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ISOZYME VARIATION IN THE RACES OF MAIZE FROM MEXICO[1]

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ABSTRACT

Twelve plants each of 94 collections of maize (*Zea mays* L. subsp. *mays*) representing 34 races from Mexico were analyzed for 13 enzyme systems encoded by 23 loci. This analysis revealed an exceptionally high level of variation within and among the races. We recorded an average of 7.09 alleles/locus and an expected heterozygosity of 0.182. Seventy-two percent of the isozyme variation resided within collections, and 27% among collections. Races from northern and northwestern Mexico tend to possess higher levels of variation than those from the south. Variation for some isozyme alleles is strongly correlated with altitude. Maize is among the most variable species that have been studied isoenzymatically. Maize has levels of variation comparable to those found in its wild relatives, the teosintes. Principal component and cluster analyses showed continuous variation among the races with no well-defined racial complexes; however, three weakly differentiated groups were apparent: 1) the high-elevation Mexican pyramidal races, 2) the northern and northwestern races, and 3) most remaining races including the southern and western low-elevation dent and flour corns.

MAIZE (*Zea mays* L. subsp. *mays*) is the third most important cereal worldwide and the single most important food plant in Latin America. In Mexico and other Latin American countries, maize has a central role in the native cultures, being displayed with prominence and reverence in both religious ceremonies and secular festivals (Anderson, 1952; Mangelsdorf, 1974). The cultural importance of maize in Mexican society testifies to its long history of cultivation in this country. The oldest known archaeological maize ears were discovered near Puebla, Mexico, and were dated to the fifth millenium B.C. (MacNeish, 1967). This and other evidence have established Mexico as the cradle of maize domestication (Mangelsdorf, 1974). Not surprisingly, Mexico is also the homeland of the only close relative and probable wild ancestor of maize, teosinte (Wilkes, 1967; Doebley, 1983).

Maize is cultivated in nearly every district of Mexico from the desert northwest to the tropical lowlands of Chiapas. Over this ecogeographic landscape, maize has diversified into a multitude of forms, each adapted to the demands of its environment and the desires of its cultivators. Because the morphological-ecological variation among these forms is continuous in nature, the classification of Mexican maize into discrete categories is difficult. Despite these difficulties, Wellhausen, Roberts and Hernández (1952) produced a useful morphological classification of Mexican maize including the description of 29 races and subraces. This work and the more recent description of five additional Mexican maize races by Hernández and Alanis (1970) provide an understanding of the racial diversity in Mexican maize.

In this paper, we examine the pattern of isozyme variation among and within the races of maize of Mexico. The isozyme data are used to examine relationships among the races and to assess the amount of genetic variation within each race. Further, the correlations between isozyme variation and ecological-geographical parameters are discussed and the amount of genetic variation in Mexican maize, teosinte, and other plants compared.

MATERIALS AND METHODS—We examined 12 plants each from 93 collections of maize from Mexico and 1 collection from Guatemala (Ta-

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TABLE 1. *Races and collections of Mexican maize studied*

