PATTERNS OF MITOCHONDRIAL DNA VARIATION IN INDIGENOUS MAIZE RACES OF LATIN AMERICA

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ABSTRACT

Mitochondrial DNAs (mtDNAs) were isolated from 93 diverse races of maize from Latin America. DNAs were examined by agarose gel electrophoresis of undigested DNA and by *Bam*HI and *Eco*RI cleavage fragment analysis. Eighteen races contained plasmid-like mtDNAs. One race contained the S-1 and S-2 molecules associated with the S cytoplasmic male-sterile, and 17 were found to have the R-1 and R-2 plasmid-like DNAs. *Bam*HI digestion of mtDNAs generated ten distinct electrophoretograms, and *Eco*RI digestion produced eight different fragment patterns. Races were assigned to one of 18 groups according to *Eco*RI and *Bam*HI fragment patterns and whether or not they contained plasmid-like DNAs. Eight races produced restriction patterns similar to one of the characterized cytoplasmic male-steriles C, T, or S. Races from Meso-America and some from South America with Meso-American affinities were separated from other South American races. South American races were placed in three general classes of related groups. There was considerable agreement among the groupings here and those based on morphological and cytological affinities.

MAIZE (*Zea mays* L.) of Latin America is distributed over a wide geographical area and is extremely varied. Diverse environments and continued movement and manipulation by man have given rise to a multitude of distinct morphological types or races, many of which are interrelated. The extent of this variation and the interrelationships of these races provides an unparalleled source of experimental material for potential use in breeding and for studying facets of evolution in this important crop species.

The thousands of indigenous strains of maize were circumscribed to a workable level by a series of monographs (the so-called "race bulletins") which described the physical characteristics of each race (BRIEGER *et al.* 1958; BROWN 1960; GRANT *et al.* 1963; GROBMAN *et al.* 1961; HATHEWAY 1957; RAMÍREZ *et al.* 1960; ROBERTS *et al.* 1957; TIMOTHY *et al.* 1961, 1963; WELLHAUSEN *et al.* 1952, 1957). These works attempted to point out known relationships between races. The race bulletins, considered to be of a preliminary nature by their authors, have been bolstered by results from cytology (KATO 1976; MCCLINTOCK 1959,

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1960, 1978; McCLINTOCK, KATO and BLUMENSCHEIN 1981) and numerical taxonomy (GOODMAN 1968; GOODMAN and BIRD 1977; GOODMAN and PATERNIANI 1969). There have also been efforts to synthesize these various results to clarify racial relationships and origins (BROWN and GOODMAN 1977; GOODMAN 1978; MANGELSDORF 1974).

These studies have largely ignored the role of the cytoplasmic genomes in the evolution of maize (HARVEY, LEVINGS and WERNSMAN 1972). Even efforts to characterize male-sterile cytoplasms (BECKETT 1971; DUVICK 1965; GRACEN and GROGAN 1974) have been dependent upon the interaction of nuclear fertility restoration genes with the various male-sterile systems.

The advent of molecular techniques for isolation and characterization of organelle DNAs has made possible the systematic examination of cytoplasms. Electrophoresis of organelle DNAs, restriction endonuclease cleavage fragment analysis and electron microscopy of organelle DNAs have demonstrated that considerable variation exists between organelle DNAs within and among the major maize cytoplasmic groups N, C, S and T (KEMBLE, GUNN and FLAVELL 1980; LEVINGS and PRING 1976, 1977; LEVINGS *et al.* 1979; PRING and LEVINGS 1978; PRING, CONDE and LEVINGS 1980; THOMPSON, KEMBLE and FLAVELL 1980). Variation has also been found (TIMOTHY *et al.* 1979) in both the chloroplast and mitochondrial DNAs of teosinte, *Zea* spp. The variation of the teosinte organelle DNAs closely paralleled groupings based on plant morphology and evolutionary affinities. Finally, a survey of mtDNAs from 81 races of Latin American maize (WEISSINGER *et al.* 1982) showed a considerable amount of mtDNA variation that appears to follow the geographical distribution of the races. Plasmid-like mtDNAs, R-1 and R-2, similar to, but distinct from, those of the *cms*-S cytoplasm (PRING *et al.* 1977) were found in "RU" mitochondrial genomes of several of the races (WEISSINGER *et al.* 1982).

