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Mitochondrial DNA variation in races of maize indigenous to Mexico

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Summary. Mitochondrial DNAs have been examined in accessions of 25 Mexican races of maize and compared with the mitochondrial DNAs previously found in inbred lines from the USA. Many variants were found. Low molecular weight DNA components, not previously found in US lines, were found in many of the accessions. Accessions classified as belonging to the same race, and plants from a single accession, sometimes had different mitochondrial genomes. Mitochondrial genomes similar to those in T and S cytoplasms were found in Mexican accessions.

A low molecular weight linear DNA species has partial homology with a sequence in the high molecular weight mitochondrial genome. All plants with a shorter version of the linear molecule had a correspondingly altered region of homology in the high molecular weight genome.

There is evidence that the geographical distribution of mitochondrial DNA types within Mexico is not random. One type, found in the oldest races, appears to be widely dispersed but another less common type appears to be confined largely to coastal regions. The potential value of these findings in maize breeding and for evolutionary studies is discussed.

Key words: *Zea mays* – Mexican races of maize – Mitochondrial DNA – Cytoplasmic male sterility

Introduction

Investigations of mitochondrial DNA (mtDNA) variation in maize have been carried out predominantly on plants with cytoplasms belonging to one of the four groups (N, T, C and S) that have been characterized genetically with respect to their effect on pollen fertility in maize stocks from the USA (Duvick 1965; Beckett 1971). Plants with N cytoplasm are fertile whereas plants with T, C and S cytoplasms are male sterile unless group-specific restorer genes are present in the nucleus. The mitochondria of each group contain a characteristic combination of low molecular weight DNA species (Kemble and Bedbrook 1980; Kemble et al. 1980). The mtDNAs of the four groups can also be distinguished after digestion with restriction endonucleases and fractionation of the DNA fragments by electrophoresis (Pring and Levings 1978). Some variation in mtDNA within groups has been detected by using restriction endonucleases (Levings and Pring 1977; Pring et al. 1980), but apart from a recent report (Weissinger et al. 1982) there has been no extensive survey of material from outside the USA. In this paper we present results of a study on variation in mtDNA species between and within 25 male-fertile races and subraces of maize that are indigenous to Mexico. The Mexican races of maize were chosen because they present an unusually wide array of recognizably different phenotypes for which there is detailed documentary evidence on their geographical distribution, probable age and evolutionary relationships (Wellhausen et al. 1952). Using this material, we have been able to detect many new low molecular weight DNA species in male-fertile cytoplasms and to learn more about mtDNA structure and variation.

Materials and methods

Plant material

Seed of 25 accessions, each representative of one of the races or subraces described by Wellhausen et al. (1952), was obtained

from CIMMYT (Table 1). In most cases, the accessions chosen for study were those defined by Wellhausen et al. (1952) as type specimens for the race. When this was not possible, accessions were selected for which there was documentary evidence in CIMMYT archives that they had been assigned to a particular race. We omitted from the study seven races that Wellhausen et al. (1952) considered to be poorly defined and we were unable to test Harinoso de Ocho because seed of this recognised race did not germinate. For a special study on variation in mtDNA within the race Celaya we also used seed of four additonal accessions (Jal. 8, Jal. 38, Mich. 111 and Mich. 390; Table 2) from the CIMMYT germplasm collection. Seed of the American inbred lines WF9C, W64AN, W64AT and B37S, which were used as controls, was obtained from commercial sources. Inbred A188N was received as a gift from Dr. B. Gengenbach (University of Minnesota).

Isolation of mtDNA

Mitochondrial DNA was isolated from samples comprising 30 to 90 dark-grown coleoptiles by the method described by Kemble et al. (1980). When single plants were analysed, mtDNA was obtained from green leaves of young plants either by the same method or a more rapid method (Kemble 1980).

Electrophoresis, blotting and hybridizations

MtDNA was fractionated by electrophoresis on 1% or 1.5% agarose gels, stained with ethidium bromide, photographed (Kemble et al. 1980), transferred to nitrocellulose (Southern 1975) and prehybridized at 65° for 5 to 20 h in 0.3 M sodium chloride, 30 mM sodium citrate, 0.1% SDS and 0.2% each of Ficoll, BSA and PVP. Hybridizations were performed at 65 °C for 18 to 24 h in fresh solution containing between 1.2×10^7 to 2.1×10^7 cpm nick-translated (Maniatis et al. 1975) cloned probes. The probes used were pZmS4, pZmS21 and pZmpT 3.52. Both pZmS4 and pZmS21 contain inserts of mtDNA from S cytoplasm maize (Thompson et al. 1980). pZmpT 3.52 contains a full length insert of the 1.94 kb mtDNA plasmid (Kemble and Bedbrook 1980; Thompson et al. unpublished). All were constructed in pBR322. An uncloned probe of the 2.35 kb linear mtDNA species (Kemble and Bedbrook 1980) from a line carrying S cytoplasm was also used. This was prepared by subjecting total mtDNA to cesium chloride- ethidium bromide gradient centrifugation followed by a 5 to 20% linear sucrose density gradient centrifugation. Fractions rich in the 2.35 kb molecules were detected by agarose gel electrophoresis and used as probes.

Results

Variation in low molecular weight mtDNA species among Mexican races

In a previous analysis of mtDNA from a collection of inbred lines from the USA carrying different cytoplasms only four different combinations of low molecular weight plasmids were distinguished by electrophoresis (Kemble et al. 1980). These combinations were characteristic of C, T, S and N cytoplasms (Kemble and Bedbrook 1980) and are shown in Fig. 1 A, C (lanes C, T, S and N). Each fractionated mtDNA displays the highly fluorescent high molecular weight mtDNA (HMW mtDNA) band and three bands of smaller size which represent the supercoiled (ccc), open circular (oc) and linear (l) conformations of the 1.94 kb mtDNA (Kemble and Bedbrook 1980). MtDNA of C, N and S cytoplasms also possess a linear mtDNA species (n) of 2.35 kb. In mtDNA from T cytoplasms this species (t) is shorter. C cytoplasm is characterized by additional circular mtDNA species of 1.57 kb (C1) and 1.42 kb (C2) (Kemble and Bedbrook 1980). S cytoplasm possesses two linear mtDNA species of about 6.2 kb (S1) and 5.2 kb (S2) (Pring et al. 1977).

MtDNA of almost all the 25 different Mexican races examined had low molecular weight nucleic acid species not previously observed in the USA lines (Fig. 1 A, C). All bands disappeared on DNAase digestion but were resistant to RNAase. There was considerable diversity for the low molecular weight linear mtDNA species between the races. Some races exhibited the 'n' band, some the 't' band. Others exhibited both bands (Fig. 1 A, C). These results are summarized in Table 1. All races, with the exception of Zapolote Grande (number 17), possessed bands corresponding to the supercoiled and open-circular conformations of the 1.94 kb mtDNA plasmid (Fig. 1 A, C). To determine if this plasmid species was totally absent from Zapolote Grande, the gels shown in Fig. 1 A, C were transferred to nitrocellulose (Southern 1975) and probed with radioactively nick-translated pZmpT 3.52, which is a full length copy of the 1.94 kb plasmid inserted into pBR 322 (Thompson et al., unpublished). In all races, except Zapolote Grande, hybridization bands corresponding to the supercoiled, open-circular and linear conformations of the plasmid are visible (Fig. 2 A, B). Zapolote Grande is the only maize examined to date which does not possess this mitochondrial plasmid. The other higher molecular weight bands showing hybridization in Fig. 2 A, B are unrelated to the pZmpT 3.52 probe; they are simply residual radioactivity from pZmS21 and pZmS4 which were used as probes on the blots in a previous experiment (see below).