Race (abbreviation)	Collections[a]	Altitudes (meters)
Apachito (APA)	CHH 138, 177, 182, 207	—
Arrocillo Amarillo (A-A)	PUE 91	2,321
Azul (AZU)	CHH 218; 70-2[b]	—
Bofo (BOF)	DGO 110, 112; NAY 191, 203	—
Bolita (BOL)	OAX 28, 40, 44, 68	1,649, 1,557, 794, 1,649
Cacahuacintle (CAC)	MEX 7	2,657
Celaya (CEL)	GTO 29, 36, 84, 88	1,802, 1,741, 1,771, 1,802
Chalqueño (CHL)	HGO 7; MEX 48; PUE 82; ZAC 4	2,137, 1,832, 2,107, 2,260
Chapalote (CHP)	SIN 2	61
Comiteco (COM)	CHS 38, 86	2,046, 1,557
Cónico (CON)	MEX 58, 72; PUE 32, 109	2,840, 2,657, 2,199, 2,107
Cónico Norteño (C-N)	AGS 7; GTO 22; QRO 2; ZAC 12	2,168, —, 1,924, 1,954
Dulcillo del Noroeste (D-N)	SON 57	—
Gordo (GOR)	CHH 131, 160, 214, 256	—
Harinoso de Ocho (H-O)	NAY 24	46
Harinoso de Ocho Occidentales (HOO)	JAL 54, 71; NAY 29, 38	1,557, 1,557, 46, 46
Jala (JAL)	JAL 44, 69; NAY 6	1,405, 1,618, 1,099
Maíz Dulce (M-D)	JAL 78	1,893
Mushito (MUS)	OAX G43	—
Nal-Tel (N-T)	YUC 7	31
Olotillo (OLO)	CHS 52B, 53, 56, 81	580, 580, —, 687
Olotón (OLN)	GUA 45[c]	—
Palomero Toluqueño (P-T)	MEX 5, 6	2,657, 2,657
Pepitilla (PEP)	GRO 3; MOR 17	748, —
Reventador (REV)	NAY 15, 39	46, 46
Tablilla de Ocho (T-O)	JAL 301; NAY 185, 189; ZAC 187	—
Tabloncillo (TAB)	JAL 42, 43, 200, 102	1,252, 1,405, 1,893, 1,282
Tabloncillo Perla (T-P)	NAY 12, 16, 41	46, 46, 46
Tehua (TEH)	CHS 29, 159, 204, 234	—, 748, —, —
Tepecintle (TEP)	CHS 26, 76, 225	107, 580, 92
Tuxpeño (TUX)	OAX 9; PUE 27; VER 39, 44	92, 153, 107, 366
Vandeño (VAN)	CHS 30A, 31, 96, 112	611, 107, 61, 107
Zapalote Chico (Z-C)	OAX 48, 50, 51, 70	46, 107, 107, 46
Zapalote Grande (Z-G)	CHS 104, 224	92, 92

[a] Unless otherwise noted, the three-letter abbreviation represents the state of Mexico in which the collection was made.

[b] Collection received from David H. Timothy, Department of Crop Science, North Carolina State University.

[c] This collection is from Guatemala.

ble 1). Each collection is based on a variable number of ears (plants), rarely less than 6 or more than 12. The collections have been increased an unknown number of times since they were procured from farmers' fields. These 94 collections represent 34 races, 29 described by Wellhausen et al. (1952) and 5 described by Hernández and Alanis (1970). Up to 4 collections were chosen to represent each race. In selecting collections to study, we chose those classified as morphologically "typical" of their race (Wellhausen et al., 1952; Hernández and Alanis, 1970). Collections within each race were selected to best represent the geographic range of the race. Kernels were obtained primarily from two sources: Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico City, Mexico, and Instituto Nacional de Investigaciones Agrícolas, Chapingo, Mexico.

Each plant was analyzed for 13 enzyme systems encoded by 23 loci (Table 2). The electrophoretic techniques and genetic analyses have been described elsewhere (Cardy, Stuber and Goodman, 1980; Stuber and Goodman, 1983; Goodman and Stuber, 1983a). Gene diversity or expected panmictic heterozygosity was calculated for collections (H_s), races (H_r) and the total sample (H_t) for each locus as follows:

$$H = 1 - \Sigma x_i^2,$$

where x_i is the frequency of the i^{th} allele. Because sampling was balanced for loci and within collections, pooling over loci and within collections was performed without weighting. Pooling over races was weighted by the number of collections used per race. Relative genetic differentiation (G_{st} or G_{sr}) was calculated according to the method of Nei (1973, 1977).

Variation among races and collections was assessed using Genetic Identity (I) as defined

by Nei (1972) and a modified Rogers' distance (Wright, 1978). The matrix of modified Rogers' distances formed the basis for an average linkage cluster analysis. Principal component analysis was performed on the among-race variance-covariance matrix of the allele frequencies.