We used electrophoresis of unrestricted DNAs and restriction endonuclease fragment analysis to examine mtDNAs from a large array of maize races. These races represent the major portion of the morphological variation in Latin American maize. It was our purpose to determine (1) the scope of cytoplasmic variability, (2) whether this variation could be organized into a classification of racial cytoplasms, and (3) the extent to which mtDNA variation reflects racial affinities delineated by more conventional criteria.

MATERIALS AND METHODS

Purification of mitochondrial DNA: Mitochondria were isolated from coleoptile and mesocotyl tissue of dark-grown maize seedlings, and mtDNA was isolated by CsCl/ethidium bromide gradient centrifugation of sarkosyl/proteinase-K lysates of mitochondria as described (PRING and LEVINGS 1978). Purified DNA was precipitated with ethanol, dried and resuspended in 10 mM Tris/0.5 mM EDTA. In some cases, DNA samples were further purified by phenol extraction.

Restriction endonuclease digestion and agarose gel electrophoresis: MtDNA samples were digested with *Bam*HI and *Eco*RI (New England Biolabs, Inc. or Bethesda Research Laboratories, Inc.) according to the manufacturer's protocol for 1 hr at 37°. In addition to electrophoresis of digested samples, undigested mtDNA from each race was electrophoresed to test for the presence of plasmid-like mtDNAs. Approximately 1-μg samples of DNA were mixed with agarose beads (SCHAFFNER *et al.* 1976), loaded on horizontal 0.8% agarose (Seakem) gels (PRING and LEVINGS 1978) and electrophoresed for 17 hours at 40 VDC. After electrophoresis, gels were stained for 15 to 30 min in an

aqueous solution containing 0.5 μg/ml of ethidium bromide, illuminated with shortwave ultraviolet light and photographed through a Wratten 23A filter on Polaroid type 55 (positive/negative) film.

Gels were scored for the presence and intensity of the 40 restriction fragments of higher molecular weight when compared to the restriction patterns from the normal maize standard, B73 × Mo17, or the cytoplasmic male-sterile standards.

Maize stocks: Maize stocks represented a broad sample of races from Meso- and South America. Open-pollinated seed was produced from collections of maize tracing back to those originally classified in the Races of Maize bulletins (BROWN 1960; GRANT *et al.* 1963; GROBMAN *et al.* 1961; HATHEWAY 1957; RAMÍREZ *et al.* 1960; ROBERTS *et al.* 1957; TIMOTHY *et al.* 1961, 1963; WELLHAUSEN *et al.* 1952, 1957). Collections were initially increased by planting ear-to-row; the identity of these ear-row progeny was maintained in subsequent generations. Because the inheritance of mitochondrial DNAs is strictly maternal (CONDE, PRING and LEVINGS 1979), ear-to-row planting should preserve cytoplasmic variation within the original collections. In most cases, data were collected on one of the ear-row progenies of each collection. Complete data were collected on two random ear-row progenies for 20 of the collections.

The single cross hybrid, B73 × Mo17, was used as a normal (N) standard on electrophoretic gels. Antoher N cytoplasm, NC7 × T204, was occasionally used for comparison. Cytoplasmic male-sterile standards included B37 × NC236 (*cms*-C), SD (*cms*-S; BECKETT 1971) and T204 × NC236 (*cms*-T).

RESULTS

The mtDNAs examined produced one of ten different electrophoretic patterns when digested with *Bam*HI endonuclease and electrophoresed (Figure 1). These patterns are designated B1 to B10. Patterns B6 and B7 were identical with *Bam*HI resttriction patterns of the Nobogame and Central Plateau races of teosinte, respectively (TIMOTHY *et al.* 1979). Similary, *Eco*RI digestion and electrophoresis of all mtDNAs studied produced one of eight different banding patterns (Figure 2), which are designated E1 to E8. Figures 1 and 2 are composites from representative photographs of the *Bam*HI and *Eco*RI restriction patterns. Because several different gels were utilized in these composite photographs, bands having similar mobilities may not be perfectly aligned. Collective interpretations of the gels are presented in Tables 1 and 2.

Agarose gel electrophoresis of mtDNAs resolved a single, broad, slowly migrating band containing high molecular weight DNA in all collections surveyed. In addition, all mtDNAs contained small, circular and linear DNA molecules less than 2500 base pairs in size (KEMBLE and BEDBROOK 1980; KEMBLE, GUNN and FLAVELL 1980; LEVINGS *et al.* 1979).

