Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7363–7366, October 1986
Genetics

Mitochondrial DNA changes in abnormal growth (nonchromosomal stripe) mutants of maize

(mitochondrial mutants)

KATHLEEN J. NEWTON* AND E. H. COE, JR.†

*Division of Biological Sciences, University of Missouri, and †U. S. Department of Agriculture and Department of Agronomy, Columbia, MO 65211

Communicated by E. R. Sears, June 4, 1986

ABSTRACT **The genetic analysis of higher plant mitochondria has been limited by a scarcity of identified mutations with known progenitors. Correspondingly, few molecular studies have been directed at types of plant mitochondrial variation other than cytoplasmic male sterility. The maternally inherited nonchromosomal stripe (NCS) mutants of maize have profound deleterious effects on plant growth and yield. We report specific alterations in mitochondrial DNA (mtDNA) for two independent, phenotypically distinct NCS mutants. NCS2 plants have a distinctive 21-kilobase *Xho* I mtDNA band and very reduced amounts of DNA in an 8-kilobase band that is present in the progenitor. NCS3 plants have a distinctive 20-kilobase *Xho* I band and a reduction in a 16-kilobase band. Our studies confirm that the affected organelle in NCS plants is the mitochondrion. Because NCS-type plants appear with a certain frequency in a particular line (WF9), this line is a potential source of additional mutations for functional and molecular analyses of maize mitochondrial genes.**

It is now well established that phenotypic variability can result from alterations in organelle genomes. Several examples have been described for lower eukaryotes, such as yeast and fungi. They include mitochondrial DNA (mtDNA) changes that correlate with premature senescence, altered colony size and morphology, and aberrant patterns of growth (1–4).

Previously, much of the research on mitochondrial variability in higher plants has focussed on cytoplasmic male sterility (CMS), a trait usually associated with mtDNA rearrangements (5, 6). In contrast with the work on CMS in maize, little effort has yet been expended on the study of other types of putative mitochondrial mutations. Nonchromosomal stripe (NCS) describes a set of maternally inherited mutations in maize, characterized by striking and variable phenotypic effects including poor growth, abnormal morphologies, and leaf striping. First extensively studied by Shumway and Bauman (7), NCS arose in a particular line of maize, WF9, carrying the T-type male-sterile cytoplasm (cms-T). Although the original NCS mutation has been lost, two other mutations, known as NCS2 and NCS3, were discovered by Bauman in T-cytoplasmic versions of inbred WF9 (NCS3) and the related line, H49 (NCS2) (8).

The results from crosses using NCS plants as either the male or female parent showed that NCS traits are maternally inherited (7, 8). Although the generation of NCS mutations may depend on the nuclear genotype, the mutations are subsequently inherited strictly through the female parent. The phenotypic expression of the NCS2 and NCS3 mutations was not suppressed in crosses with several inbred lines: Ky21, K55, W23, Mo17, Tr, Oh51a, and A619 (8). The effects of fertility-restoring genes on NCS phenotypes were tested by crossing NCS2 and NCS3 plants by pollen from the Ky21 line, which carries dominant nuclear genes that restore pollen shedding to cms-T plants. The nuclear genes that suppress the male-sterile phenotype had no effect on the expression of either NCS2 or NCS3 in the resulting progeny (8).

Both NCS2 and NCS3 can drastically affect plant height, vigor, and yield (8). In each case, an individual cross generates a continuum of phenotypes, which appear to be quantitatively different sectorial expressions of the same basic defect. Such a situation could arise if the mutant plants actually carry a mixture of defective and normal organelles. Somatic segregation of an initial mixture of mutant and normal organelles should lead to sectors of defective and normal growth. Progeny resulting from a cross of an affected ear would be expected to inherit a varying number of affected organelles. A few progeny might receive no abnormal organelles and would be normal in appearance. In fact, we observe normal plants in affected families; among these are individuals that no longer transmit the NCS phenotypes to their progeny. The progeny from such normal plants are referred to as normal derivatives.

The two mutants are phenotypically distinct from each other (8). While the leaves on both types of mutant plants tend to be narrow and asymmetric, NCS2 plants often exhibit broad pale green stripes while NCS3 leaves have fine, necrotic striations correlated with the narrowing of leaf surface. While extreme individuals of both NCS2 and NCS3 are small, extreme NCS3 plants also have a twisted, highly distorted morphology (8).