RESULTS AND DISCUSSION—*General genetic variation*—Table 3 lists the 23 loci examined, alleles observed at these loci, the frequency of each allele, and the number of collections and races in which each allele was observed. A total of 163 alleles was recorded with an average of 7.09 alleles per locus. For a sample of Bolivian maize (31 races: 101 collections) equivalent to our sample of Mexican maize, Goodman and Stuber (1983b) found many fewer alleles (5.17 per locus). Among a much smaller sample (245 plants) of *Z. mays* var. *parviglumis* (Balsas teosinte), the genetically most maizelike teosinte, Doebley, Goodman and Stuber (1984) recorded an average of 6.62 alleles per locus. Wendel and Parks (1985) report an average of 6.1 alleles/locus in *Camellia japonica.* Most plants examined have far fewer—2–4.8 alleles/locus (Gottlieb, 1981). While these data suggest Mexican maize is rich in allelic diversity as compared to other plants, caution is necessary because both variation in electrophoretic techniques and sample size strongly influence reported allele number.

The distribution of the 163 alleles among the 23 loci is not uniform. Three loci (*Got2, Mdh4, Mmm*) have only 3 alleles each, but *Glu1,* the most variable locus, has 18 alleles (Table 4). If one defines a polymorphic locus to be one at which the most common allele has a frequency less than 0.99 (Gottlieb, 1981), then 21 of the 23 loci examined (91.3%) are polymorphic. For *Z. mays* var. *parviglumis,* all (100%) loci were polymorphic by this criterion. The average for outcrossers is 51% (Gottlieb, 1981).

Most of the 163 recorded alleles are rare, being found in five or fewer collections and having frequencies of 0.01 or less (Fig. 1; Table 3). Conversely, few (17) alleles are ubiquitous and fewer still (10) have overall frequencies greater than 0.90. About 68 alleles have frequencies between 0.05 and 0.90 and occur in many but not all collections and races (Table 3). Variation in the frequencies of these alleles among races should be taxonomically informative, and, because there are many such alleles, the taxonomic information they provide should be meaningful.

The mean expected heterozygosity per collection (H_s) varied from 0.014 for *Got2* to 0.607 for *Glu1*. The average overall loci is 0.182. This value is substantially higher than the mean value for outcrossing species (0.086) reported by Gottlieb (1981), but somewhat lower than the closest wild relatives of maize, *Z. mays* var. *parviglumis* (0.231), and *Z. mays* subsp. *mexicana* (0.218) (Doebley et al., 1984). Curiously, the value of H_s for these Mexican maize collections obtained from germplasm banks is noticeably lower than H_s (0.229) for five collections of maize obtained directly from farmers' fields in Mexico and Guatemala (Doebley et al., 1984).

TABLE 2. *Loci studied and their chromosomal locations (from Goodman and Stuber, 1983a)*

Locus	Chromosome[a]	Locus	Chromosome[a]
Acp1	9	*Mdh1*	8
Adh1	1L	*Mdh2*	6L
Cat3	?	*Mdh3*	3L
Enp1	6L	*Mdh4*	1L
E8	3S	*Mdh5*	5S
Glu1	10	*Mmm*	1L
Got1	3L	*Me1*	3L
Got2	5L	*Pgd1*	6L
Got3	5S	*Pgd2*	3L
Idh1	8L	*Pgm1*	1L
Idh2	6L	*Pgm2*	5S
		Phi1	1L

[a] L = Long arm; S = Short arm.