MtDNA isolated from Celaya showed the presence of two bands corresponding in mobility to S1 and S2 which are characteristic of plants carrying S cytoplasm (Fig. 1 C, lane 23). To determine if these, and the other new minor bands, had any sequence homology with S1 and S2, Southern blots of the gels shown in Fig. 1 A, C were probed with radioactively nick-translated pZmS21 and pZmS4 (Thompson et al. 1980). pZmS4 has an insert of about 2.1 kb complementary to S2 only, whereas pZmS21 has an insert of about 4 kb and is complementary to S1 and S2 (Thompson et al. 1980; Lonsdale et al. 1981). Fig. 1 D shows that the bands in Celaya (lane 23) are homologous with those in the S cytoplasm sample (lane S), indicating that the mtDNA from race Celaya contains S1 and S2 DNA species. Hybridization is also seen in Fig. 1 D to two bands which have similar mo-

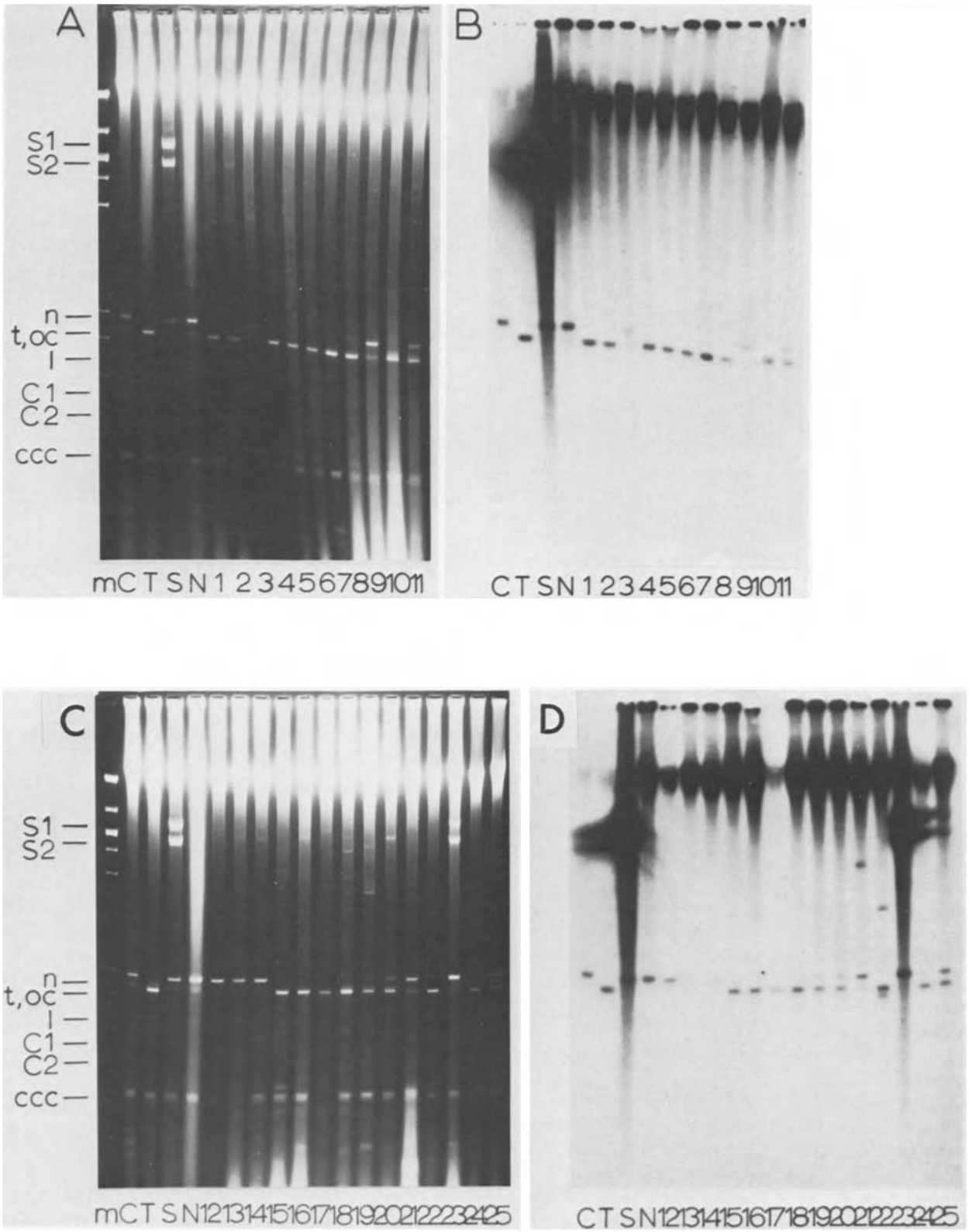

Fig. 1. A and C Electrophoresis on 1.5% agarose gels of mtDNA isolated from inbred lines carrying C, T, S and N cytoplasm and 25 Mexican races. The lane numbers correspond to the number designated to each Mexican race in Table 1. m is a marker lane of independent digests of λDNA with Eco RI and Hae III (Daniels et al. 1980; Kemble and Bedbrook 1980). **B and D** Autoradiographs of Southern blots of the gels after hybridization to a radioactive probe of pZmS21 and pZmS4

Table 1. Classification of mtDNA banding patterns in Mexican races of maize

Our code	Accession no.[a]	Race	Bands detected by		
			EthBr	pZmS21+4	
			on un-digested gels	on un-digested gels	on Bam HI gels
Ancient indigenous races[b]					
1	Mex. 5[c]	Palomero Toluqueno	t	t	6.55 kb
2	Sin. 2[c]	Chapalote	t	t	6.55 kb
3	Yuc. 7[c]	Nal-Tel	n+t	n+t	6.55+6.85 kb
4	Pue. 91[c]	Arrocillo Amarillo	t	t	6.55 kb
Pre-Columbian exotic races					
5	Mex. 7[c]	Cacahuacintle	t	t	6.55 kb
6	Chis. 35[c]	Oloton	t	t	6.55 kb
7	Jal. 78[c]	Maiz Dulce	t	t	6.55 kb
Prehistoric hybrid races					
8	Mex. 3[c]	Conico	t	t	6.55 kb
9	Nay. 15[c]	Reventador	n	n	8.00 kb
10	Jal. 43	Tabloncillo	t	t	6.55 kb
11	Nay. 12[c]	Tabloncillo perla	n+t	n+t	6.55+6.85 kb
12	Chis. 109[c]	Tehua	n	n	6.85 kb
13	Chis. 26[c]	Tepecintle	n	n	6.85 kb
14	Chis. 46	Comiteco	n	n	6.85 kb
15	Jal. 76	Jala	t	t	6.55 kb
16	Oax. 50[c]	Zapalote Chico	t	t	6.55 kb
17	Chis. 224[c]	Zapalote Grande	t	t	
18	Mor. 17[c]	Pepitilla	t	t	6.55 kb
19	Chis. 52[c]	Olotillo	n+t	n+t	6.55+6.85 kb
20	Ver. 39[c]	Tuxpeno	n+t	n+t	6.55+6.85 kb
21	Chis. 167	Vandeno	n+t	n+t	6.55+6.85 kb
Modern incipient races					
22	Pue. 464	Chalqueno	t	t	6.55 kb
23	Gto. 79	Celaya	n	n	6.85 kb
24	Gto. 49[c]	Conico Norteno	t	t	6.55 kb
25	Oax. 40[c]	Bolita	n+t	n+t	6.55+3.5 kb

[a] Accession number in CIMMYT collection
[b] Race names and groups from Wellhausen et al. (1952)
[c] Type specimen accession of Wellhausen et al. (1952)

bility to S1 and S2 in races Conico Norteno and Bolita (lanes 24 and 25). Separate experiments (results not shown) showed that this was due to sample spill-over from lane 23. In these additional experiments there was evidence of a minor DNA species in Conico Norteno and Bolita which migrated to a position between S1 and S2 and which showed weak hybridization to pZmS21 and pZmS4.

There is sequence homology between S1 and S2 and the low molecular weight linear mtDNA species termed 'n' and 't' (Thompson et al. 1980). Fig. 1 B, D show this and also verify the heterogeneity for these two low molecular weight species between the Mexican races. Races which exhibited either 'n' bands, 't' bands or both on the ethidium bromide stained gels (Fig. 1 A, C) showed a similar pattern regarding hybridization to either 'n' bands, 't' bands or both in Fig. 1 B, D (summarized in Table 1). The poor hybridization in Fig. 1 D lanes 13 and 14 was due to poor DNA transfer from the gel to the nitrocellulose on this particular blot; other blots clearly show that there was hybridization to the 'n' band only. Only two of the minor DNA bands visible in Fig. 1 A, C not previously found in stocks with N, T, S or C cytoplasms hybridized to the probes (Fig. 1 D, lanes 21 and 22).