It has previously been argued that the affected organelle in NCS mutations is the mitochondrion rather than the chloroplast, despite the existence of "stripes" (8). In order to test this hypothesis we isolated mtDNA from affected NCS individuals and analyzed restriction enzyme patterns. One advantage in working with NCS in maize is that the progenitor cytoplasm, cms-T, is known. In addition, we have available nonmutant plants (normal derivatives) from NCS lineages. Therefore we could compare mtDNA restriction digests among progenitor, mutant, and normal derivatives.

MATERIALS AND METHODS

Plant Materials. Pedigreed sources of NCS2 and NCS3 cytoplasms were originally provided by L. F. Bauman. They were supplied as hybrids from successive crosses with different inbred lines as pollen parents, as described previously (8). Thus, all materials used in this study had mixed nuclear backgrounds but constant maternal-parent (cytoplasmic) sources. The mutants were further propagated at the University of Missouri. Our tests involved progeny from crosses of mutant plants with pollen from the following inbred lines: B37, B73, Ky21, Mo17, A619, and NY821.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NCS, nonchromosomal stripe; kb, kilobase(s).

Progeny were scored for the degree of phenotypic expression based upon reductions in plant height and vigor, asymmetric growth, degree of pale striping (NCS2), necrotic sectors (NCS3), and leaf narrowness. For the purposes of this study, we classified plants into the following categories: normal, slightly affected, moderately affected, and extreme. For comparison, normal families (normal derivatives) arising from NCS materials in crosses with B37, A619, and Ky21 pollen were available. Cms-T in the inbred B37 line was used to represent the progenitor cytoplasm, in which the mutations arose.

DNA Isolation and Analysis. mtDNA was isolated from unfertilized ear shoots (within 2 days after silks first appeared) of plants scored for the degree of mutant expression. It had been found that this material is a good source of mtDNA from individual plants (9), although variable amounts of contaminating plastid DNA are present in our preparations. mtDNA was prepared by DNase I treatment of a 10,000 $\times$ *g* pellet according to the procedure of Kemble *et al.* (10). In control experiments, we also isolated mtDNA from the following sources: (*i*) normal-appearing sib plants (normal sibs) in families containing mutant plants; (*ii*) normal plants in NCS-derived families that have lost the mutant phenotype (normal derivatives); (*iii*) cms-T plants, the progenitor cytoplasm in which the NCS mutations occurred. In addition, to rule out that the changes we found might be due to contaminating chloroplast DNA, we isolated plastid fractions from a few of the ear shoot preparations used for mtDNA isolations. Plastid DNA was prepared by DNase I treatment of an initial 1000 $\times$ *g* pellet as previously described (9). The plastid DNAs prepared from ear shoots in this manner are also contaminated with mtDNAs. Highly purified chloroplast DNA from young green leaves was included in some of the analyses to identify the plastid DNA fragments. The DNA preparations were digested with 10–20 units of *Xho* I per μg of DNA (Bethesda Research Laboratories). Gel electrophoresis was conducted in 0.7% agarose using Tris·HOAc buffers (11). Gels were stained with ethidium bromide and photographed on a UV light box using Polaroid Type 55 film.

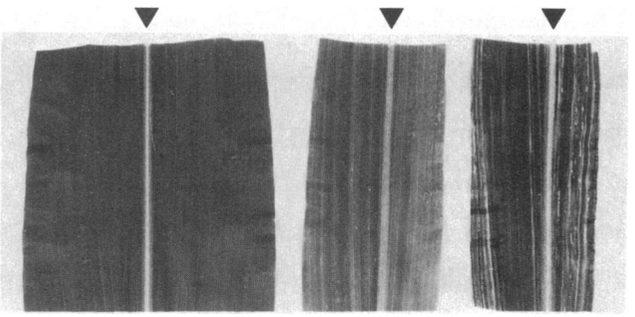

FIG. 1. Portions of leaves from: a normal plant from an NCS2 lineage (A); a moderately affected NCS2 plant (B); and a moderately affected NCS3 plant (C). The position of the midrib of each leaf is indicated by the arrowheads. Both types of mutant leaves can be asymmetric (note the displacement of the midribs from the center of the leaves). The stripes on NCS2 plants are pale green and broader than the necrotic striations on NCS3 plants.