The *Bam*HI and *Eco*RI digests and whole-mtDNA electrophoretograms formed a particular combination reflecting the organization of the mitochondrial genome of each race. These combinations were designated to indicate the *Bam*HI and *Eco*RI patterns and types of plasmid-like DNAs. For example, a group designated (B7, E5, R-1/R-2) produced *Bam*HI pattern B7 (Figure 1), *Eco*RI pattern E5 (Figure 2), and contained the R-1 and R-2 plasmid-like elements. Similarly, (B8, E1, 0) designates a mtDNA type that produces a *Bam*HI pattern B8, and *Eco*RI pattern E1, and contains no plasmid-like DNAs. In all, 18 such combinations were identified, each of which is associated with one or more cytoplasmic sources. Each group has been given a number (Tables 3 and 4) to facilitate discussion. Five of these combinations are associated with male-sterile cytoplasms (Table 3), which includes *cms*-S: Conico Norteño; *cms*-C: Araucano, Canilla Venezolano, Dzit Bacal and Nal-Tel BTA; and *cms*-T: Cariaco, Cuzco

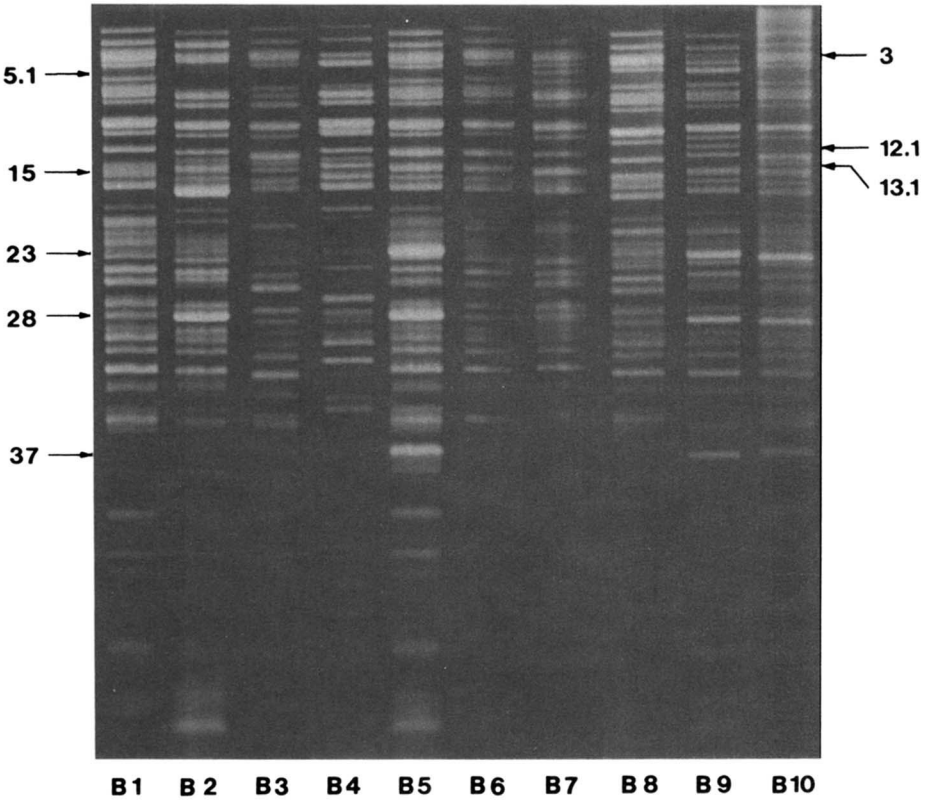

FIGURE 1.—Electrophoretograms produced by *Bam*HI restriction and electrophoresis of mtDNAs. B1, Unique pattern produced by B73 × Mo17. All numbering of *Bam*HI patterns based on this pattern. B2, Unique pattern produced by *cms*-S cytoplasm. Has brightly fluorescent bands 17 and 28. B3, Unique pattern produced by *cms*-C mtDNA. Represents substantial departure from pattern of N cytoplasm at several band positions. B4, Unique pattern produced by *cms*-T cytoplasm. Varies from pattern of N cytoplasm at several band positions. B5, Typical RU mtDNA pattern. Note absence of band 15 and bright fluorescence of bands 23, 28 and 37. B6, Identical with B5 except has bands 23, 28 and 37 of normal fluorescence. B7, Differs from B1 by presence of 5.1 band and absence of band 15. B8, Differs from B1 by slight difference in intensity of band 15. Characteristic of NC7 × T204, an N cytoplasm. B9, Coroico pattern. Note bright 23, 28 and 37, different mobility of band 3, and unique band 12.1. B10, Pollo pattern. Differs from B6 by presence of unique band 13.1.

and Kulli. The remaining groups more closely resemble normal (fertile) cytoplasms (Table 4).