Variation in high molecular weight mtDNA between and within Mexican races: a correlation between sequences in high molecular weight and low molecular weight forms

Bam HI digests of total mtDNA isolated from the Mexican races revealed some heterogeneity between races although most produced a fragment pattern more similar to that from N cytoplasm than that from S, C or T cytoplasm (Fig. 3 A, C). The exceptions were Zapolote Grande (lane 17), which produced a pattern similar to

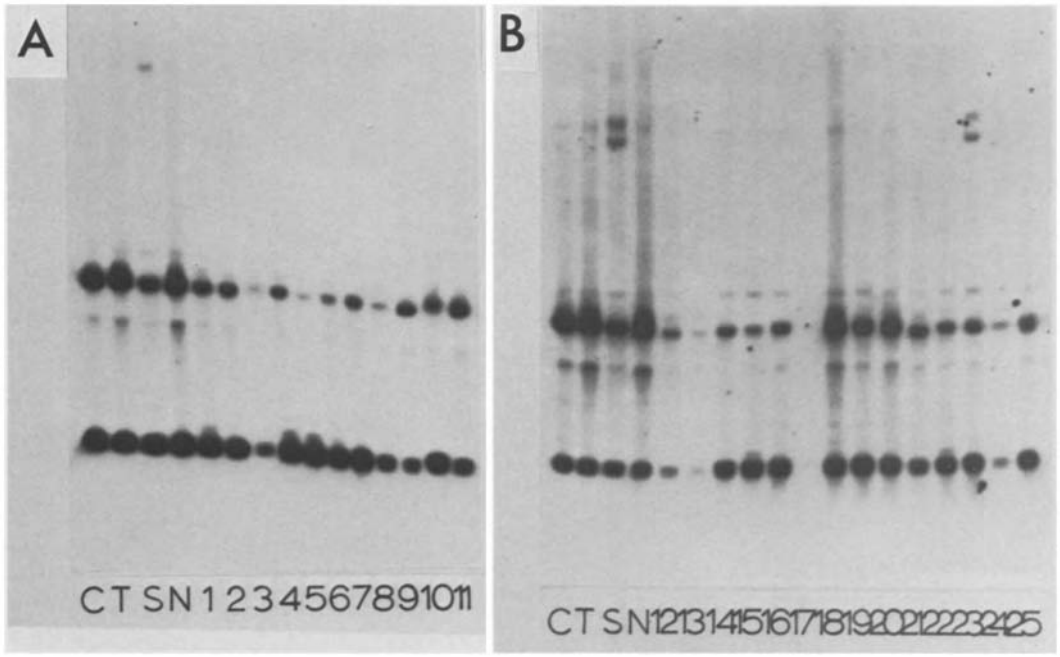

Fig. 2. A, B Autoradiographs of the nitrocellulose filters of Fig. 1 B and 1 D (after allowing 3 months for radioactive decay) reprobed with radioactive pZmpT 3.52

that from T cytoplasm, and Celaya (lane 23) which produced a N cytoplasm-like pattern but contained highly fluorescent bands corresponding to S1, in which there is no Bam HI site, and the 3.7 kb fragment derived from S2 (see above).

The principal mitochondrial genome of N cytoplasm contains some sequences homologous to S1 and S2. Some of these sequences are absent from T, C and S cytoplasms (Thompson et al. 1980; Lonsdale et al. 1981). To investigate the presence and structures of these sequences in the Mexican races, the Bam HI digests (gels in Fig. 3 A, C) were transferred to nitrocellulose and probed with pZmS21. The two heavily hybridizing bands which are characteristic of N cytoplasm, i.e. 4.4 kb, which is related to S2, and 6.85 kb and/or 6.55 kb which is related to S1, were found in all races except Zapolote Grande (lane 17), Celaya (lane 23) and Reventador (lane 9) (see later for further discussion on these races) (Fig. 3 B, D). The data for the 6.85 and 6.55 kb bands are summarized in Table 1. Of special interest is the observation that races which possess the 't' linear DNA species, identified by ethidium bromide staining and hybridization to pZmS21 and pZmS4 in Fig. 1, possess a hybridizing Bam HI fragment of 6.55 kb in Fig. 3. Likewise, races possessing the larger 'n' linear DNA species in Fig. 1 exhibited the larger Bam HI fragment of 6.85 kb in Fig. 3, and those races possessing both 'n' and 't' linear DNA species exhibited both the 6.55 kb and 6.85 kb hybridizing bands. There were only three exceptions. (1) Race Reventador (Fig. 3 B, lane 9) which has the 'n' linear DNA species and does not have a Bam HI fragment of 6.85 kb which hybridizes strongly to pZmS21. Instead it has a strong hybridizing fragment of 8.0 kb, not seen in any of the other races or inbred lines. This 8.0 kb fragment is visible as an additional band after ethidium bromide staining (Fig. 3 A, lane 9) and no ethidium bromide staining band is visible at the position corresponding to 6.85 kb. This suggests that in this mtDNA one of the Bam HI sites which create the 6.85 kb fragment has been lost. (2) Race Zapolote Grande (lane 17) which showed no major hybridization to pZmS21 – a characteristic of mtDNA from T and C cytoplasms (Thompson et al. 1980) (Fig. 1 D, lane 17). (3) Race Bolita (lane 25) which has both 'n' and 't' linear DNA species but does not have Bam HI fragments of 6.85 kb which hybridize to pZmS21. Hybridization is seen instead to fragments of 6.55 kb and 3.5 kb.

The correlation between the lengths of the partially homologous 'n' and 't' linear DNA species and of the high molecular weight mtDNA sequences prompted a further investigation of the relationship between the linear DNA species and those in high molecular weight mtDNA. This was done by probing a Southern blot of Bam HI digested mtDNA from representative Mexican races with a radioactively nick-translated preparation of band 'n' (Fig. 4 A, B). The sequence homology between band 'n' and S1 and S2 is confirmed (Fig. 4 B, lane S) since bands corresponding to S1, the 3.7 kb S2 fragment, band 'n' (no Bam HI site in 'n') and a fragment of

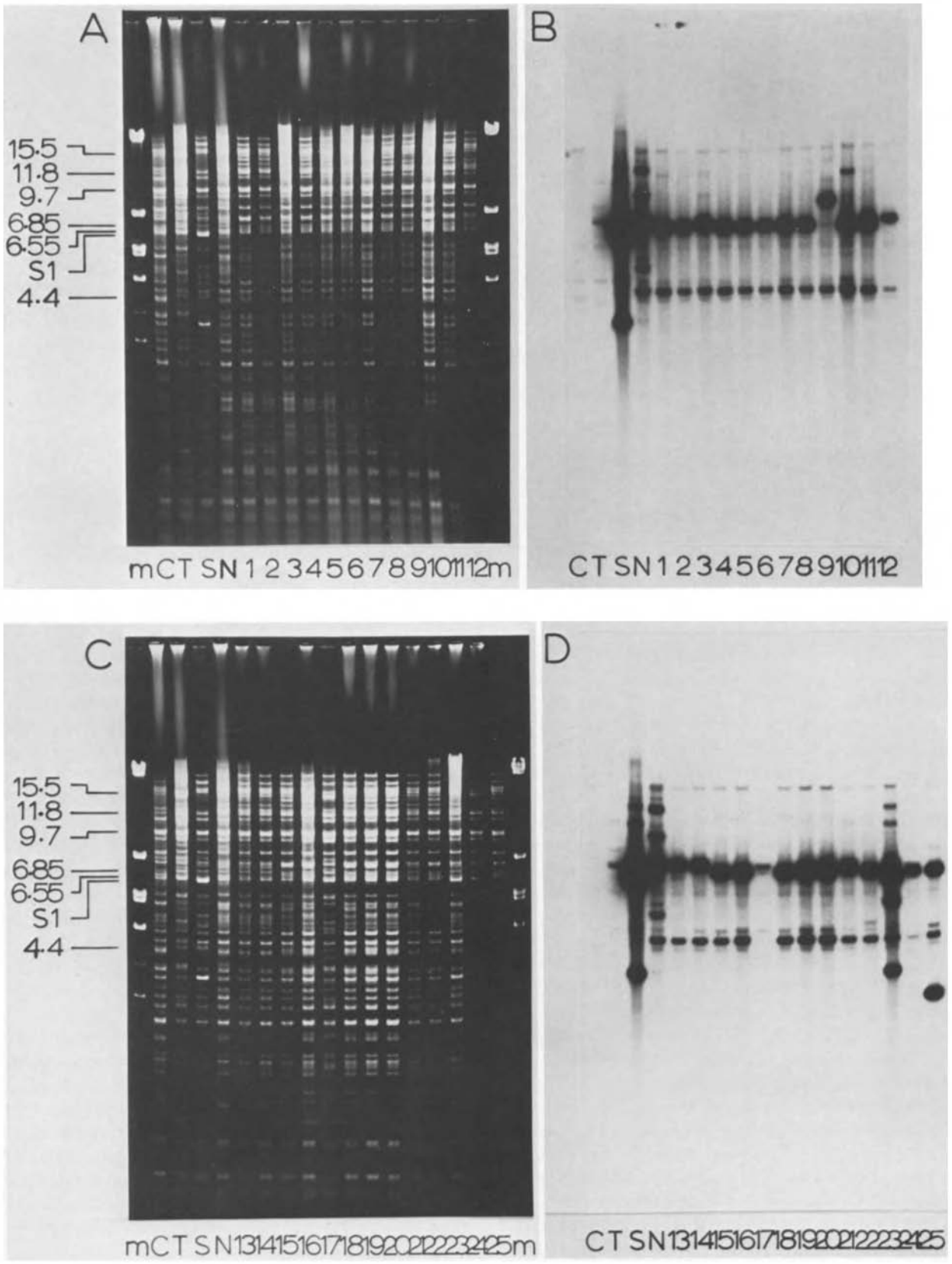

Fig. 3. A and C Electrophoresis on 1% agarose gels of Bam HI digests of mtDNA from the same lines as in Fig. 1. Sizes are in kb; all other labelling as in Fig. 1. **B and D** Autoradiographs of Southern blots of the gels after hybridization to pZmS21