Total panmictic heterozygosity (H_t) varied from 0.018 for *Mmm* to 0.841 for *Glu1* with the average overall loci being 0.251 (Table 4). Among other outcrossing plant species, values of this magnitude are found rarely even among the highly heterozygous conifers (Yeh, 1979). H_t is slightly higher for *Z. mays* var. *parviglumis* (0.311), *Z. mays* subsp. *mexicana* (0.304), (Doebley et al., 1984), and *Camellia japonica* (0.302) (Wendel and Parks, 1985) than it is for Mexican maize. *Coreopsis grandiflora* has a slightly lower value for H_t (0.223) (Crawford and Smith, 1984).

The single locus estimates of relative genetic differentiation (G_{st}) vary from 0.167 for *Mmm* to 0.419 for *Me1* (Table 4). The weighted average of the single locus values is 0.277. This value is high but within the range of values reported for F_{st}, a similar statistic (see Guries and Ledig, 1982; Wendel and Parks, 1985). This statistic indicated that the collections of Mexican maize are well differentiated from one another. Most conifers, which, like maize, have high levels of isozyme variation, have values for F_{st} below 0.05 (Guries and Ledig, 1982). This indicates that, unlike Mexican maize, conspecific populations of conifers are not well differentiated isoenzymatically.

TABLE 3. *List of alleles observed, their frequencies (p), and number of collections (NC) and number of races (NR) in which they were observed*

Locus-allele	p	NC	NR
Acp1-n	0.003	1	1
1	0.003	1	1
2	0.432	85	32
3	0.161	65	28
3.5	0.002	1	1
4	0.395	90	33
5	*	1	1
5.5	0.002	2	1
6	0.002	1	1
Adh1-n	0.011	3	3
4	0.986	94	34
5	*	1	1
6	0.003	1	1
Cat3-n	0.029	7	4
5	0.003	1	1
7	0.003	3	2
9	0.959	94	34
10.4	0.002	2	2
10.6	*	1	1
11.2	0.002	1	1
12	0.002	2	2
Enp1-n	0.056	11	10
2	0.004	4	4
4	0.080	40	21
5	0.004	3	3
6	0.781	94	34
6.2	0.001	1	1
8	0.025	20	15
10	0.042	19	15
14	0.007	6	5
E8-n	0.004	4	4
3	0.002	2	2
4	0.900	94	34
5	0.032	19	13
5.8	0.008	5	5
6	0.051	26	19
7	0.003	2	2
Glu1-n	0.110	28	19
1	0.191	13	9
2	0.255	73	30
2.5	0.030	24	15
3	0.040	24	14
3.2	0.002	2	2
3.5	0.003	2	2
4	0.006	3	3
5	0.036	23	15
6	0.206	71	29
7	0.166	67	32
7.8	0.008	7	7
8	0.008	7	6
10	0.091	44	23
11	0.004	1	1
12	0.003	2	2
13	0.009	11	6
16	0.003	2	2
Got1-n	0.003	1	1
1	0.003	2	2
1.2	0.003	3	3
4	0.890	94	34
5.8	0.012	8	8
6	0.089	44	24
7.5	0.001	1	1
Got2-2	0.003	3	3
4	0.991	94	34
6	0.006	4	3
Got3-n	0.003	1	1
2	0.006	3	3
3	0.003	1	1
4	0.971	94	34
6	0.019	9	7
7	0.001	2	2
Idh1-2	0.006	3	3
3	0.007	1	1
4	0.810	94	34
6	0.158	62	29
8	0.019	11	10
Idh2-2	*	1	1
3.8	0.007	5	4
4	0.675	94	34
4.1	0.004	1	1
4.2	0.006	2	2
5	*	1	1
6	0.304	77	31
7	0.001	1	1
7.5	*	1	1
7.8	0.003	2	2
Mdh1-n	0.014	3	3
0.05	0.006	4	3
0.1	0.002	2	2
1	0.054	23	15
2.8	0.002	3	3
6	0.898	93	34
6.4	0.011	2	2
9.2	0.003	5	5
10.5	0.024	17	11
Mdh2-0.4	*	1	1
3	0.402	92	33
3.5	0.14	55	28
3.8	0.004	3	3
5	0.004	1	1
5m	0.002	1	1
Mdh2-5.5	*	1	1
5.6	*	1	1
5.9	0.043	22	15
6	0.393	88	34
6.2	0.033	2	1
7.7	0.002	2	2
Mdh3-n	0.008	5	5
11.5	0.010	2	2
15.8	*	1	1
16	0.882	94	34
16.9	0.005	2	2
18	0.094	38	23
Mdh4-9	0.007	1	1
12	0.967	94	34
14.5	0.026	10	6
Mdh5-n	0.003	1	1
5.5	*	1	1
12	0.849	93	34

