasmid species appears to be a general phenomenon. In our view, this close connection between cytoplasmic reversion and mtDNA plasmid disappearance represents the first unequivocal evidence that the genetic determination for *cms-S* male sterility is located in *mtDNA*.

The association of an intramitochondrial transpositional event with cytoplasmic reversion strongly suggests that the S1 and S2 plasmids correspond to the earlier postulated F fertility episome. If so, are the nuclear reversions described earlier associated with corresponding integrations of plasmic sequences into nuclear chromosomal sites? This question is now being investigated in a number of laboratories, including our own, but so far the results are inconclusive.

Additional evidence is now available on the occurence of other lower molecular weight plasmid-like elements in the mtDNA of normal and male-sterile maize. As indicated above, the S1 and S2 plasmids are found in *cms-S* strains and are now implicated in cytoplasmic reversion to fertility (LEVINGS et al. 1980). Mitochondria of all four cytoplasms, that is, normal, *cms-T*, *cms-C*, and *cms-S*, appear to contain a 1.94 kilobase-pair supercoiled circular DNA species (LEVINGS et al. 1980; KEMBLE & BEDBROOK 1980). In addition, normal C and S mitochondria contain a DNA species of about 2.35 kb. pairs that is not present in T cytoplasm; and C mitochondria contain two circular DNA species, having about 1.57 and 1.46 kb. pairs, that are not found in the other cytoplasmic types (KEMBLE & BEDBROOK 1980). All the latter-mentioned plasmid-like species are smaller than S1 (6.5 kb. pairs) and S2 (5.5 kb. pairs). Summarizing, at least six plasmid-like mtDNA species have been identified among the four described maize cytoplasms. Except for S1 and S2, none of these is implicated so far in cytoplasmic reversion from male-sterile to male-fertile phenotype. In fact, it remains to be seen whether the characteristic distribution of the four smaller plasmid species found among the normal and male-sterile mitochondrial genomes is functionally related to the male-fertile and male-sterile phenotypes. Alternatively, these differences in plasmid species may represent independent mutations that have occurred during the isolated evolutionary histories of the various male-sterile strains, and that may therefore have no role in determination of the male-sterile phenotype.

Before departing the subject of mtDNA plasmids in maize we take note of an interesting case of variation in character of the S1 plasmid. Among 44 South American maize accessions whose mtDNA was examined by agarose gel electrophoresis, endonuclease fragment analysis and electron microscopy, twelve strains with unusual plasmid characteristics were identified (WEISSINGER et al. 1981). In each of these strains the mtDNA carried a plasmid that appeared to be identical to the S2 plasmid carried by *cms-S* strains; each also exhibited another plasmid whose molecular weight exceeds that of S1 (7.5 kb. pairs vs. 6.5 kb.

pairs) but which has a high degree of homology with S1 including the terminal inverted repeats. Since male sterility has so far not been identified in these strains, the significance of the presence of the S2-like plasmid and of the modified S1 plasmid in these sources remains obscure, but it is anticipated that further studies of these strains may provide important clues to the molecular basis for male sterility in S-type cytoplasms.

Are the differences in mtDNA exhibited by the several *cms* strains of maize reflected in unique patterns of messenger RNA and protein synthesis? In particular, do plasmids such as S1 and S2 have coding function, and are they associated with identifiable functional products? Very little is known about altered transcriptional patterns in *cms* maize strains but such studies are under way. Meanwhile, studies involving biosynthesis in isolated mitochondria (FORDE, OLIVER & LEAVER 1978; FORDE et al. 1980) have identified discrete differences in mitochondrially synthesized polypeptides in normal maize and in the several cytoplasmic male-sterile strains, in both their restored and nonrestored versions. Mitochondria from each of the male-sterile cytoplasms C, T, and S synthesize unique polypeptides, and in the case of *cms-S*, mitochondria isolated from both unrestored and restored strains synthesize eight characteristic high-molecular weight polypeptides that are not synthesized by mitochondria from *cms-T*, *cms-C* or normal male-fertile strains. Recent analyses of six cytoplasmic male-fertile revertant strains originating from *cms-S* male-sterile sources in our laboratory indicate (LEAVER, personal communication) that mitochondria isolated from these revertant strains no longer synthesize the eight high molecular weight polypeptides characteristically produced by *cms-S* mitochondria. There can be little doubt that these polypeptide are specified by mtDNA and it is exciting to speculate that they are encoded by the S1 and/or S2 plasmids of *cms-S* mtDNA. According to LEAVER (personal communication) the S1 and S2 plasmids are transcribed, and they carry sufficient nucleotide information to code for the eight *cms-S* polypeptides, but at this time there is no direct evidence of this relationship. Sequence information on both plasmids and polypeptides would, of course, provide unequivocal evidence for or against this hypothesis, but these data are not available. In any case, the finding of what appears to be an absolute connection between S1 and S2 mtDNA plasmids and eight unique polypeptides in *cms-S* strains provides the first evidence of a mtDNA-protein product association in this system and we may expect that its further study will enhance our understanding of the functional basis for S-type male sterility.