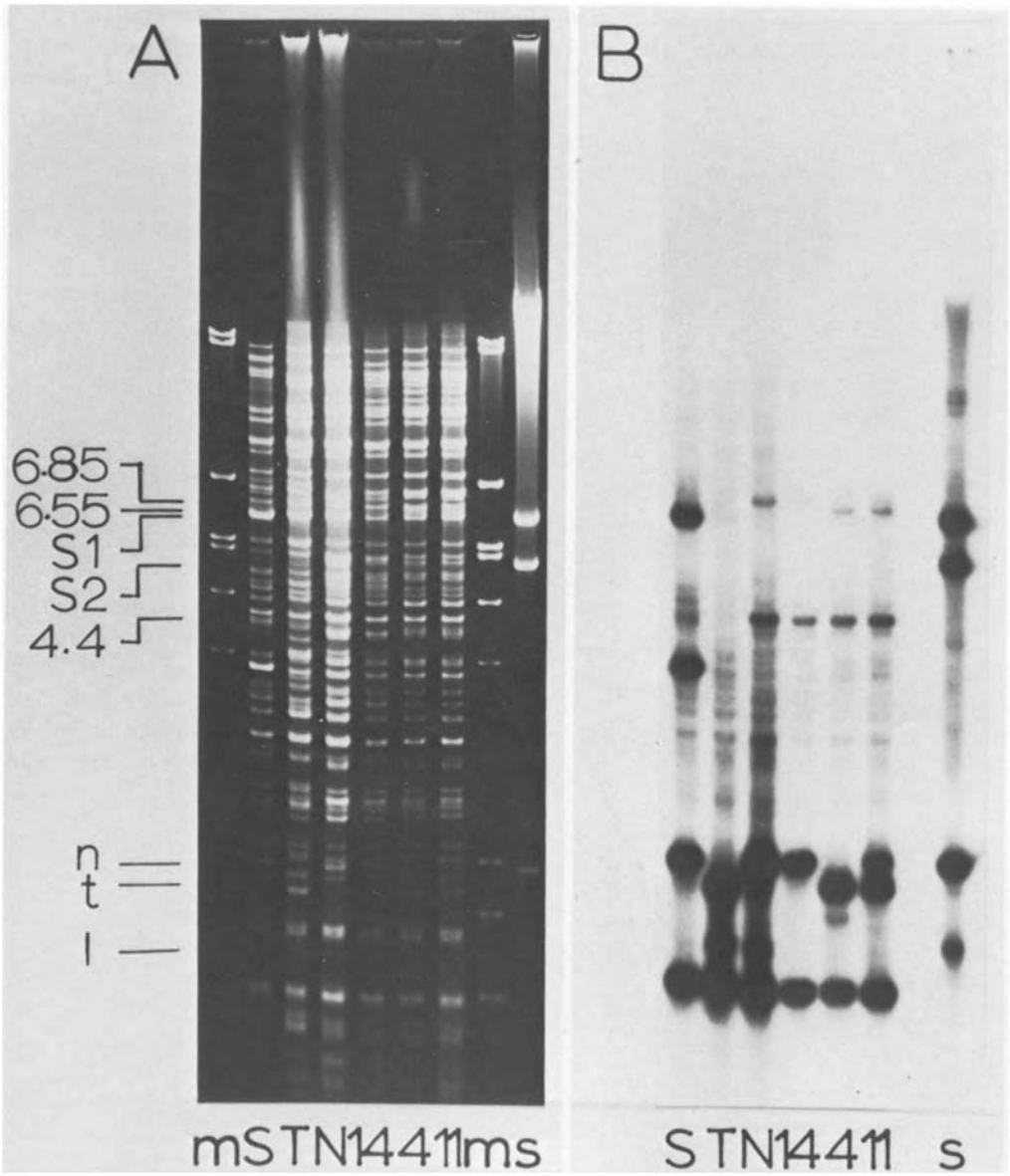

Fig. 4. A Electrophoresis on 1% agarose gel of Bam HI digests of mtDNA from 3 different cytoplasm types and 3 Mexican races. Labelling as in Figs. 1 and 2. s is an undigested lane of S cytoplasm mtDNA as a hybridization marker. **B** Autoradiograph of a Southern blot of the gel after hybridization to a radioactive probe of the 'n' DNA species

1.7 kb all hybridize to the probe. The 1.7 kb fragment hybridizes in all the Bam HI digested samples but does not hybridize in an undigested sample (lane s). The T cytoplasm sample (lane T) shows the predicted pattern of hybridization to band 't' and it also hybridizes to a band of about 1.94 kb which corresponds to the linear conformation of the common mitochondrial plasmid, suggesting that the probe was contaminated with this mtDNA species. The N cytoplasm sample (lane N) shows hybridization to 6.85 kb and 4.4 kb fragments which are the same ones that hybridize with pZmS21 (Fig. 3 B, D). Race Comiteco (lane 14) which showed hybridization to 6.85 kb and 4.4 kb fragments with pZmS21, shows hybridization with the 'n' band probe to fragments of 6.85 and 4.4 kb as well as to the 'n' DNA species itself. Race Arrocillo Amarillo (lane 4), which showed hybridization to fragments of 6.55 kb and 4.4 kb with pZmS21, showed hybridization to those same fragments and also to the 't' DNA species. Race Tabloncillo subrace Perla (lane 11) which showed hybridization to fragments of 6.85 kb, 6.55 kb and 4.4 kb and the 'n' and 't' DNA species with pZmS21 displays the same hybridization pattern with the 'n' band probe. The less intense hybridization to other bands

seen in all lanes of Fig. 4 B probably does not represent important sequence homology with the 'n' band but is due to contaminating DNA in the probe which was not purified by cloning.

The hybridization results in Fig. 4 strengthen further therefore the correlation between the forms of the low molecular weight linear molecules and sequences in the high molecular weight mitochondrial genome.

The presence of 'n' and 't' DNAs in a single race is due to variation between plants

The presence of both 'n' and 't' DNA species, together with 6.85 and 6.55 kb Bam HI fragments, in 6 of the 25 races analysed raised several important questions. For example, do individual mitochondria possess both 'n' and 't' DNA species, do individual plants possess more than one type of mitochondrion or are the race accessions heterogeneous and contain plants with different mitochondria? These questions could not be answered from the experiments already described because each mtDNA preparation was made from a bulk sample of 30 to 90 seedlings. Consequently, mtDNA preparations were made from individual plants from the races numbered 3, 19, 20 and 25 in Table 1 (Fig. 5).

The two Nal-Tel (race 3) plants analysed differed from one another. One had the 'n' DNA species (Fig. 5) and showed hybridization with pZmS21 to 6.85 kb fragments after Bam HI digestion. The other had the 't' DNA species and showed hybridization to 6.55 kb fragments. However, both had similar plasmid-like DNA species of 5.9 and 5.0 kb. All five of the Olotillo (race 19) plants analysed had 't' bands only and showed hybridization to 6.55 kb Bam HI fragments. Four out of the five had the 3.6 kb major DNA species (Fig. 5) but differed for larger molecular weight DNA species. The fifth displayed no plasmid-like DNAs larger than the 't' species.

The individual Tuxpeno (race 20) plant analysed had the 't' DNA species, and a higher molecular weight species (5.5 kb) which was also detected in the pooled seedlings (Fig. 1, lane 20).