RESULTS

The sizes of ear shoots correlated generally with the size and vigor of mutant plants. The most extremely affected NCS3 plants are tiny and highly distorted, with very little leaf material, and they produce no ear shoots. Moderately affected NCS3 plants are generally a maximum of half as tall as normal sib plants and have pronounced necrotic sectors on the leaves (Fig. 1). They regularly produce ear shoots, which are usually reduced in size and often twisted. If ear shoots from NCS3 plants are pollinated, sectors with poor seed set are often seen (Fig. 2). NCS2 plants are very different. The most extreme cases are small and mostly pale green with very small ear shoots, but they grow upright. Moderately affected NCS2 plants may be relatively tall and quite vigorous despite the presence of large pale green sectors (Fig. 1). The ear shoots from such plants can be quite large. If the ear shoots are pollinated, sectors of small kernels are seen on the

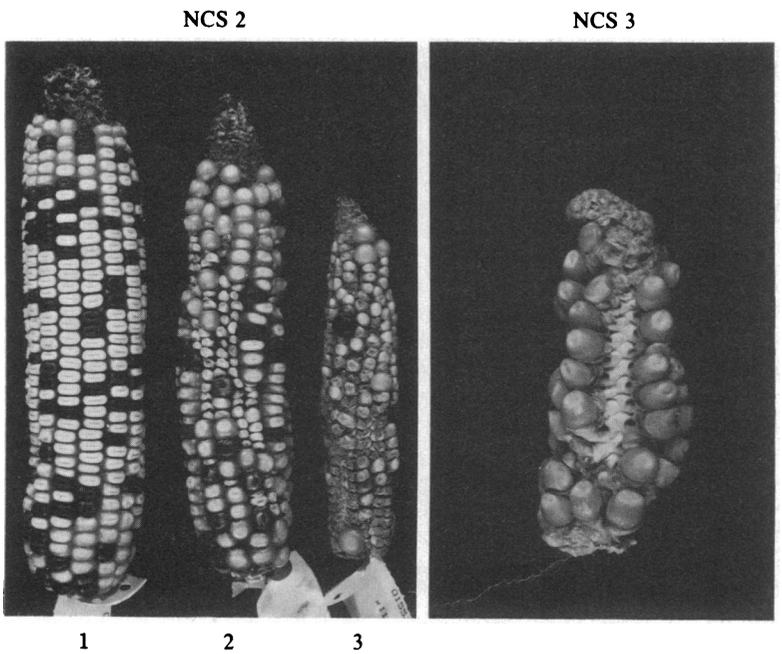

FIG. 2. Ears from NCS2 and NCS3 plants. Ears 1, 2, and 3 are from sib NCS2 plants (grown from kernels taken from the same ear) crossed by pollen from the same male parent plant (inbred A619). The plants were similar in height but differed greatly in the extent of striping. Ear 1 came from a normal plant, ear 2 from a moderately striped plant, and ear 3 from a highly striped plant. Ear 1 lacks defective kernels, while ear 2 shows clonal regions with small and defective kernels. Ear 3 has many small and defective kernels and occasional large kernels. NCS3 ears often have clonal sectors where kernels are absent.