NUCLEAR CONTROL OVER *cms-S* REVERSION

Suppression of the cytoplasmic male-sterile phenotype by nuclear genes (restorers) is widely encountered in high plants, but we were surprised at the extent to which *cms-S* reversion is subject to nuclear influence. It has been known for some time that reversions to male fertility in *cms-S* strains occur more frequently in some inbred line backgrounds than in others (LAUGHNAN & GABAY 1975, 1978). For example, in inbred line

M825, crosses of *cms-S* female parents with isogenic nonrestoring (maintainer) plants produce progenies in which about 11 percent of the plants exhibit tassel fertility; most of these (90%) occur as fertile sectors in the tassel while fewer (10%) occur as exceptional plants with entirely fertile tassels. On the other hand, most *cms-S* male-sterile inbred lines we have examined exhibit a relatively low frequency of reversion.

Among the genetic backgrounds that exhibit rather high frequencies of reversion there are striking differences in the proportions of these that are cytoplasmic versus nuclear in origin. The reversions in inbred line M825, mentioned above, are primarily cytoplasmic (95%), but in inbred line WB4 they are predominantly nuclear in origin. In a third *cms-S* strain that exhibits a high frequency of reversion, the M825/Oh07 singlecross hybrid, cytoplasmic and nuclear reversions occur with about equal frequencies.

Since the inbred lines examined for reversions represent striking differences in nuclear genotype but carry a common *cms-S* cytoplasm, it is already apparent from these observations that nuclear genes must exert some considerable influence over the frequency and type of reversion event. In order to get a better idea of the relative influence of cytoplasm and nucleus on both the frequency and nature of the reversion event, we undertook a backcrossing experiment involving a number of inbred lines carrying different subgroups of S cytoplasm as the nonrecurrent female parents, and the inbred line M825, whose nuclear background apparently promotes high-rate reversion, as the recurrent male (maintainer) parent. Several inbred lines that do not restore *cms-S*, that is WF9, 38-11, N6, K55, Ml4, Il53, and IllA, and five subgroups of S cytoplasm, S, VG, I, ML and RD, were represented among the nonrecurrent female parents. In all, 14 different line-cytoplasm combinations were employed (see Table 1 for pedigrees). Each of these, as a male-sterile inbred line, is relatively stable, with reversion frequencies ranging from 0.0 to 2.5%. By contrast, 10.9% of M825 *cms-VG* (a subgroup of *cms-S*) plants exhibit reversion.

The conversion program referred to above was undertaken without selection for high or low reversion rate, male-sterile plants in each generation being selected at random for crosses by M825 to produce the successive backcross progenies. The experiment is now in the tenth backcross. In 1978 the second backcross (3 crosses with M825) and sixth backcross (7 crosses with M825) generations were grown in numbers and plants were scored for reversion events. Plants from the 14 inbred line-cytoplasm combinations, representing the original nonrecurrent parents (no crosses with M825) were also searched for reversions. On the basis of pollen records and subsequent analysis of the revertants it was determined whether the change occurred at the cytoplasmic or nuclear level. For some revertants it was not possible to make this determination.

Continued backcrossing by the M825 male parent maintainer line is expected to produce plants that have most of the nuclear genes of M825 (high frequency reversion), while retaining

the cytoplasmic genotypes of the original nonrecurrent S male-sterile inbred lines (low frequency reversion). This provides the opportunity to determine the relative influence of cytoplasm and nucleus on reversion rates. Table 1 summarizes data on reversion frequencies in the original line-cytoplasm sources, and Tables 2 and 3 present corresponding data for the 2nd and 6th backcross generations, respectively.

Table 1. Frequencies and types of male-fertile revertants in inbred lines representing fourteen line-cytoplasm combinations of source pedigrees in the conversion experiment.

Inbred line	*cms*-type cytoplasm	Number of plants analyzed	Male-fertile revertants				
			Number	Type[1]			%
				N	SS	No test	
WF9	S	201	0	0	0	0	0.0
	Vg	93	1	0	1	0	1.1
	I	227	1	0	0	1	0.4
	ML	165	4	2	1	1	2.4
	RD	244	2	0	0	2	0.8
38-11	S	158	4	0	4	0	2.5
	VG	240	2	0	2	0	0.8
N6	S	118	0	0	0	0	0.0
	VG	173	0	0	0	0	0.0
K55	VG	360	0	0	0	0	0.0
M14	S	241	1	0	1	0	0.4
	ML	193	1	0	1	0	0.5
I153	VG	199	0	0	0	0	0.0
I11A	S	361	0	0	0	0	0.0
		2973	16	2	10	4	0.54

[1] Pollen records: N = all pollen normal (cytoplasmic reversion indicated); SS = semisterile, ca. 50% aborted pollen (nuclear reversion indicated); pollen records could not be obtained for 4 of the revertants.