The two Bolita (race 25) plants analysed differed from one another, one having the 'n' DNA species and the other the 't' DNA species. When total mtDNA was digested with Bam HI, the plant with the 't' DNA species exhibited hybridization with pZmS21 to fragments of 6.55 kb. The plant with

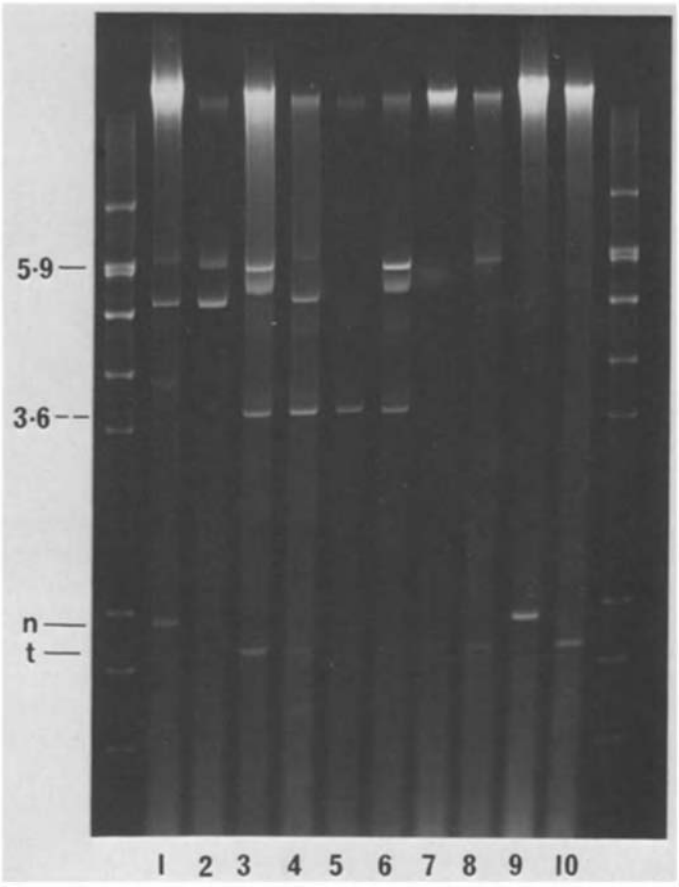

Fig. 5. Electrophoresis on 1.5% agarose of mtDNA isolated from individual plants of four different races. Lanes 1 and 2, Nal-Tel; lanes 3–7, Olotillo; lane 8, Tuxpeno; lanes 9 and 10, Bolita

the 'n' DNA species hybridized strongly not with 6.85 kb or 6.55 kb fragments but with 3.5 kb fragments. Thus this plant has a major structural difference in its mtDNA from most other Mexican races. This plant type is not a rare component in the seed batch of Bolita (race 25) because the 3.5 kb fragments related to pZmS21 were in a similar concentration to the 6.55 kb fragments in the analyses of the pooled seedlings (Fig. 2 D, lane 25).

These studies on individual plants imply that the presence of 'n' and 't' DNA species and of more than one high molecular weight DNA sequence related to S1 in pooled seedling batches, are due to variation between seedlings and not due to plants having different types of mitochondria or mitochondria with both 'n' and 't' DNA species and both 6.85 and 6.55 kb Bam HI fragments.

Different Celaya accessions have different mitochondrial genomes; some 'Celaya' plants have a cytoplasm similar to that of S cytoplasm

To determine if the Celaya trait of possessing a HMW mtDNA characteristic of N cytoplasm together with S1 and S2 mtDNA species was restricted to the one accession analysed, seed of four additional accessions was obtained (Table 2). Fig. 6 A shows the electrophoretic separation of the mtDNA species obtained for each accession, and Fig. 6 B shows the hybridization pattern obtained with pZmS21 and pZmS4. DNA from each accession showed hybridization to the HMW mtDNA characteristic of N cytoplasm, although Gto. 79 (lane A) was the only one to show the presence of S1 and S2. It was also the only accession to possess the 'n' DNA species alone. Jal. 38 exhibited both 'n' and 't' DNA species, whereas the other three accessions possessed the 't' DNA species only. There was also heterogeneity between the acessions in terms of minor bands visible in Fig. 6 A. Bam HI digestion of the mtDNA from these five accessions together with hybridization to pZmS21 confirmed that Gto. 79 was the only one possessing S1 and S2 species (Fig. 7 A, B). The hybridization to fragments about 11.8 and 9.7 kb in Gto. 79 is also characteristic of S cytoplasm mtDNA (Thompson et al. 1980).

Table 2. The five Celaya accessions used for mtDNA analysis

Accession no.	Designation in Figs. 6 and 7
Gto. 79	A
Jal. 8	B
Jal. 38	C
Mich. 111	D
Mich. 390	E

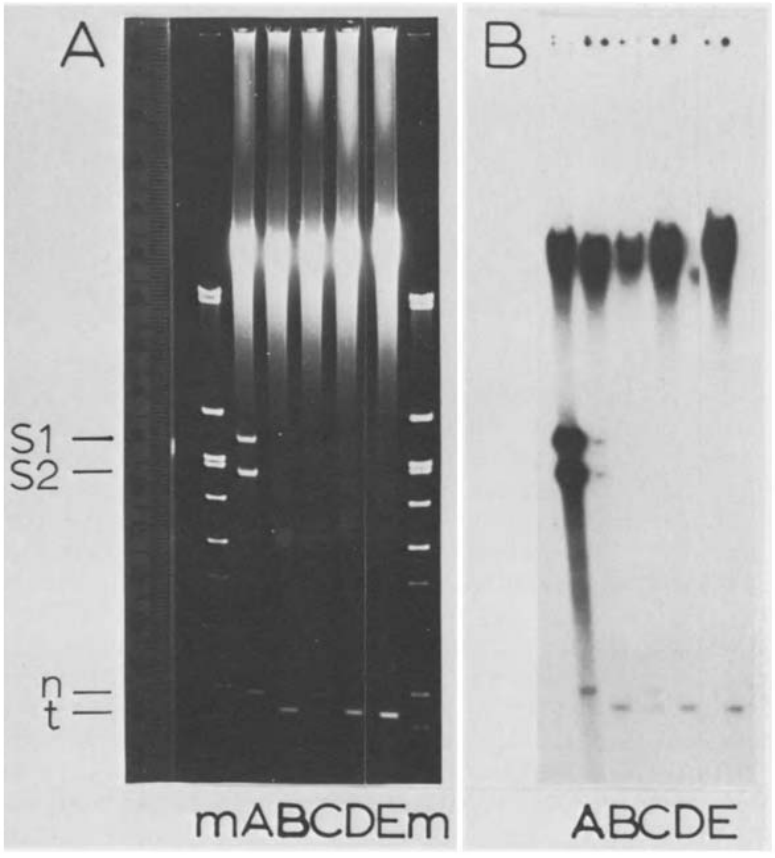

Fig. 6. A Electrophoresis on 1% agarose gel of mtDNA isolated from 5 different accessions of race Celaya. The lane lettering corresponds to the letter designated to each collection in Table 2. Other labelling as in previous figures. **B** Autoradiograph of a Southern blot of the gel after hybridization to a radioactive probe of pZmS21 and pZmS4