The occurrence of the R-1 and R-2 plasmid-like DNAs previously characterized (WEISSINGER *et al.* 1982) has been extended to now include the races Chutucuno Chico, Kcello Ecuatoriano, Confite Puneño, Araucano and Serrano (Table 4). Other plasmid-like DNAs larger than 2500 bp were not demonstrated except for S-1 and S-2 of *cms*-S Conico Norteño (Table 3).

In 17 of the 20 cases, for which complete data were collected on two random ear-row samples, results were identical for both samples. In three, the samples differed, and the races were listed in two groups.

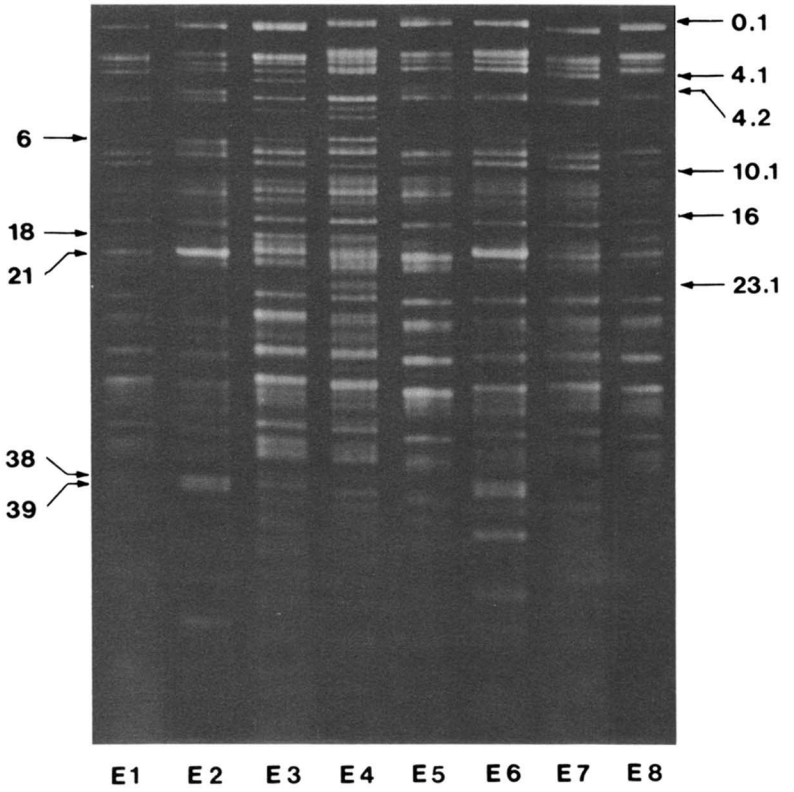

FIGURE 2.—Electrophoretograms produced by *Eco*RI restriction and electrophoresis of mtDNAs. E1, Unique pattern produced by mtDNA of (N) B73 × Mo17 mtDNA. Numbering of bands in all patterns based on E1. E2, Unique pattern produced by *cms*-S mtDNA. Note brightly fluorescent bands 21, 38 and 39. E3, Unique pattern produced by *cms*-C mtDNA. E4, Unique pattern produced by *cms*-T mtDNA. Note that E2, E3 and E4 represent substantial departures from the N mtDNA restriction patterns, suggesting substantial differences in mtDNA sequence arrangement. E5, Pattern characterized by presence of bands 0.1, 4.2 and 23.1. E6, Pattern produced by mtDNA of typical RU cytoplasm with R-1 and R-2 mtDNAs. Note absence of bands 6 and 18 and bright fluorescence of bands 21, 38 and 39. E7, Pattern typical of NC7 × T204. Differs from E1 by absence of bands 6 and 18. E8, Pattern produced only by Meso-American races Dzit Bacal and Nal-Tel BTA. Note presence of bands 4.1, 10.1 and faint band 23.1. Bands 6, 16 and 19 are absent.