Table 2. Frequencies and types of male-fertile revertants in the 2nd backcross generation produced by crossing the Table 1 inbred line sources as nonrecurrent female parents by the recurrent M825 male parent.

Inbred line	*cms*-type cytoplasm	Number of plants analyzed	Male-fertile revertants				
			Number	Type[1]			%
				Cyto-plasmic	Nuclear	No Test	
WF9	S	159	1	0	1	0	0.6
	VG	106	0	0	0	0	0.0
	I	101	4	1	2	1	4.0
	ML	95	5	5	0	0	5.3
	RD	100	7	6	0	1	7.0
38-11	S	120	2	0	0	2	1.7
	VG	102	0	0	0	0	0.0
N6	S	201	2	0	0	2	1.0
	VG	107	3	1	0	2	2.8
K55	VG	306	5	2	1	1	1.6
M14	S	312	1	0	0	1	0.3
	ML	141	1	1	0	0	0.7
I153	VG	127	2	1	0	1	1.6
I11A	S	309	15	8	4	3	4.9
Totals		2286	48	25	8	15[2]	2.10

[1] On the basis of progeny tests, 25 revertants were confirmed as cytoplasmic and 8 as nuclear in origin.

[2] Progeny tests could not be obtained for the remaining 15 cases, but pollen readings available for 11 of these revertants indicate that 3 were cytoplasmic and 8 were nuclear in origin.

The data indicate that after 3 generations of crosses with M825 the mean reversion frequency is increased almost four-fold, from 0.54% (16/2973) in the inbred line backgrounds to 2.10% (48/2286) in the 2nd backcross progenies. After four more crosses with M825, in the 6th backcross generation, the rever-

sion frequency has risen to 8.27% (169/2044), which represents a 15-fold increase over the mean rate in inbred line backgrounds. Contingency tests indicate that the difference in rates between the inbred line sources and 2nd backcross progeny,

Table 3. Frequencies and types of male-fertile revertants in the 6th backcross generation produced by crossing the Table 1 inbred line sources as nonrecurrent female parents by the recurrent M825 male parent.

Inbred line	*cms*-type cytoplasm	Number of plants analyzed	Male-fertile revertants				
			Number	Type[1]			%
				Cyto-plasmic	Nuclear	No test	
WF9	S	140	4	3	0	1	2.9
	VG	135	19	11	0	8	14.1
	I	100	7	5	0	2	7.0
	ML	72	6	5	0	1	8.3
	RD	112	23	16	1	6	20.5
38-11	S	166	9	7	0	2	5.4
	VG	20	1	1	0	0	5.0
N6	S	38	3	2	0	1	7.9
	VG	159	25	17	0	8	15.7
K55	VG	254	26	20	1	5	10.2
M14	S	221	8	6	0	2	3.6
	ML	196	19	18	1	0	9.7
I153	VG	162	10	8	0	2	6.2
I11A	S	269	9	6	1	2	3.3
Totals		2044	169	125	4	40[2]	8.27

[1] On the basis of progeny tests, 125 revertants were confirmed as cytoplasmic and 4 as nuclear in origin.

[2] Progeny tests could not be obtained for the remaining 40 cases, but pollen readings available for 32 of these revertants indicate that 29 were cytoplasmic and 3 were nuclear in origin.

as well as between the 2nd and 6th backcross progenies, are highly significant. While statistical analysis indicates that the value 8.27% for the 6th backcross generation is still significantly lower than the previously established reversion rate of 10.9% for the *cms-VG* M825 strain, it closely approaches it. Moreover, two of the 14 pedigrees, WF9-RD and N6-VG, after 7 crosses with M825 (Table 3) had reversion rates that were significantly higher than the M825 strain, and three others had rates that were not significantly different from M825.

Table 4. Mean reversion rates in inbred lines and in progenies resulting from the 2nd and 6th backcrosses with M825 as recurrent male parent.[1]

Generation	Number of male-sterile plants scored	Number of Revertants	Reversion frequency %
Inbred lines	2973	16	0.54
2nd backcross	2286	48	2.10
6th backcross	2044	169	8.27

[1] 14 line-cytoplasm combinations were involved as nonrecurrent female parents and as the sources of cytoplasms in ensuing backcross generations (See Tables 1, 2 and 3).

To summarize (Table 4), the near replacement of the inbred line nuclear genomes of 14 line-cytoplasm sources with the M825 nuclear genome results in an increase in spontaneous reversion rate from below 1% to an average rate of around 8%, which is close to the frequency characteristic of the M825 strain itself. The results indicate a preponderant influence of the M825 nuclear genome on reversion frequency.