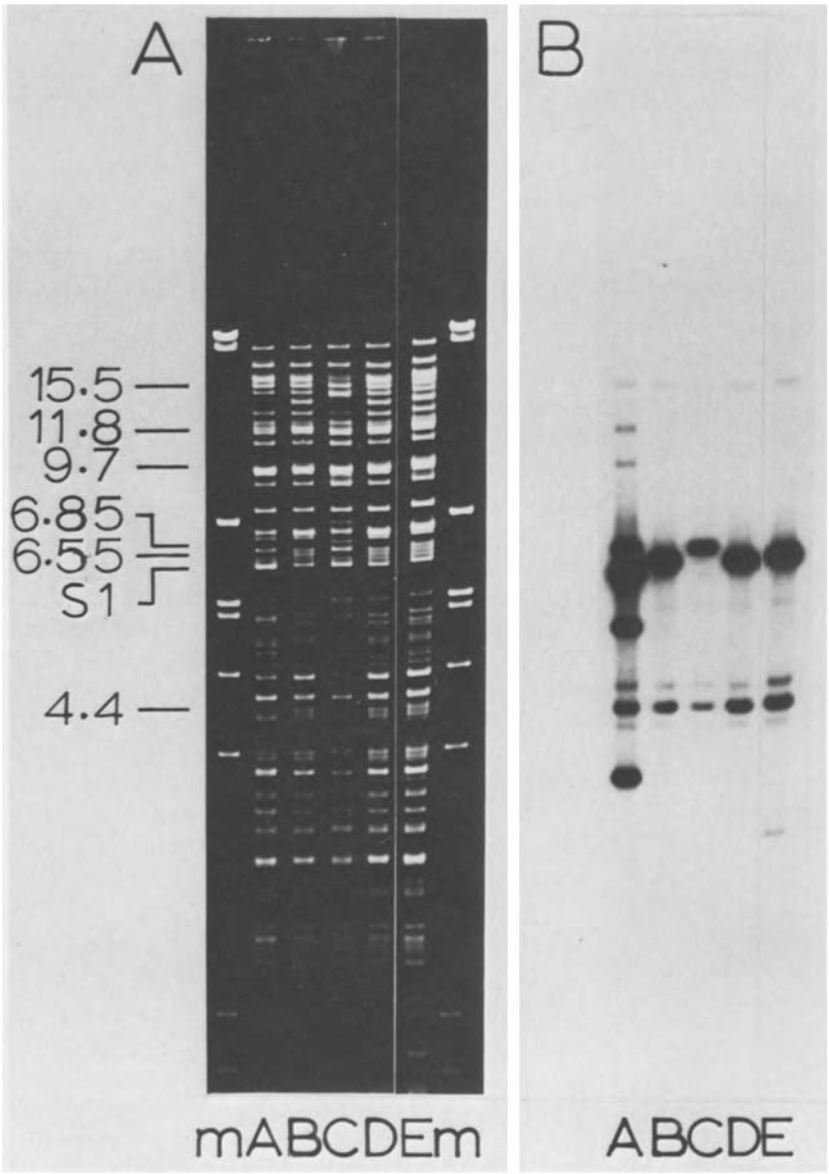

Fig. 7. **A** Electrophoresis on 1% agarose gel of Bam HI digests of mtDNA from the same races as in Fig. 6. Labelling as in previous figures. **B** Autoradiograph of a Southern blot of the gel after hybridization to pZmS21

Jal. 8, Mich. 111 and Mich. 390 had identical restriction patterns, but Gto. 79 and Jal. 38 differed from them and from each other (Fig. 7 A). The correlation between the presence of 'n' and 't' DNAs and the 6.85 kb and 6.55 kb Bam HI fragments related to S1 predicts that Gto. 79 DNA should have 6.85 kb fragments, Jal. 8, Mich. 111 and Mich. 390 should have 6.55 kb fragments and Jal. 38 should have both. These predictions were confirmed by the hybridization results (Fig. 7 B) except for Jal. 38 which exhibited hybridization to the 6.85 kb fragment only. Mich. 390 also showed weak hybridization to a band of about 3.5 kb (lane E). This hybridization pattern is similar to that observed for Bolita and resolved into two separate mitochondrial types. Thus these studies provide another example of heterogeneity in mtDNA of different accessions of the same race.

pZmS21 hybridized strongly not only to S1 and S2-like DNA species and 11.8 and 9.7 kb fragments (characteristic of S cytoplasm) in Gto. 79 but also to 6.85 kb and 4.4 kb fragments characteristic of N but not S cytoplasm (Thompson et al. 1980). To establish whether this hybridization pattern was typical for individual plants or was due to a mixture of mitochondrial types in the seed collection, mtDNA was analysed from 13 individual plants of Gto. 79, 10 plants of Jal. 8, 8 plants of Jal. 38, 15 plants of Mich. 111 and 11 plants of Mich. 390. The only plants exhibiting S1 and S2-like DNAs were two plants of Gto. 79, suggesting that this accession was a heterogeneous seed mixture. A more sensitive detection method was also employed to confirm this. The gels were transferred to nitrocellulose and probed with radioactively nick-translated pZmS21 and

pZmS4. The hybridization data indicated that only the same two plants from Gto. 79 exhibited S1 and S2 (data not shown).

The mtDNA of the U.S. inbred A188N is similar to the most common mtDNA in Mexican races

All the USA lines of the N cytoplasm group, characterized previously, possessed the 6.85 kb Bam HI fragment and the 'n' DNA species. It was therefore a surprise to find the self-fertile inbred line A188N to be considerably different. This inbred has the 't' DNA species and also two prominent DNA species which migrate slightly faster than S1 and S2 (Fig. 8). Although not easily visible on Fig. 8, A188N possessed the common 1.94 kb mitochondrial plasmid; a result which was confirmed by hybridization with pZmpT 3.52 (data not shown). To determine if these two DNA species share any homology with authentic S1 and S2, the DNA from the gel in Fig. 9A was transferred to nitrocellulose and probed with radioactive pZmS21 and pZmS4 (Fig. 9B). The lanes marked 'A' represent mtDNA prepared from self-pollinated seed from different harvests. Both lanes show the presence of the two DNA species, the difference in mobility between lanes is probably due to the difference in DNA concentration in the two lanes (Fig. 9A). The hybridization data (Fig. 9B) show that the two DNA species do not share homology with S1 and S2, and that the 't' band is similar to 't' bands of other lines in that it has homology to S1 and S2 sequences.

Bam HI digested mtDNA from A188N exhibited a fragment pattern more characteristic of N cytoplasm than T or S cytoplasms (Fig. 10A). Probing the Southern blot with pZmS21 revealed that A188N had a hybridizing fragment of 6.55 kb rather than of 6.85 kb (Fig. 10B). Further evidence that the S1 and S2-like molecules observed in the mitochondria of A188N bear no sequence homology to authentic S1 and S2 is the lack of hybridization to Bam HI fragments of similar size to those of S cytoplasm (Fig. 10B).

Thus A188N mtDNA is similar to the mtDNAs of many Mexican races in possessing (1) low molecular weight DNA species larger than 'n', (2) the 't' DNA species, and (3) 6.55 kb Bam HI fragments.

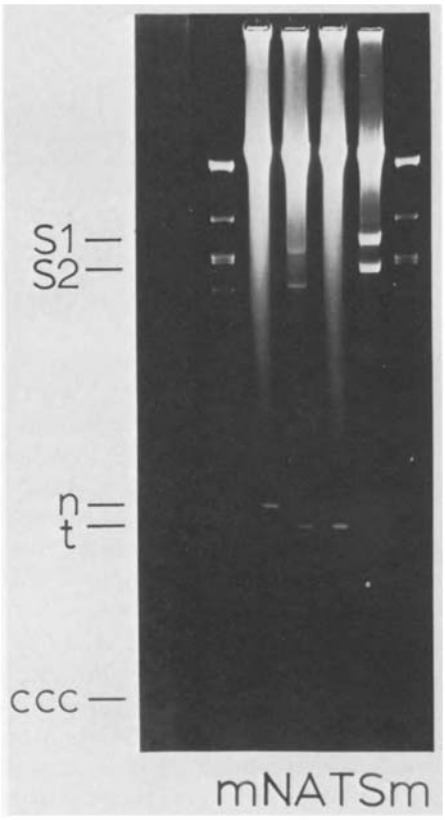

Fig. 8 Electrophoresis on 1.5% agarose gel of mtDNA isolated from A188N (lane A) and lines carrying N, T and S cytoplasm. Labelling as in previous figures

Discussion

Mitochondrial DNA variation in maize

In previous studies of US maize stocks, four groups of mtDNAs have been recognised by scrutiny of the electrophoretic patterns formed after treatment with restriction endonucleases (Pring and Levings 1978; Thompson et al. 1980). There are variants within some groups but each group is distinguished from the other three by many differences. These groups (N, T, C and S) coincide with the cytoplasm groupings made on the basis of pollen fertility restoration patterns against different nuclear backgrounds (Duvick 1965; Beckett 1971). Most of the mtDNAs of the Mexican accessions surveyed in this paper belong in the N group but there were three exceptions: (1) the accession of the race Zapalote Grande (No. 17) which appears to have a mtDNA similar to that of the Texas (T) group, (2) the accession of the race Conico Norteno (No. 24) whose Bam HI digest has major characteristics of T mtDNA but which possesses 6.55 kb and 4.4 kb fragments (related to the S1 and S2 sequences respectively) which are absent from the mtDNAs of T cytoplasms but present in the mtDNAs of N cytoplasms, (3) some individual plants of accession Gto. 79 of race Celaya which contained a mitochondrial genome characteristic of S cytoplasm. We have shown that these Celaya plants were derived from a heterogeneous seed batch in which the majority of seeds gave rise to plants possessing mitochondrial genomes characteristic of N cytoplasm.