TABLE 3. *Continued*

Locus-allele	p	NC	NR
Mdh5-14.4	0.002	1	1
15	0.145	51	25
16	0.004	1	1
Me1-n	0.013	3	3
S	0.002	4	4
R	0.985	94	34
F	*	1	1
Mmm-m	0.991	94	34
mmm-m1	0.007	4	3
mmm-m3	0.002	1	1
Pgd1-n	0.010	6	6
0.5	0.002	1	1
1	*	1	1
1.8	0.008	6	4
2	0.324	87	33
3.8	0.655	94	34
Pgd2-n	*	1	1
2.8	0.004	1	1
5	0.989	94	34
8	0.003	2	2
10	0.002	1	1
11	0.001	1	1
Pgm1-1	0.005	2	2
7	0.015	5	5
9	0.819	94	34
9.5	0.001	1	1
16	0.123	65	31
16.5	0.035	26	19
19	0.002	1	1
21	*	1	1
Pgm2-0.45	0.004	2	2
0.5	0.006	3	3
3	0.048	21	11
4	0.919	94	34
7.2	0.003	2	2
7.3	0.001	1	1
7.5	0.008	2	2
8	0.010	6	5
12	*	2	2
Phi1-1	0.003	2	2
2	0.074	38	23
3	0.006	4	4
4	0.894	94	34
5	0.025	15	12

* $p < 0.0005$ but >0.0.

One locus, *Glu1*, greatly exceeds the other loci in both heterozygosity and number of alleles (Table 4). *Glu1* is also atypical in having a high frequency of null alleles (Table 3). In some cases, these "nulls" do not behave as true nulls in crosses, but instead revert to activity. In other cases, F_1's with one null parent will themselves be null, expression of the allele from the active parent having been inhibited. The mechanism(s) controlling nulls at *Glu1* is currently under study by one of us (C.W.S.).

TABLE 4. *Measures of genetic diversity in Mexican maize for 23 loci including number of alleles, mean expected heterozygosity per collection (H_s), total panmictic heterozygosity (H_t) and relative genetic differentiation (G_{st})*

Locus	Number of alleles	H_s	H_t	G_{st}
Acp1	9	0.461	0.631	0.269
Adh1	4	0.019	0.028	0.321
Cat3	8	0.053	0.080	0.338
Enp1	9	0.261	0.377	0.308
E8	7	0.149	0.187	0.203
Glu1	18	0.607	0.841	0.278
Got1	7	0.150	0.200	0.250
Got2	3	0.014	0.019	0.263
Got3	6	0.044	0.055	0.200
Idh1	5	0.244	0.318	0.233
Idh2	10	0.325	0.453	0.283
Mdh1	9	0.125	0.190	0.342
Mdh2	12	0.492	0.661	0.256
Mdh3	6	0.136	0.213	0.362
Mdh4	3	0.041	0.064	0.359
Mdh5	6	0.165	0.258	0.361
Me1	4	0.018	0.031	0.419
Mmm	3	0.015	0.018	0.167
Pgd1	6	0.339	0.466	0.273
Pgd2	6	0.019	0.025	0.240
Pgm1	8	0.240	0.313	0.233
Pgm2	9	0.108	0.154	0.299
Phi1	5	0.153	0.195	0.215
Mean	7.09	0.182	0.251	0.277[a]

[a] Mean weighted by H_s (Nei, 1977).