The pedigrees involving inbred line N6 provide what is perhaps the most dramatic evidence of nuclear control over reversion frequency. Table 1 indicates that no revertants were found among a total of 291 plants in the N6-S and N6-VG male-sterile inbred line pedigrees. We have also looked for reversions in other N6 *cms-S* pedigrees not involved in the conversion experiment including the I, ML, RD and J forms; no reversions were found among the 654 additional plants scored. So the N6 pedigrees are the most stable we have encountered so far. By contrast, in the 6th backcross M825-converted progenies (Table 3), there were 29 reversions among 197 plants in the N6-S and N6-VG pedigrees; the reversion frequency, 14.2%, is the highest encountered among the 7 inbred lines involved in the experiment.

There can be no doubt that this shift from the stable condition to high-rate revertability is the result of nuclear gene substitution in the course of conversion.

The M825-VG strain, whose maintainer counterpart was used as the recurrent parent in this experiment, produces overwhelmingly cytoplasmic reversions, these being estimated at 95% of the revertants in experiments involving in excess of 100 reversion events. As indicated in Table 1, a minority of the reversions (2/12=16.7%) occurring spontaneously in the inbred line male-sterile sources are cytoplasmic. After three crosses with M825 (Table 2) the proportion of cytoplasmic revertants rises to 75.8% (25/33), and after four more M825 crosses (Table 5) the proportion of revertants that are cytoplasmic, 96.9% (125/129), has reached or exceeded the frequency in the M825-VG strain itself.

Table 5. Origin of male-fertile revertants summarized in Table 4.

Generation	Number of[1] revertants analyzed	Number of cytoplasmic revertants	Number of nuclear revertants	% cytoplasmic revertants
Inbred lines[2]	12	2	10	16.7
2nd backcross[3]	33	25	8	75.8
3rd backcross[3]	129	125	4	96.9

[1] Not all revertants could be analyzed. [2] Cytoplasmic vs. nuclear origin of revertants determined by pollen analysis. [3]Cytoplasmic vs. nuclear origin of revertants established by progeny test.

It should be noted that this shift in proportion of cytoplasmic to nuclear revertants results from both an increase in frequency of cytoplasmic cases and a decrease in frequency of nuclear cases. Note also that the 14 male-sterile sources used as the nonrecurrent parents in the conversion (Table 1) probably differ in reversion characteristics. For example, no reversions have yet been identified in six of these sources, and in the remaining eight sources reversion frequencies range from 0.4% to 2.5%, which is to say that the total numbers of cases in individual sources are too few to characterize them in regard to a cytoplasmic versus nuclear origin. In the 2nd and 6th backcross progenies (Tables 2 and 3) the origins of a substantial number of revertants could not be established by progeny test, but the pollen records available for most of these revertants (footnote 2, Tables 2 and 3) again indicate a marked shift in favor of cytoplasmic revertants with increasing numbers of M825 backcrosses.

In summary, the data indicate that the nuclear genome has a predominant influence not only over the frequency of the reversion event, but over the site (cytoplasm vs. nucleus) of reversion as well.

NUCLEAR CONTROL OVER REPLICATION OF THE S1 AND S2 mtDNA PLASMIDS

In most inbred lines of maize carrying one or another version of S-type cytoplasm the S1 and S2 mtDNA plasmids occur in equimolar amounts. This was the finding of PRING et al. (1977) in a study that involved eight different inbred lines and five different subgroups of S-type cytoplasm. Restriction endonuclease analyses indicated that the replication of these plasmids is amplified about five-fold in comparison with the main high molecular weight mitochondrial genome. Seven other inbred lines, carrying a number of versions of S-type cytoplasm, that were investigated in our laboratory were also found to have equimolar amounts of S1 and S2.

Recently an interesting exception to this pattern was reported (LEVINGS et al. 1980). Agarose gel electrophoresis analysis of the mtDNA from a *cms* control source in this study revealed a striking reduction in the amount of the S2 plasmid compared with S1. The control source was the single-cross hybrid

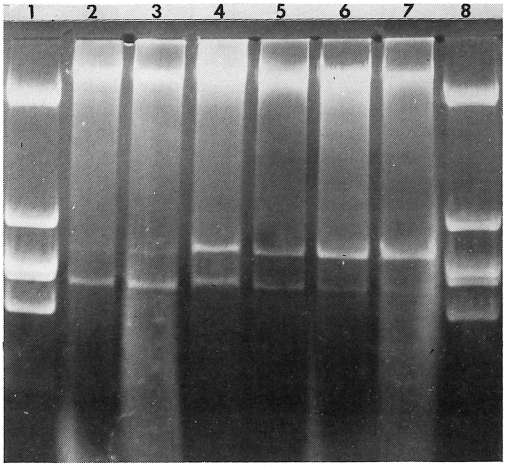


Figure 1. Agarose gel electrophoresis patterns of undigested DNA from crude mitochondrial lysates prepared from 38-11-S (lane 2), 38-11-VG (lane 3), W23-S (lane 4), W23-VG (lane 5), M825-S (lane 6) and M825-VG (lane 7). Corresponding lysates of mitochondria from the normal cytoplasm versions of 38-11, W23 and M825 (not shown here) do not exhibit the S1 and S2 plasmids. Lane 1 and 8 exhibit marker fragments resulting from EcoR1 digestion of lambda virus DNA.