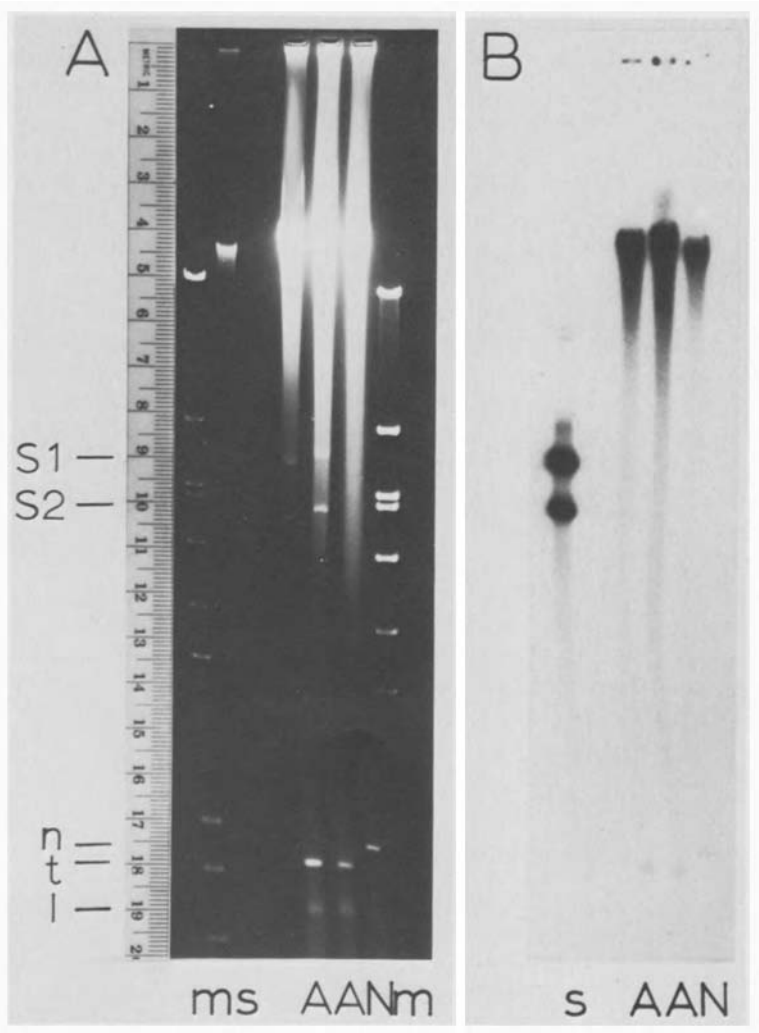

Fig. 9. A Electrophoresis on 1% agarose gel of mtDNA isolated from different seed batches of A188N (lanes A) and W64AN (lane N). Labelling as in previous figures. **B** Autoradiograph of a Southern blot of the gel after hybridization to a radioactive probe of pZmS21 and pZmS4

The predominance of the N type of cytoplasm in the ancient Mexican races is expected because US races evolved from material introduced from Central and South America. The finding of T and S types of mtDNA in Mexican races suggests that the T and S mtDNAs found in US stocks also came from Central America. The mitochondrial DNAs of the four cytoplasm groups are sufficiently different that it is highly unlikely that they evolved one from another in the US over very short time periods, e.g. as single generation mutants.

Several differences between the N type mtDNAs can be discerned from the patterns generated by Bam HI. Major subgroupings established in this paper are those based upon the lengths of the Bam HI fragment related to sequences in S1. Fragments of 6.85, 6.55 or 8.0 kb have been detected. Other variant mtDNAs have a 3.5 kb Bam HI fragment homologous to S2 instead of the more common 4.4 kb fragment. A recent study has indicated that mtDNAs isolated from Latin American maize races show restriction fragment patterns similar to, but not exactly the same as, those derived from US inbred lines (Weissinger et al. 1982).

In analyses of the US corn lines several low molecular weight, plasmid-like DNAs were found, viz. 'n', 't', S1 and S2 linear DNAs, the common 1.94 kb supercoiled plasmid and two circular plasmids in C cytoplasm (Kemble and Bedbrook 1980; Kemble et al. 1980). However, in the male fertile N group, no DNA species larger than 'n' were found. In the Mexican self-fertile lines of the N group many new low molecular weight DNA species are present. This is a very interesting collection of molecules worthy of detailed study. S1 and S2 probably originated from the main mitochondrial genome (Thompson et al. 1980). The finding of some homology between 'n', 't' and the HMW mtDNA raises the similar possibility that 'n' and 't' arose from sequences in the HMW mitochondrial genome. Alternatively it is formally possible that the HMW sequences showing homology to 'n' and 't' were originally integrated from low molecular weight DNA species. The corre-

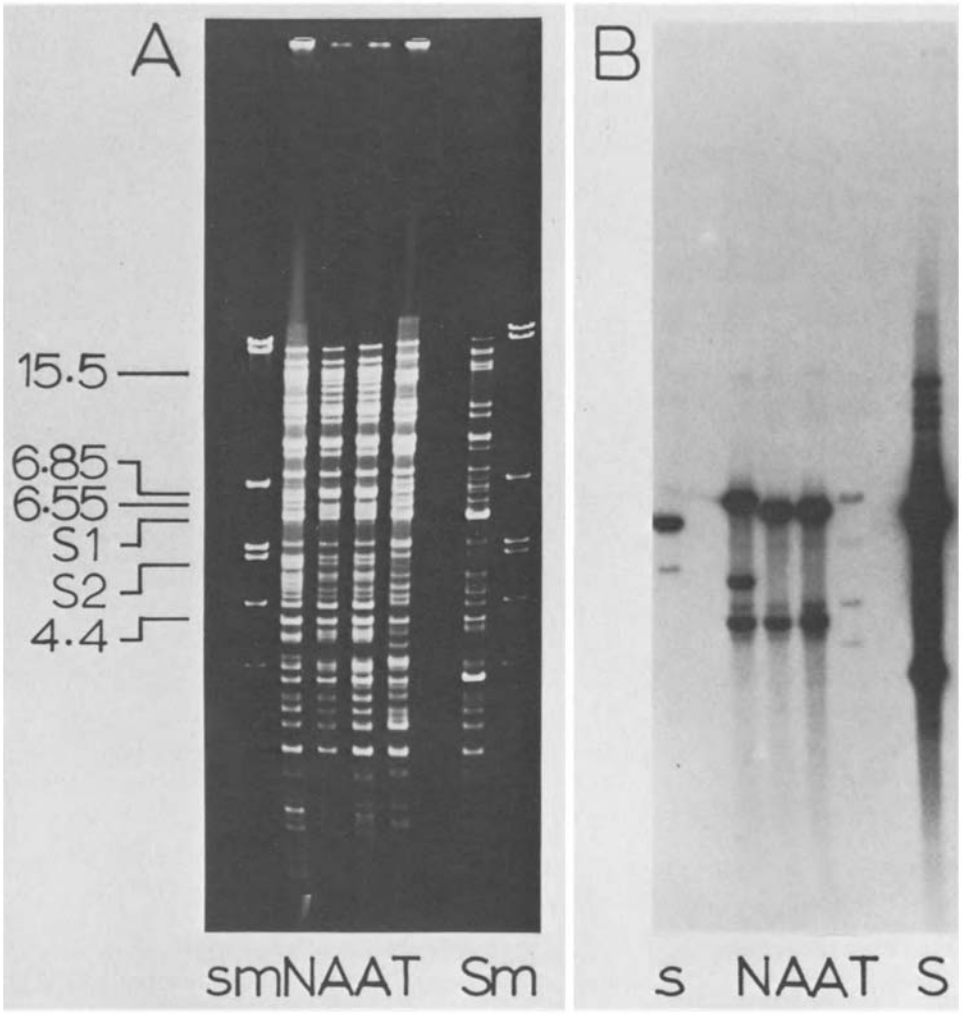

Fig. 10. **A** Electrophoresis on 1% agarose gel of Bam HI digests of mtDNA isolated from different seed batches of A188N and lines carrying N, T and S cytoplasm. Labelling as in previous figures. **B** Autoradiograph of a Southern blot of the gel after hybridization to pZmS21

lation between the 6.85 kb Bam HI fragment and 'n' and the 6.55 kb Bam HI fragment and 't' can be explained if the cytoplasmic lineages carrying the different HMW mtDNAs also happened to possess different linear DNA species or vice versa. Alternatively the correlation may be the result of some sort of recombination event between the related high and low molecular weight sequences.