DISCUSSION

We have examined mtDNAs from 93 races of Latin American maize, three known sources of cytoplasmic male sterility, and two normal standards. These diverse germplasms have been divided into 18 distinct groups on the basis of mtDNA restriction pattern data and the presence of plasmid-like mtDNAs. Because a broad survey has not been attempted previously for the mtDNAs of any single plant species, it is in order to examine the groupings. Differences among groups are more reliable than is the inclusion of a race within a specific group because differences in restriction patterns unequivocally denote differences in mtDNA sequence organization, but identical patterns do not prove that

TABLE 1

mtDNA BamHI *restriction fragment patterns of normal (fertile) races*

	BamHI restriction pattern						
Band	B1	B5	B6	B7	B8	B9	B10
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1
3	1	1	1	1	1	A	1
4	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1
5.1	0	0	0	1	0	0	0
6	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1
12.1	0	0	0	0	0	1	0
13	1	1	1	1	1	1	1
13.1	0	0	0	0	0	0	1
14	1	1	1	1	1	1	1
15	1	0	0	0	A	0	0
16	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1
22.1	1	1	1	1	1	1	1
23	1	B	1	1	1	B	B
24	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1
28	1	B	1	1	1	B	B
29	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1
37	1	B	1	1	1	B	B
38	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1

All of the mtDNAs examined produced one of ten different electrophoretic patterns. Bands below 40 could not always be scored unambiguously because of diffusion and are, therefore, excluded. Symbols: 1, band present; 0, band absent; A, band with slightly altered migration; B, band with exceptionally brilliant fluorescence.

TABLE 2

mtDNA EcoRI restriction fragment patterns of normal (fertile) races

	EcoRI restriction pattern				
Band	E1	E5	E6	E7	E8
0.1	0	1	0	0	0
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	1	1	1
4	1	1	1	1	1
4.1	0	0	0	0	1
4.2	0	1	0	0	0
5	1	1	1	1	1
6	1	0	0	0	0
7	1	1	1	1	1
8	1	1	1	1	1
9	1	1	1	1	1
10	1	1	1	1	1
10.1	0	0	0	0	1
11	1	1	1	1	1
12	1	1	1	1	1
13	1	1	1	1	1
14	1	1	1	1	1
15	1	1	1	1	1
16	1	1	1	1	0
17	1	1	1	1	1
18	1	0	0	0	0
19	1	1	1	1	1
20	1	1	1	1	1
21	1	1	B	1	1
22	1	1	1	1	1
23	1	1	1	1	1
23.1	0	1	0	0	1
24	1	1	1	1	1
25	1	1	1	1	1
26	1	1	1	1	1
27	1	1	1	1	1
28	1	1	1	1	1
29	1	1	1	1	1
30	1	1	1	1	1
31	1	1	1	1	1
32	1	1	1	1	1
33	1	1	1	1	1
34	1	1	1	1	1
35	1	1	1	1	1
36	1	1	1	1	1
37	1	1	1	1	1
38	1	1	B	1	1
39	1	1	B	1	1
40	1	1	1	1	1

All of the mtDNAs examined produced one of eight different electrophoretic patterns. Bands below 40 could not always be scored unambiguously because of diffusion and are, therefore, excluded. Symbols: 1, band present; 0, band absent; B, band with exceptionally brilliant fluorescence.

TABLE 3

Groups of male-sterile cytoplasms in Latin American maize races

Group	Electrophoretic patterns	Male-sterile cytoplasm or race	Accession no.	Country
		cms-S		
1	B2, E2, S-1/S-2	SD		
		Conico Norteño	GTO 22	Mexico
		cms-C		
2	B3, E3, 0	B37 × NC236		
		Araucano[a, b]	CHI 325	Chile
3	B3, E7, 0	Canilla Venezolano	VEN 604	Venezuela
4	B3, E8, 0	Dzit Bacal[a]	GUA 131	Guatemala
		Nal-Tel BTA[a]	GUA 161	Guatemala
		cms-T		
5	B4, E4, 0	T204 × NC237		
		Cariaco[a, b]	COR 338	Colombia
		Cuzco	BOV 725	Bolivia
		Kulli	BOV1004	Bolivia

Groups are designated according to *Bam*HI and *Eco*RI patterns and the presence and type of plasmid-like mtDNAs. Example: Group 3 designates mtDNAs producing *Bam*HI pattern B3 (Figure 1), *Eco*RI pattern E7 (Figure 2) and which is devoid of plasmid-like DNAs. S-1/S-2 indicates the presence of the S-1 and S-2 plasmid-like mtDNAs characteristic of the *cms*-S cytoplasm.

[a] Complete data were obtained for two random samples of that collection.

[b] Collection was placed into another group on the basis of data from a different random sample (see text).

the mtDNA structures are identical. The use of additional enzymes might further divide homogeneous groups.