M825/Oh07 and it carried VG-type cytoplasm which is another member of the *cms-S* group that we have employed extensively in reversion studies. This reduction in the relative amount of S2 was at first attributed to the cytoplasmic background (*cms-VG*). The studies indicating that cytoplasmic reversion is strongly influenced by nuclear genotype, however, led us to further investigate this difference, and we have shown recently that M825 strains involving two other S-type cytoplasms, *cms-S* and *cms-RD*, also exhibit reduced levels of S2, so it seems reasonable to conclude that the reduction in amount of S2 plasmid, in comparison with S1, is not due to *cms-VG* as opposed to *cms-S*, but is characteristic of the M825 inbred line, and is therefore almost certainly under nuclear control. Measurements of the S1 and S2 plasmids in mtDNA isolated from M825 S-male-sterile plants indicate a five-fold difference in the amounts of these two plasmids. This is illustrated in Figure 1 where electrophoretograms of unrestricted mtDNA from M825 *cms-S* and *cms-VG* strains may be compared with those from inbred line W23 *cms-S* and *cms-VG* strains which characteristically exhibit equimolar amounts of the S1 and S2 plasmids.

We recently discovered another instance in which the nuclear genotype influences differential replication of the S1 and S2 mtDNA plasmids. In this case, the inbred line 38-11 is involved and the situation is reversed; measurements of amounts of plasmids in mtDNA isolated from 38-11 *cms-S* and *cms-VG* strains indicated a reduction in S1 so that the amount of S2 exceeds that of S1 by a factor of 3 or more. This is shown in Figure 1, where comparisons may be made with electrophoretograms of mtDNA isolated from the corresponding M825 and W23 strains mentioned previously.

Recent experiments carried out in our laboratory provide additional evidence that S1 and S2 plasmid replication is influenced by nuclear genotype. Agarose gel electrophoresis studies of unrestricted mtDNA from various *cms-S* strains recurrently back-crossed with inbred line M825 (see preceding section) confirm the role played by the nucleus in general, and by inbred line M825 in particular, in differential plasmid replication. When inbred line WF9 *cms-RD*, whose mtDNA exhibits equimolar amounts of S1 and S2 plasmids (Figure 2, lane headed 0), is crossed with M825 as the recurrent male parent, there is a decrease in proportionate molar amounts of S2 as compared with S1 (Figure 2, lanes headed 0-4, and lane headed 8). Microdensitometer measurements of the S2 mtDNAs indicate that the first cross of WF9-RD x M825 produces no reduction in the amount of S2 (compare lanes headed 0 and 1), that a second cross with M825 produces an intermediate level of S2 (see lane headed 2), and that after the third M825 cross the amount of S2 reaches a level typical of that for *cms-S* versions of M825 itself (see lane headed 3), after which additional crosses by M825 produce no further diminution in amount of the S2 plasmid. The delay in S2 response observed in progeny from the first generation cross with M825, as compared with progeny from later crosses, may be due to dominance of the nuclear gene(s) controlling this plasmid replication characteristic; alternatively, it could represent a delay in response of mitochondrial elements to a changed genotype.

Studies on the responses of the S1 and S2 mtDNA plasmids of *cms-S* strains to changes in nuclear genotype have also been carried out with inbred lines IllA *cms-S* and 38-11 *cms-S* as nonrecurrent female parents and inbred line M825 as the recurrent male parent. In the case of IllA-S which, like WF9-RD, has equimolar amounts of S1 and S2, successive crosses with M825 produce corresponding decreases in amounts of the S2 plasmid relative to S1, the final result being similar to that of the WF9-RD conversion crosses described above. A similar first-generation lag phenomenon was also observed in the IllA-S conversion experiment.

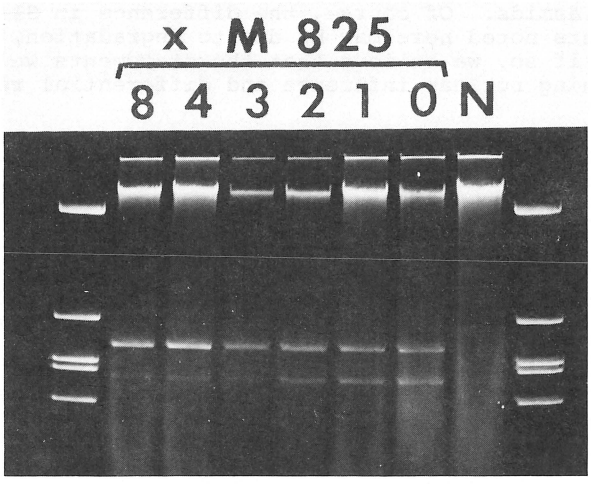


Figure 2. The effect of nuclear substitution on relative amounts of S1 and S2 mtDNA plasmids. Agarose gel electrophoresis patterns of undigested DNA from crude mitochondrial lysates prepared from: SF9 inbred line with normal cytoplasm (lane N), WF9-RD (lane 0), WF9-RD as nonrecurrent parent crossed in conversion program 1, 2, 3, 4 and 8 times with recurrent male parent M825 (lanes 1, 2, 3, 4 and 8, respectively). The outside lanes exhibit the characteristic EcoRl digestion fragments of lambda phage DNA.