Apportionment of genetic variation—Nei (1973, 1977) describes a means of assessing the apportionment of variation among hierarchical categories within a species. We applied this method to assess the apportionment of variation among the races (r) and collections (s) of our total (t) sample of Mexican maize. H_s/H_t is 0.723, indicating that 72% of total variation resides within collections. Among conifers this value usually exceeds 0.90 (O'Malley, Allendorf and Blake, 1979; Yeh and Layton, 1979; Yeh and O'Malley, 1980; Wheeler and Guries, 1982; Guries and Ledig, 1982; Dancik and Yeh, 1983). H_s/H_t equals 0.88 for *Camellia japonica* (Wendel and Parks, 1985) and 0.68 for *Coreopsis grandiflora* (Crawford and Smith, 1984). G_{st} for Mexican maize equals 0.277, indicating that 28% of the variation occurs among collections. This may be further divided into variation among races ($G_{rt} = 0.124$) and variation among collections within races ($G_{sr} = 0.153$). These values indicate that there is perhaps slightly more variation among collections within races than among races. With other plants, researchers have also found greater variation within subspecific categories than among them (Wheeler and Guries, 1982; Crawford and Smith, 1984; Wendel and Parks, 1985).

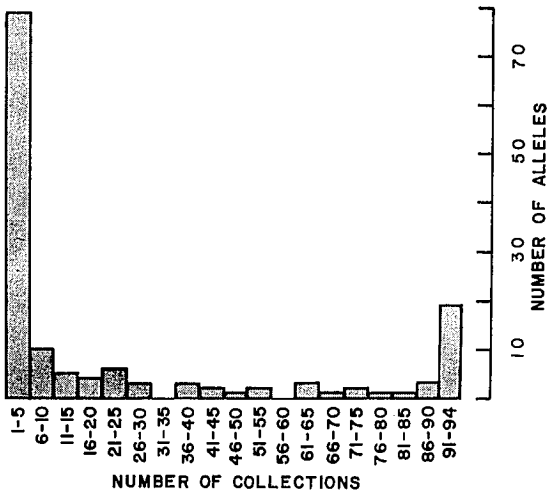

Fig. 1. Histogram showing the number of collections of Mexican maize in which the 163 recorded alleles occurred. For example, 79 alleles occurred in 1–5 collections and 20 alleles occurred in 91–94 collections.

Variation within races—Table 5 lists several measures of isozyme variation within each of the 34 races. These measures help identify those races that are rich in genetic variation. The average number of alleles per collection and the total number of alleles are highest in races Apachito, Celaya, Chalqueño, and Tablilla de Ocho. Olotillo, Tuxpeño and Zapalote Chico are the lowest. The former are mostly northern and high elevation races, and the latter mostly southern and low elevation. For the mean percentage of polymorphic loci per collection (PLP), the least variable races are the specialized ones known only from a few collections (Olotón, Palomero Toluqueño, Pepitilla, Reventador). Races with the largest proportion of polymorphic loci include Apachito, Cacahuacintle, Chapalote, and Comiteco (Table 5). Mean expected heterozygosity (H_s) is lowest in races Reventador (0.084), Azul (0.117), Naltel (0.137), Zapalote Grande (0.147), and Harinoso de Ocho (0.149). H_s is highest within races Apachito (0.219), Arrocillo Amarillo (0.214), Cacahuacintle (0.232), and Chapalote (0.247) (Table 5). Again, the general trend appears to be that the northern and high-elevation races are the most variable. Panmictic heterozygosity for each race (H_r) shows a similar pattern (Table 5).