Another question is whether our study adequately samples both the races in question and the total mtDNA variation present in Latin American races of maize. To sample racial variation so extensively, it was necessary in most cases to sample only a single representative collection of each race. Data taken on second ear-rows of almost a quarter of the collections surveyed suggest that collections are relatively homogeneous, so it is likely that little variation within collections had gone undetected.

This study does not address the frequency of male-sterile plants or whether their occurrence in a race is introduced or *de novo*. However, the finding of *cms*-C in Araucano and *cms*-T in Cariaco is important, in that these races are two of the three exceptions to complete agreement of the two random ear-row samplings from the 20 accessions. Araucano and Cariaco are included in groups 14 and 15, respectively, because fertility reflects the normal condition.

The only other case of nonagreement of the two samples involves a Colombian accession of Pollo in groups 7 and 18. Both groups contain the R-1 and R-2 plasmids but differ in digestion pattern.

The finding that a cytoplasmic male-sterile system can persist in indigenous

TABLE 4

Designation of cytoplasmic affinities among Latin American maize races

Group	Electrophoretic patterns	Race or genotype	Accession no.	Country
6	B1, E1, 0	B73 × Mo17 (N)		
7	B5, E6, R-1/R-2	Aragüito	VEN 678	Venezuela
		Chirimito[a]	VEN 529	Venezuela
		Chutucuno Chico	CHI 360	Chile
		Enano	BOV 1036	Bolivia
		Guaribero	VEN 653	Venezuela
		Kcello	BOV 948	Bolivia
		Kcello	ECU 768	Ecuador
		Marrón[a]	ARG 468	Argentina
		Mochero[a]	LBQ 14	Peru
		Morotí[a]	BOL II	Bolivia
		Pollo[a, b]	CUN 443	Colombia
		Racimo de Uva[a]	ECU 398	Ecuador
8	B6, E1, 0	Camba	BOV 1131	Bolivia
9	B6, E5, 0	Altiplano	BOV 903	Bolivia
		Chake-Sara	BOV 413	Bolivia
		Chullpi	HCA 69	Peru
		Confite Puneño	BOV 1002	Bolivia
		Granada	ANC 57	Peru
		Huayleño	ANC 181	Peru
		Karapampa	BOV 961	Bolivia
		Kculli	JUN 58	Peru
		Morado Canteño[a]	LIM 34	Peru
		Niñuelo	BOV 1088	Bolivia
		Pardo	LIM 19	Peru
10	B6, E5, R-1/R-2	Confite Puneño	PUN 6	Peru
11	B6, E7, 0	Blanco Blandito	ECU 523	Ecuador
		Cacao	SAS 335	Colombia
		Capio Chico Chileno	CHI 382	Chile
		Chococeño	CHO 314	Colombia
		Enano Gigante	ECU 969	Ecuador
		Güirua[a]	MAG 469	Colombia
		Huandango	ECU 623	Ecuador
		Nal-Tel ATB	GUA 281	Guatemala
		Pira	BOY 462	Colombia
		Pira	VEN 485	Venezuela
		Pojoso Chico	ECU 611	Ecuador
		Sabanero	SAN 329	Colombia
		Uchuquilla	BOV 303	Bolivia
12	B6, E7, R-1/R-2	Mishca[a]	ECU 321	Ecuador
13	B7, E5, 0	Andaquí[a]	CAQ 327	Colombia
		Arrocillo Amarillo[a]	PUE 91	Mexico
		Cacahuacintle	MEX 7	Mexico
		Camelia[a]	CHI 411	Chile
		Candela	ECU 699	Ecuador
		Canguil Grueso	ECU 447	Ecuador