The 38-11 *cms-S* conversion is somewhat more interesting because, as indicated earlier, *cms-S* versions of this inbred line have reduced amounts of the S1 plasmid relative to those of S2. After the first cross of 38-11-S with inbred line M825 as male parent, there is little change in the S2>S1 plasmid situation characteristic of 38-11-S, which again is consistent with a first generation lag in response. After the second cross with M825 the S1 and S2 plasmids have reached roughly equimolar status, which persists after the third M825 cross as well. A relative diminution in S2 is noted among progeny of the fourth M825 cross, and after five crosses with M825 the 5:1 ratio for S1:S2 that is characteristic of M825 *cms-S* strains has been reached.

The studies indicating an influence of nuclear genotype on

replication of the S1 and S2 mtDNA plasmids do not, of course, provide details on how that control is exercised at the molecular level. They do tell us, however, that replication of both S1 and S2 is subject to some control by nuclear genes, and that in different genotypes replication of either S1 or S2 may be suppressed relative to the other. These observations, combined with those from the conversion experiments described above, indicate that with some genotypes the responses are differential rather than coordinate as they appear to be in most inbred line backgrounds where equimolar amounts of S1 and S2 are encountered. Presumably S1 and S2 have unique sequences at a site or sites, that, with certain genotypes, lead to differential replication of the two plasmids. Of course, the difference in S1 and S2 plasmid amounts noted here may be due to degradation, rather than replication; if so, we believe that the statements we have made above concerning nuclear influence and differential response still apply.

It is tempting to consider that the striking reduction in amount of the S2 plasmid in the mtDNA of M825 *cms-S* strains is somehow related to the high reversion rate characteristic of this inbred line background. It may be that there is an equilibrium between autonomous and integrated plasmid sequences in *cms-S* strains, and that in the M825 nuclear background this equilibrium is shifted to favor the integrated state for S2. This in turn may increase the likelihood of integration events leading to loss of the plasmids as identifiable free mtDNAs and to associated reversion to male fertility at the plant phenotypic level. On this scheme the first generation lag phenomenon described above could represent an epigenetic effect based on a delay, at the level of plasmid synthesis, in attainment of a changed equilibrium called for by a changed genotype. Of course this is speculation and even so it does not approach an answer to the more fundamental question of the underlying molecular and functional bases for reversion. Even after all the desired nucleotide sequencing has been done, and gene products have been identified, there will still be questions concerning which products are involved in the *cms-S* male-sterility:male-fertility system and how they function in the expression of male fertility in maize.

CONCLUDING STATEMENTS

The studies indicating an influence of nuclear genotype on plasmid replication indicate that other mtDNA elements may also be subject to nuclear regulation and that we may expect the same for the chloroplast genome in plants. This suggests that caution be exercised in drawing conclusions about differences in mtDNA (or chloroplast DNA) patterns in cases where the DNAs are isolated from sources that are not isogenic at the nuclear level. The hazard involved is surely greatest when comparisons are made between different but related species, where nuclear evolution would be expected to have gone, so to speak, hand in hand with organellar evolution. In numbers of such studies observed differences in mtDNA characteristics have formed the basis for conclusions about evolution of mitochondrial genomes with no attention paid to differing nuclear genotypes.

We have called attention here to the evidence (LEVINGS et al. 1980) that cytoplasmic reversion of *cms-S* strains is associated with disappearance of the S1 and S2 plasmids to separate mtDNA entities and with transposition of at least some plasmid sequences into the high molecular weight mtDNA of revertant strains. We have also presented evidence supporting the conclusion that the nuclear genotype has a decided influence on replication (or degradation) characteristics of the S1 and S2 plasmids and plays a major role in determination of the frequency and type of the *cms-S* reversion event. It is apparent therefore that the molcular events associated with cytoplasmic reversion are influenced by nuclear genes. It is possible that such genes specify proteins that facilitate transposition but at this time there is only circumstantial evidence to support this notion; the S1 and S2 plasmids, like the IS elements of bacterial transposons, carry terminal inverted repeats.