Relative genetic differentiation (G_{sr}) measures the extent of heterogeneity among collections within races or the correlation between random gametes within collections relative to those of the entire race (Nei, 1977). The lowest values for G_{sr} are found among races from northwestern Mexico including Apachito (0.078), Azul (0.115), Gordo (0.085), and Tablilla de Ocho (0.097). G_{sr} is highest within races Jala (0.240), Tehua (0.251), Tepecintle (0.233), Tuxpeño (0.302), Vandeño (0.244), and Zapolote Grande (0.256). These are mostly lowland southern races. The disparate ecologies of these two groups of races may be associated with the apparent differences in the genetic structuring of their collections.

The large values of G_{sr} for the individual races is noteworthy. For most outcrossing plant species (Guries and Ledig, 1982; Wendel and Parks, 1985), values for G_{st} do not approach the 0.20 level seen commonly in Mexican maize races. This observation demonstrates that there is a surprisingly high level of isozyme variation in most of the Mexican maize races. In this sense, a single collection of a race does not fully represent the isozyme variation present in the race.

Variation among races—As mentioned above, 12.4% of the total variation resides among the races. A large part of this variation reflects differences in the frequencies of 68 alleles that are neither ubiquitous nor rare. To summarize this variation, a principal component analysis based on the variance-covariance matrix among the mean allele frequencies for each race was performed. Figure 2 is a graph of the first two components from that analysis. On this graph, the races do not segregate into well-defined complexes, but rather they form a continuum with several outliers. The most extreme outlier, Olotón, is represented by a single apparently inbred collection. This collection probably does not portray the array of variation present within this race, which is widely grown in Chiapas and neighboring Guatemala.

Within the main cluster of races in Fig. 2, there are some associations that agree well with morphological and ecogeographic data. First, races Arrocillo Amarillo (A-A), Cacahuacintle (CAC), Cónico (CON), Palomero Toluqueño (P-T), and Chalqueño (CHL) all lie near one another. Four of these races also segregate together in an average linkage cluster analysis based on the allele frequencies (Fig. 3). These are high-elevation races that share in common red hairy leaf sheaths, sparsely branched tassels, and conical-shaped ears (Wellhausen et al., 1952). This group has long been noted as a natural assemblage and was designated "Mexican Pyramidal" maize by Anderson and Cutler (1942). McClintock, Kato Y. and Blumenschein (1981) demonstrate that these races share a common pattern of chromosome knobs.

TABLE 5. *Measures of genetic diversity for Mexican maize races including mean alleles per collection (APC), total number of alleles, mean proportion of polymorphic loci per collection (PLP), mean expected heterozygosity per collection (H_s), total panmictic heterozygosity per race (H_T), and relative genetic differentiation (G_{ST})*

Race	APC	Total alleles	PLP[a]	H_s	H_T	G_{ST}
Apachito	45.5	61	0.58	0.219	0.237	0.076
Arrocillo Amarillo	43.0	43	0.49	0.214	0.214	—
Azul	35.5	43	0.37	0.117	0.132	0.114
Bofo	40.3	63	0.55	0.179	0.228	0.215
Bolita	38.3	55	0.48	0.187	0.236	0.207
Cacahuacintle	44.0	44	0.65	0.232	0.232	—
Celaya	42.3	66	0.55	0.187	0.232	0.194
Chalqueño	41.5	64	0.50	0.199	0.247	0.195
Chapalote	41.0	41	0.57	0.247	0.247	—
Comiteco	41.0	53	0.59	0.166	0.192	0.135
Cónico	40.0	61	0.57	0.191	0.235	0.188
Cónico Norteño	39.8	57	0.54	0.206	0.245	0.160
Dulcillo de Noroeste	37.0	37	0.52	0.181	0.181	—
Gordo	42.8	55	0.52	0.198	0.217	0.088
Harinoso de Ocho	37.0	37	0.48	0.149	0.149	—
Harinoso de Ocho Occidentales	39.8	57	0.53	0.205	0.246	0.167
Jala	36.7	49	0.42	0.181	0.238	0.240
Maíz Dulce	36.0	36	0.44	0.161	0.161	—
Mushito	38.0	38	0.35	0.164	0.164	—
Nal-Tel	33.0	33	0.35	0.137	0.137	—
Olotillo	37.0	52	0.48	0.171	0.222	0.230
Olotón	33.0	33	0.39	0.106	0.106	—
Palomero Toluqueño	38.0	44	0.39	0.166	0.200	0.169
Pepitilla	37.5	46	0.37	0.164	0.202	0.188
Reventador	31.0	37	0.30	0.084	0.103	0.185
Tablilla de Ocho	45.3	64	0.54	0.200	0.221	0.095
Tabloncillo	37.3	52	0.44	0.173	0.202	0.144
Tabloncillo Perla	37.7	51	0.45	0.167	0.201	0.169
Tehua	39.0	62	0.50	0.188	0.251	0.251
Tepecintle	38.0	52	0.52	0.183	0.238	0.231
Tuxpeño	36.3	57	0.45	0.161	0.230	0.302
Vandeño	37.8	58	0.49	0.201	0.266	0.244
Zapalote Chico	37.8	54	0.47	0.184	0.231	0.203
Zapalote Grande	37.0	43	0.44	0.147	0.198	0.258