TABLE 4—Continued

Group	Electrophoretic patterns	Race or genotype	Accession no.		Country
		Canguil	ECU	500	Ecuador
		Cateto	MG	II	Brazil
		Clavo	CHO	311	Colombia
		Conico	PUE	166	Mexico
		Curagua	CHI	301	Chile
		Cuzco	JUN	33	Peru
		Huevito	VEN	396	Venezuela
		Maíz Dulce	JAL	78	Mexico
		Montaña	NAR	426	Colombia
		Montaña	ECU	631	Ecuador
		Nal-Tel BTB	GUA	280	Guatemala
		Nal-Tel BTB	GUA	765	Guatemala
		Nal-Tel	YUC	7	Mexico
		Olotón	GUA	653	Guatemala
		Palomero Toluqueño	MEX	6	Mexico
		Pepitilla	MOR	17	Mexico
		Perola	BOV	350	Bolivia
		Pisankalla	BOV	344	Bolivia
		Quicheño Late[a]	GUA	945	Guatemala
		Salpor	GUA	476	Guatemala
		San Marceño	GUA	565	Guatemala
		Tabloncillo	JAL	42	Mexico
		Tepecintle	CHS	76	Mexico
		Tusilla	ECU	581	Ecuador
		Uchima[a]	ECU	746	Ecuador
		Zapalote Chico	OAX	48	Mexico
		Zapalote Chico	OAX	50	Mexico
		Zapalote Chico	OAX	51	Mexico
14	B7, E5, R-1/R-2	Araucano[a,b]	CHI	325	Chile
		Serrano	GUA	940	Guatemala
15	B8, E1, 0	NC7 × T204 (N)			
		Cabuya	SAN	317	Colombia
		Cariaco[a,b]	COR	338	Colombia
		Cariaco	VEN	408	Venezuela
		Dzit Bacal	GUA	127	Guatemala
		Harinoso de Ocho	NAY	24	Mexico
		Olotillo	CHS	56	Mexico
		Pira Naranja	NAR	369	Colombia
		Pollo	VEN	336	Venezuela
		Pororo	BOV	583	Bolivia
		Yunga	ECU	923	Ecuador
16	B8, E7, 0	Clavito[a]	ECU	884	Ecuador
17	B9, E6, R-1/R-2	Coroico	BOV	1063	Bolivia
18	B10, E6, R-1/R-2	Pollo[a,b]	CUN	443	Colombia

Groups are designated according to *Bam*HI and *Eco*RI restriction patterns, and the presence and type of plasmid-like mtDNAs. Example: Group 13 designates mtDNAs that produce *Bam*HI pattern B7 (Figure 1), *Eco*RI pattern E5 (Figure 2), and is devoid of plasmid-like DNAs. R-1/R-2 indicates the presence of the R-1 and R-2 plasmid-like molecules.

[a] Complete data were obtained for two random samples of that collection.

[b] The collection was placed into another group on the basis of data from a different random sample (see text).

maize races (WEISSINGER *et al.* 1982) is confirmed. It is noteworthy that these results were determined by examination of mtDNAs rather than by inference based upon the frequency of male gametes containing fertility restoration.

The frequency, 8.6%, of male-sterile accessions found was not unexpected (see DUVICK 1965). Eight races have been classified as cytoplasmic male-steriles on the basis of similarity between their mtDNA restriction patterns and cms standards (Table 3). Four of the eight have been tested for sterility and response to various nuclear fertility restoration alleles in the field. Conico Norteño (GTO 22) has the *cms*-S cytoplasm (WEISSINGER *et al.* 1982); Araucano (CHI 325) and Dzit Bacal (GUA 131) have the *cms*-C cytoplasm, and Kulli (BOV 1004) has the *cms*-T cytoplasm (S. NOBLE, Pioneer Hi-Bred International, personal communication).

The mtDNAs of the sterile cytoplasms are unique. The *Bam*HI pattern B2 has been produced by only *cms*-S cytoplasms and has only been found in conjunction with *Eco*RI pattern E2. The plasmid-like DNAs, S-1 and S-2, have been found only with the B2 and E2 patterns, *i.e.*, *cms*-S. The *Bam*HI pattern B4 has only been found in connection with *Eco*RI pattern E4, and neither has been associated with any cytoplasm other than *cms*-T. The *Bam*HI endonuclease fragment pattern B3 is associated with the *cms*-C cytoplasm, but B3 has been found in conjunction with *Eco*RI patterns E3, E7 and E8. Both E3 and E8 are uniquely associated with B3, but E7 is also associated with two other *Bam*HI patterns, B6 and B8. Neither B6 nor B8 has been associated with male-sterile cytoplasms. The heterogeneity found among the *cms*-C cytoplasms in this study is in keeping with the findings of PRING, CONDE and LEVINGS (1980), who demonstrated heterogeneity among *cms*-C cytoplasms from various sources.

There is considerable conservation of mtDNA sequence in Zea. Some differences in restriction patterns may reflect nucleotide changes in cleavage sites, but rearrangements appear to play a significant role in the evolution of the mitochondrial genome (SEDEROFF *et al.* 1981; SPRUILL, LEVINGS and SEDEROFF 1981).