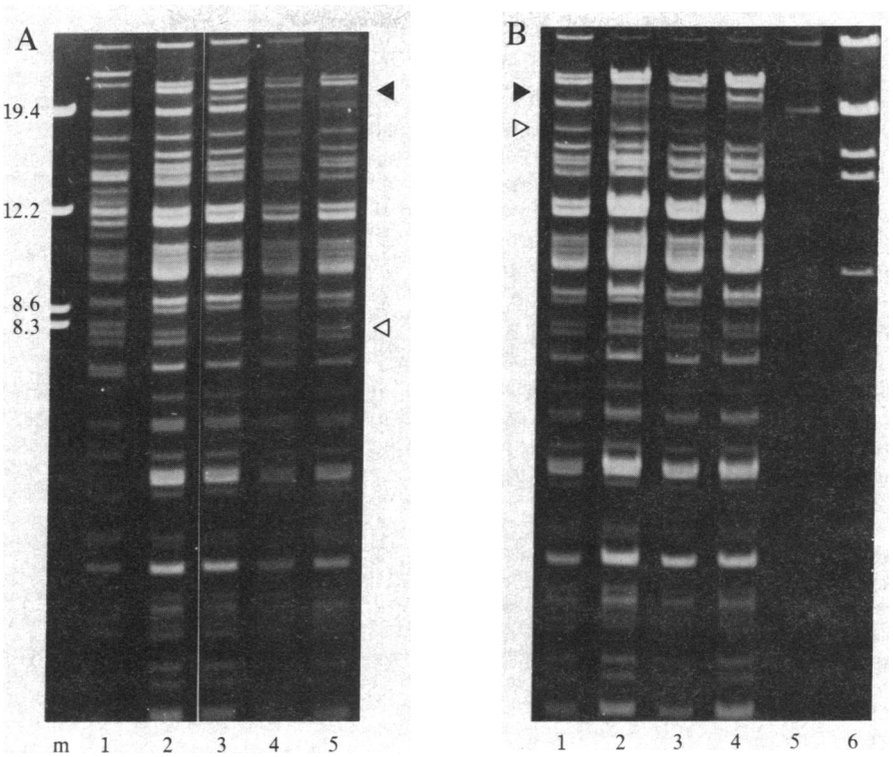

FIG. 3. *Xho* I restriction digest fragment patterns. (*A*) Mitochondrial DNA from ear shoots: An N-type cytoplasm from the inbred Ky21 line (lane 1); cms-T in inbred B37, representing the progenitor cytoplasm (lane 2); moderately affected NCS2 individuals (lanes 3 and 4); and a normal derivative plant from an NCS2 lineage (lane 5). *Sma* I-digested λ DNA is in the marker (m) lane. The positions of the distinctive 21-kb NCS2 band (◀) and the progenitor 8-kb (◁) are shown at the right. (*B*) Mitochondrial DNA from ears of normal derivative plants from an NCS3 lineage (lane 1), a slightly affected NCS3 individual (lane 2), a moderately affected NCS3 individual (lane 3), and a more severely affected NCS3 individual (lane 4); plastid DNA from the more severely affected NCS3 plant (lane 5); highly purified chloroplast DNA from young green leaves (lane 6). The positions of the distinctive NCS3 20-kb band (▶) and the 16-kb band (▷) are shown at the left.

resulting ears (Fig. 2). The amount of striping on the leaves generally correlates with the size of the small kernel sectors.

Because of the difficulty in obtaining sufficient material from the most severely affected plants, our samples from NCS2 and NCS3 were biased toward moderately and slightly affected plants. However, even if a plant were only slightly affected—i.e., tall and vigorous with only a few discernible stripes—characteristic alterations were always visible following restriction endonuclease digestions of the mtDNA from its ear shoot.

For both mutants, several restriction enzymes reveal alterations in one or more mtDNA bands. While discrete changes can be seen with *Pst* I, *Sma* I, *Sal* I, *Kpn* I, *Hin*dIII and *Bam*HI, the clearest visible changes are seen for both mutants with *Xho* I. Consequently, we have used this enzyme in our screen of mtDNA isolated from NCS plants. mtDNAs isolated from NCS2 plants have an additional, ≈21-kilobase (kb) fragment and greatly reduced amounts of DNA in an 8-kb band, relative to the progenitor or normal derivative genotypes (Fig. 3*A*). mtDNAs from NCS3 plants exhibit an extra, ≈20-kb band and have reduced amounts of DNA in a 16-kb band (Fig. 3*B*).

The crude mitochondrial pellets from ear shoots are contaminated with plastids. Thus, digests of the mtDNA are contaminated with plastid DNA bands. However, comparisons with plastid DNAs, from the same preparations used for mtDNA isolations, demonstrated that the distinctive bands were present in mtDNA (Fig. 3*B*). There is no evidence for plastid DNA alterations in the NCS mutants.