Based on the evidence presented here, nuclear reversion events are also strongly influenced by nuclear genotype, but in this case the corresponding evidence for a connection between reversion to male fertility and underlying molecular events is not available. It is worth reviewing why, on the basis of genetic field studies alone, the cytoplasmic and nuclear revertants were considered to have a common origin (LAUGHNAN & GABAY 1973; 1978). For one thing, most of the nuclear revertant strains carried similar deleterious side effects that were associated with the newly-arisen restorer loci and this was easier to reconcile with the idea that these reversions resulted from a common male-fertility element with episomal properties, than with the presumption that the reversions occurred as mutations at scattered pre-existing restorer gene loci. A common origin for both types of reversions was also suggested by the observation that in some *cms-S* strains both kinds of male-fertile exceptions occurred, and that both could occur initially as plants with entirely fertile tassels or as plants with fertile sectors in their tassels. Finally, in several cases of reversion, genetic analyses of the original male-fertile revertant indicated that it involved both cytoplasmic and nuclear events, thus providing additional evidence that these two are associated. We have since encountered additional male-fertile exceptions that support the idea of a common origin for the two kinds of reversions and for this reason, and the other cited above, we feel there are still compelling reasons to test this hypothesis at the molecular level. One obvious approach is to employ labelled probes carrying S1 and/or S2 plasmid sequences in attempts to identify homologous sequences in nuclear DNA from strains carrying nuclear revertants; another is to use similar probes in *in situ* hybridization experiments involving nuclear revertant strains in which the chromosomal location of the newly-arisen restorer gene has been established, and in which a closely-linked cytological marker, such as a translocation breakpoint, is provided. Such experiments are underway in our laboratory.

As suggested above the terminal reverse repeat sequences carried by the S1 and S2 mtDNA plasmids may well be the basis for transposition of plasmid sequences into high molecular weight mtDNA in association with cytoplasmic reversion to male fertil-

ity. If it is shown that a similar event involving transposition of S1 and/or S2 sequences into nuclear chromosomal sites occurs in association with nuclear reversion, various other opportunities are presented. An established case involving transposition of a naturally occurring genetic element from mitochondrion to nucleus would suggest the use of the S1 and S2 plasmids, and perhaps other plasmids that have now been identified in maize mtDNA, as vehicles for DNA transfer in maize and probably other plant species as well. Their use as such in experiments designed to improve plant productivity, and to gain a better understanding of organelle biogenesis and evolution, would be greatly enhanced if it were established that they are capable of interorganellar transposition.

ACKNOWLEDGEMENTS

These investigations were supported in part by research grants from the Department of Energy #DE-AC02-81ER10873, Pioneer Hi-Bred International, Inc., Johnston, Iowa, and Illinois Foundation Seeds, Inc., Tolono, Illinois.

The authors express appreciation to Janet Day for technical assistance with field and laboratory studies, and to Susan Harvey who assisted with the laboratory analysis of some of the cytoplasmic revertants.

* John E. Carlson is currently Graduate Research Assistant in the School of Life Sciences, University of Illinois, Urbana, Illinois.

This presentation is dedicated to Victor, whose memory sustains us.

LITERATURE CITED

BATESON, W., and A. E. GAIRDNER 1921 Male-sterility in flax. J. Genet. 11: 269-275.

BECKETT, J. B. 1971 Classification of male-sterile cytoplasms in maize (*Zea mays L.*). Crop Science 11: 724-727.

BOND, D. A., J. L. FYGE and G. TOWNBEE-CLARKE 1966 Male sterility in field beans (*Vicia faba L.*) III. Male sterility with a cytoplasmic type of inheritance. J. Agr. Sci. Camb. 66: 359-367.

BRETTELL, R. I. S., E. THOMAS and D. S. INGRAM 1980 Reversion of Texas male-sterile cytoplasm maize in culture to give fertile, T-toxin resistant plants. Theor. Appl. Genet. 58: 55-58.

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

CASSINI, R., A. CORNU, A. BERVILLE, E. VUILLAUME and A. PANOUILLE 1977 Hérédité et caractéristiques des sources de resistance à *Helminthosporium maydis* race T, obtenues par mutagenèse chez des mais à cytoplasme mâle stérile Texas. Ann. Amélior. Plant. 27: 753-766.

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

DUVICK, D.N., R. J. SNYDER and E. G. ANDERSON 1961 The chromosomal location of Rf_1, a restorer gene for cytoplasmic pollen sterile maize. Genetics 46: 1245-1252.

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

FORDE, B. G., and C. J. LEAVER 1980 Nuclear and cytoplasmic genes controlling synthesis of variant mitochondrial polypeptides in male-sterile maize. Proc. Natl. Acad. Sci. USA 77: 418-422.

FORDE, B. G., R. J. C. OLIVER and C. J. LEAVER 1978 Variation in mitochondrial translation products associated with male-sterile cytoplasms in maize. Proc. Natl. Acad. Sci. USA 75: 3841-3845.

FORDE, B. G., R. J. C. OLIVER, C. L. LEAVER, R. E. GUNN and R. J. KEMBLE 1980 Classification of normal and male-sterile cytoplasms in maize I. Electrophoretic analysis of variation in mitochondrially synthesized proteins. Genetics 95: 443-450.