The influence of the nuclear background on plasmid-like DNA content is well documented (LAUGHNAN and GABAY 1978; LAUGHNAN, GABAY-LAUGHNAN and CARLSON 1981; LEVINGS *et al.* 1980). It is possible that the small groups (10, 12, 14, 17, and 18) associated with the R-1 and R-2 plasmid-like DNAs reflect these phenomena. For example, in groups 10, 12 and 14, the brightly fluorescing bands of R-1 and R-2 digestion products noted in other digestion fragment patterns were not visible. Moreover, in one instance, there was appreciable and repeatable difference in intensity of electrophoretograms of the undigested plasmid-like DNAs between the two ear-row samples of Mishca. These distinctions appear to be due to differences in relative abundance of the plasmid-like molecules and could be mediated by nuclear genes.

Much of the mitochondrial genome may have noncoding functions (WARD, ANDERSON and BENDICH 1981). This situation may be analogous to that of chromosome knobs. Knobs have no known function, with the possible exception of abnormal–10, but they are extremely useful in studies of classification, introgression and migration of nuclear components (MCCLINTOCK 1959).

The patterns of mtDNA variation in teosinte closely parallel those of whole-

plant and cytological variation (TIMOTHY *et al.* 1979). Our data suggest a similar phenomenon in maize. The mtDNA groups show substantial agreement with racial affinities based on conventional methodologies.

The racial groupings by cytoplasmic distinctions provide several insights into maize evolution/migration. All Meso-American collections but one, Nal-Tel-ATB, are found only in three groups (13, 14 and 15). In maize, the *Bam*HI pattern B7 has been found only in association with *Eco*RI pattern E5 (groups 13 and 14). This *Bam*HI patterns is identical with that found in the teosinte races Central Plateau of Mexico and Huehuetenango of Guatemala (TIMOTHY *et al.* 1979). It is noteworthy that the only Meso-American accession found to contain R-1 and R-2 was Serrano in group 14. Groups 13 and 14 differ only by the presence of the plasmid-like DNAs. Furthermore, all of the pointed popcorns (MANGELSDORF 1974) and three of the four postulated ancient indigenous races from Mexico (WELLHAUSEN *et al.* 1952) were found only in the former group. Finally, most of the South American races in groups 13 and 14 have strong Meso-American affinities (BROWN 1960; BROWN and GOODMAN 1977; MANGELSDORF 1974; MCCLINTOCK 1978, MCCLINTOCK, KATO and BLUMENSCHEIN 1981; ROBERTS *et al.* 1957; WELLHAUSEN *et al.* 1957).

The predominant *Bam*HI South American mtDNA pattern (WEISSINGER *et al.* 1982) was divided by *Eco*RI digestion into two geographical/biological groups, 9 and 11. Group 9 corresponds to the Central Andean Complex (BROWN and GOODMAN 1977). It is a subset of the Andean Complex (MCCLINTOCK 1959) to which most of group 11 also belong. Currently, there is no apparent interpretation for the heterogeneous group 15 and the single-member groups 8 and 16.

Evolutionary and taxonomic implications: One factor that could contribute to apparent discord between cytoplasmic (mtDNA) groupings and presumed racial affinities is introgressive hybridization (introgression) between races with dissimilar cytoplasms. Hybrids made on "alien" females would carry the alien cytoplasm but would have a hybrid nuclear component. By repeated backcrossing to the native race, such progeny would more and more resemble the native race but would continue to have the alien cytoplasm because of strict maternal inheritance. If it is assumed that the alien cytoplasm was reasonably well adapted and that such crosses would likely be heterotic or might be selected by cultural preference, a substantial proportion of the individuals in the native race could eventually carry the alien cytoplasm. Individuals in this introgressed population might now appear identical with the native race but might be placed with the alien race in a classification based only on cytoplasmic characteristics, *e.g.*, mtDNA structure.

The cytoplasmic catalog: Hazards of genetic vulnerability have emphasized the need for genetic diversity to minimize the susceptibility of crops to the danger of epiphytotics. More recently, consideration has been given to the need for cytoplasmic diversity. The process of incorporating diverse cytoplasms into breeding materials has been hindered by lack of criteria by which cytoplasmic variation might be evaluated except by tests of fertility restoration in male-sterile cytoplasms. These tests fail to depict the range of cytoplasmic variation present and are limited to sterile cytoplasms.

Techniques now available offer a way in which fertile cytoplasms can be

classified. We have examined representatives of what is probably the most variable assemblage of maize extant, *i.e.*, the Latin American maize races. The study has disclosed a considerable range of cytoplasmic variation. The catalog thus produced (Tables 3 and 4) is the most comprehensive currently available to geneticists and breeders.

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