For each mutant, we have now analyzed mtDNA from >20 affected individuals and several normal plants. The correlation between the expression of the mutant phenotype in the plant and the presence of the extra bands in mtDNA is thus far absolute (Table 1). In addition, in the case of NCS3, there appears to be a good correlation between the extent to which the plant is affected and the amount of DNA in the 20-kb *Xho* I band in the mtDNA from its ear shoots (Fig. 3*B*). The more DNA in the 20-kb band, the less is present in the 16-kb band. Other bands are altered neither in migration nor in intensity.

DISCUSSION

The conclusion supported by these results is that NCS plants contain a mixture of two different types of mitochondrial genomes, normal and mutant. The mutant genomes are not extensively rearranged, but NCS2 and NCS3 each have a specific change. The mutations are highly deleterious to plant and kernel growth. Thus, sorting out of normal and mutant mitochondria in affected plants can result in some totally normal progeny, but germ cell lineages containing only mutant mitochondria would give no viable progeny.

The mechanism by which the WF9 nuclear constitution leads to mutations in mtDNA remains to be established. While the mitochondrial genome can be very stable in some

Table 1. Correlation of distinctive mtDNA restriction fragments with phenotypic expressions of NCS2 and NCS3

	NCS2		NCS3	
	Samples	*Xho* I 21-kb band	Samples	*Xho* I 20-kb band
mtDNA source				
Affected plants	26	26	30	30
Normal sibs	2	0	5	0
Normal derivatives	7	0	5	0
Plastid DNA				
Affected plants	3	0	6	0

nuclear backgrounds (9), nuclear genes are also known to control cytoplasmic reversions to fertility in S-type male sterile cytoplasms (12). Cytoplasmic reversions of cms-S in the M825 nuclear constitution are accompanied by rearrangements of the mitochondrial genome (13). NCS-type plants (also referred to as wsp) have been observed at a low but workable frequency (up to approximately 1%; D. N. Duvick, personal communication) in the WF9 nuclear background (14). While the present studies have concerned mutations arising in the male sterile T-type mitochondrial genome, the WF9 genotype can induce NCS-like mutations in other types of male sterile and non-male-sterile (N) cytoplasms (14). We have shown that different modifications to the mitochondrial genome occur in the two defective growth mutations examined to date. Therefore, the WF9 line provides a source of mitochondrial mutants that can be used to identify and analyze maize mitochondrial genes.

The technical assistance of A. Fajen and S. Shuckman is gratefully acknowledged. We thank V. Walbot, in whose laboratory preliminary experiments were conducted, D. Stern for a generous gift of purified maize chloroplast DNA, and E. Zimmer for a critical reading of the manuscript. This work was supported by National Science Foundation Grant DCB-8408687 and a Missouri Research Council Grant.

1. Bertrand, H., Chan, B. S.-S. & Griffiths, A. J. F. (1985) *Cell* **41,** 877–884.
2. Gross, S. R., Hsieh, T. S. & Levine, P. H. (1984) *Cell* **38,** 233–239.
3. Wright, R. M., Horrum, M. A. & Cummings, D. J. (1982) *Cell* **29,** 505–515.
4. Lazarus, C. M., Earl, A. J., Turner, G. & Kuentzel, H. (1980) *Eur. J. Biochem.* **106,** 633–641.
5. Hanson, M. R. & Conde, M. F. (1985) *Int. Rev. Cytol.* **94,** 214–267.
6. Pring, D. R. & Lonsdale, D. M. (1985) *Int. Rev. Cytol.* **97,** 1–46.
7. Shumway, L. K. & Bauman, L. F. (1967) *Genetics* **55,** 33–38.
8. Coe, E. H., Jr. (1983) *Maydica* **28,** 151–167.
9. Oro, A. E., Newton, K. J. & Walbot, V. (1985) *Theor. Appl. Genet.* **70,** 287–293.
10. Kemble, R. J., Gunn, R. E. & Flavell, R. B. (1980) *Genetics* **95,** 451–458.
11. Palmer, J. D. (1982) *Nucleic Acids Res.* **10,** 1593–1605.
12. Laughnan, J. R., Gabay-Laughnan, S. & Carlson, J. E. (1983) *Stadler Genet. Symp.* **13,** 93–114.
13. Schardl, C. L., Pring, D. R. & Lonsdale, D. M. (1985) *Cell* **43,** 361–368.
14. Duvick, D. N. (1965) *Adv. Genet.* **13,** 1–56.