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

JONES, D. F. 1956 Genic and cytoplasmic control of pollen abortion in maize. Genetics and Plant Breeding. Brookhaven Symp. Biol. 9: 101-112.

KEMBLE, R. J. and J. R. BEDBROOK 1980 Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm. Nature 284: 565-566.

KIM, B. D., M. F. CONDE, S. J. GABAY-LAUGHNAN, J. R. LAUGHNAN, C. S. LEVINGS, III, R. J. MANS and D. R. PRING 1980 Integration of plasmid sequences into mitochondrial chromosomal DNA upon reversion of S-type cytoplasmic male-sterile plants to fertility in maize. In Mobilization and Reassembly of Genetic Information, W. A. Scott, R. Werner, D. R. Joseph and J. Schultz (eds.). Academic Press, New York.

KIM, B. D., R. J. MANS, M. F. CONDE, D. R. PRING and C. S. LEVINGS, III (1981) Physical mapping of homologous segments of mitochondrial episomes from S male-sterile maize (submitted).

LAUGHNAN, J. R. and S. J. GABAY 1973 Mutations leading to nuclear restoration of fertility in S male-sterile cytoplasm in maize. Theor. Appl. Genet. 43: 109-116.

LAUGHNAN, J. R. and S. J. GABAY 1975 An episomal basis for instability of S male sterility in maize and some implications for plant breeding. In Genetics and the Biogenesis of Cell Organelles, C. W. Birky, Jr., P. S. Perlman and T. J. Byers (eds.). Columbus: Ohio State Univ. Press.

LAUGHNAN, J. R. and S. J. GABAY 1978 Nuclear and cytoplasmic mutations to fertility in S male-sterile maize. In Maize Breeding and Genetics, B. D. Walden (ed.). John Wiley, New York.

LEVINGS, C. S., III, and B. D. KIM, D. R. PRING, M. F. CONDE, R. J. MANS, J. R. LAUGHNAN and S. J. GABAY-LAUGHNAN 1980 Cytoplasmic reversion of *cms-S* in maize: Association with a transpositional event. Science 209: 1021-1023.

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.

LEVINGS, C. S., III and D. R. PRING 1978 The mitochondrial genome of higher plants. Stadler Genetics Symposia 10: 77-93.

LEVINGS, C. S., III and D. R. PRING 1979 Molecular bases of cytoplasmic male sterility in maize. In Physiological Genetics, J. G. Scandalios (ed.). Academic Press, New York.

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.

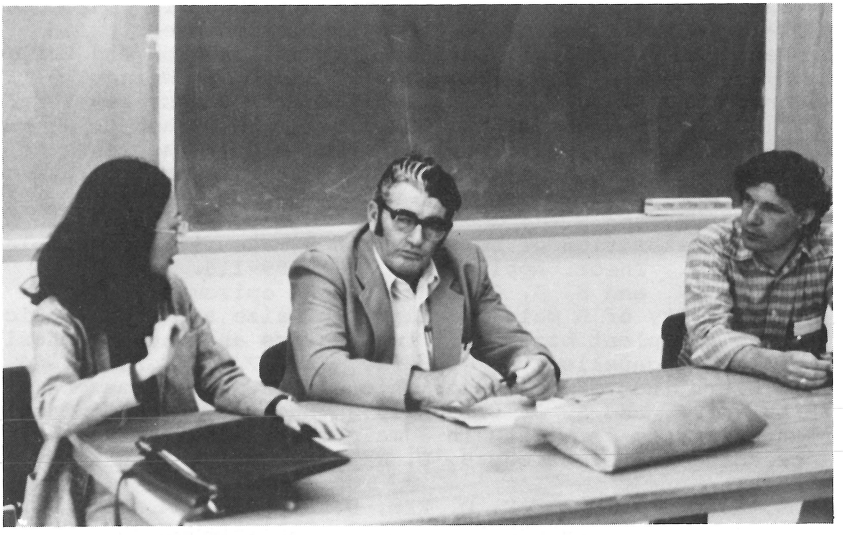
RHOADES, M. M. 1931 Cytoplasmic inheritance of male sterility in *Zea mays*. Science 73: 340-341.

RHOADES, M. M. 1933 The cytoplasmic inheritance of male sterile ity in maize. J. Genet. 27: 71-93.

SINGH, A. and J. R. LAUGHNAN 1972 Instability of S male-sterile cytoplasm in maize. Genetics 71: 607-620.

SNYDER, R. J. and D. N. DUVICK 1969 Chromosomal location of Rf_2, a restorer gene for cytoplasmic pollen sterile maize. Crop. Sci. 9: 156-157.

WEISSINGER, A. K., D. H. TIMOTHY, C. S. LEVINGS, III, W. W. L. HU and M. M. GOODMAN 1981 Plasmid-like mitochondrial DNAs from Latin American maize races. Maize Genet. Coop. News Letter 55: 84-86.



Frances Burr, John Laughnan and Stuart Kauffman