Three other interesting aspects of maize mtDNA organization have also been reported for the first time here: (1) In maize from the USA, the linear DNA species 't' has been found only in mitochondria from plants with T cytoplasm. However, in Mexican races it is present in mitochondria with DNAs that resemble those of plants with N cytoplasm. (2) With the exception of Zapalote Grande, all of the maize stocks examined to date have the 1.94 kb supercoiled plasmid. The absence of this plasmid from Zapalote Grande implies that it is not indispensable. (3) Nineteen of the 25 Mexican races analysed showed no heterogeneity for 'n' or 't' DNA species or the integrated Bam H1 fragments homologous with them. The other six races were heterogeneous and in those cases where we sought the cause of heterogeneity we found evidence that it was between plants of the same accession. We also found variation in mtDNA between different accessions of the race Celaya, some of which appeared to contain S1 and S2. Further evidence of variation in mtDNA between different accessions of the same race has been found in Conico Norteno. Weissinger et al. (1982) found a restriction fragment pattern characteristic of S cytoplasm in an unidentified accession of this race, but we found no sign of S1 or S2 in the accession (Gto. 49) that we examined. It is perhaps significant that both of the races

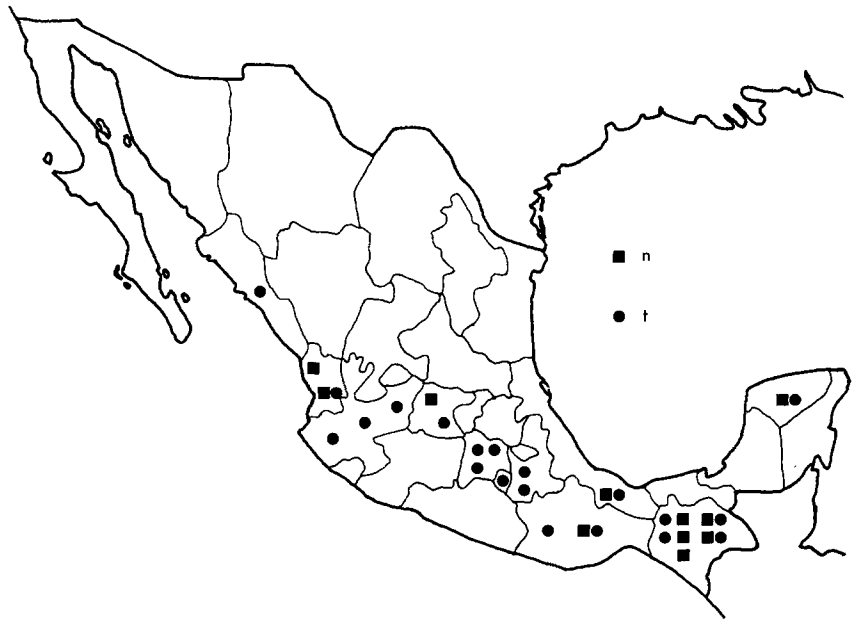

Fig. 11. A map of Mexico showing the sites where the accessions were collected and whether they contain the 'n' or 't' mtDNA species. The positions marked indicate the state in which the accession was found and not the exact location within the state

(Celaya and Conico Norteno) in which S type mtDNAs have been found evolved within historic times and there is evidence of introgressive hybridisation between them (Wellhausen et al. 1952). The existence of heterogeneity in mtDNA among plants of the same accession and between different accessions of the same race suggests that these particular DNA species have little or no effect on the characters that Wellhausen et al. (1952) used to define the races.

The geographical distribution of mtDNA variation

Twenty-one of the 25 races analysed possess mtDNA with the 6.55 kb Bam H1 fragment and the 't' DNA species (Table 1). This mtDNA structure is therefore probably the predominant one in Mexican races. Noting that it is present in all of the Ancient Indigenous and PreColumbian Exotic races, we conclude that it is probably also the oldest mtDNA structure in Mexico.

The states in which the accessions studied were collected are shown in Fig. 11. The number of accessions examined is small, especially those having the 'n' DNA species and 6.85 kb Bam H1 fragment. Therefore, any conclusions drawn from the geographical distributions must be treated with caution. However, it appears that plants with the 't' species and 6.55 kb Bam H1 fragment are distributed over most of the country, while plants with the 'n' DNA species and 6.85 kb Bam H1 fragment are concentrated in the coastal states. Only one of the accessions (Gto. 49, Celaya) from a central upland state had 'n' DNA species. It seems probable therefore that the cytoplasm characterised by the 6.85 kb Bam H1 fragment and 'n' DNA species spread northwards by the same sea routes that McClintock et al. (1981) described when they studied the geographical distribution of chromosome knobs in Mexican maize. This hypothesis is worthy of further investigation by studying many more accessions of known geographical origin from Central and South America.

mtDNA and racial-pedigrees

Using a combination of geographical, morphological, physiological and cytological data, Wellhausen et al. (1952) constructed pedigrees for many of the races of maize found in Mexico. In some cases they obtained additional information from observations made on artificially-produced hybrids between the putative parents of a race. The pedigrees of nineteen races are given in Table 3 which also indicates the mtDNA n/t and 6.85/6.55 Bam H1 fragment type of each race and its direct ancestors. These data show that the mtDNA types of 14 races (Conico, Arrocillo Amarillo, Pepitilla, Chalqueno, Conico Norteno, Jala, Bolita, Vandeno, Celaya, Tuxpeno, Zapalote Chico, Zapalote Grande, Comiteco and Tabloncillo perla) are in every case consistent with the mtDNA type of at least one of the probable parents of the race.

The finding of different mtDNAs within a race complicates this kind of analysis. However, the level of agreement between the few mtDNA components assayed here and the pedigrees determined by other characters suggests that it should be possible, using many more mtDNA sequences, to trace the evolution of mtDNA through the races. Four races we studied (Reventador, Tepecintle, Tehua and Olotillo) probably had teosinte (*Zea mexicana*) as a parent (Wellhausen et al.

Table 3. Relationship between mtDNA type and racial pedigrees

Parent race	mtDNA	Parent race	mtDNA	Derived race	mtDNA
Palomero Toluqueño	t	Cacahuacintle	t	Conico	t
Palomero Toluqueño	t	not known	–	Arrocillo Amarillo	t
Polomero Toluqueño	t	Vandeño	n+t	Pepitilla	t
Conico	t	Tuxpeño	n+t	Chalqueño	t
Conico	t	Celaya	n	Conico Norteño	t
Tabloncillo	t	Comiteco	n	Jala	t
Tabloncillo	t	Zapalote Chico	t	Bolita	n+t
Tuxpeño	n+t	Zapalote Grande	t	Vandeño	n+t
Tuxpeño	n+t	Tabloncillo	t	Celaya	n
Olotillo	n+t	Tepecintle	n	Tuxpeño	n+t
Nal-Tel	n+t	Tepecintle	n	Zapalote Chico	t
Zapalote Chico	t	Tehua	n	Zapalote Grande	t
Tehua	n	Oloton	t	Comiteco	n
Reventador	n	Tabloncillo	t	Tabloncillo subrace perla	n+t
Harinoso de Ocho	–	Reventador	n	Tabloncillo	t
teosinte[a]	–	Chapalote	t	Reventador	n
teosinte	–	"Harinoso de Guatamala"	–	Tepecintle	n
teosinte	–	"Harinoso de Guatamala"	–	Tehua	n
teosinte	–	"Harinoso Flexible"	–	Olotillo	n+t

[a] *Zea mexicana*

1952). The presence of 'n' mtDNA in all of these races (Table 3) suggests to us that teosinte will be found to have 'n' mtDNA. Tabloncillo probably arose from the hybridisation of Harinoso de Ocho with Reventador. We were unable to determine the mtDNA type of Harinoso de Ocho because seed of this race did not germinate, but our results (Table 3) indicate that it has 't' mtDNA.

Concluding remarks

Ever since the epidemic of southern corn-leaf blight (*Dreschlera maydis*) in 1969–70 when North American hybrid maize crops were devastated by a new race of the fungus that was adapted to all genotypes containing Texas (T) male-sterile cytoplasm, there has been concern to introduce more genetic diversity into the crop to protect it from future attacks by cytoplasm-specific pathogens (Ullstrup 1972). Texas male-sterile cytoplasm may no longer be in use for hybrid seed production, but the crop will remain just as potentially vulnerable to attack while the male- fertile (N) cytoplasms of hybrid varieties are so uniform in their content of mtDNA. Our finding that there is considerable variation in mtDNAs of Mexican races of maize indicates that the means of cytoplasmic diversification are at hand. The full extent of variation in mtDNA and its geographical distribution will not be known until a comprehensive survey of germplasm from all countries in which maize is indigenous has been made. It would be a major undertaking to carry out such a task, but the potential rewards are great, both for maize breeders and for students of the evolution of *Zea mays*.

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