[a] 99% criterion.

A similar group was identified by Cervantes et al. (1978) in their study of general combining ability and genotype by environment interactions. However, their group also included race Pepitilla, which is clearly different in isozyme pattern. It is important to note that Pepitilla occurs at a lower elevation than the other members of this complex. Brown and Goodman (1977) also define a racial complex that agrees in general with our isozyme high-altitude group. However, they also include Pepitilla, but they note that it is atypical and may include germplasm from lower altitudes.

A second, less well-defined group of races apparent in Fig. 2 includes Cónico Norteño (C-N), Gordo (GOR), Apachito (APA), and Azul (AZU). Three members of this group also segregate together in the cluster analysis (Fig. 3). These races are all from northern or northwestern Mexico. The plants are small, short-seasoned, and, with the exception of Cónico Norteño, have long slender ears that are tapered at the base. In Fig. 2, races Nal-Tel (N-T) and Maíz Dulce (M-D) lie near this northern complex. Nal-Tel is certainly out-of-place here. This race occurs only in the lowlands of southern Mexico and Guatemala. Probably, the single collection of Nal-Tel we have examined does not accurately reflect the affinities of this race. Maíz Dulce is also represented by a single collection, which may not accurately represent its relationship to other races. Some have suggested that Maíz Dulce belongs to the high-altitude racial complex (Cervantes et al., 1978; Yakoleff et al., 1982).

Reventador (REV) and Harinoso de Ocho (H-O), which are thought to be related, lie near one another in both Fig. 2 and 3. These two races are presumed to be related to the northern complex just discussed (Brown and Goodman, 1977). However, the isozyme data suggest that they are more divergent from the northern races

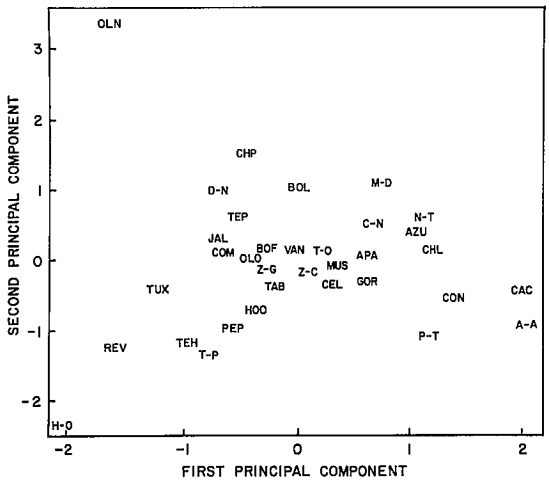

Fig. 2. Graph of the first two axes from a principal component analysis based on racial means of the allele frequencies.The first component contains 21.8% of the total variation and the second 12.1%. For key to abbreviations, see Table 1.

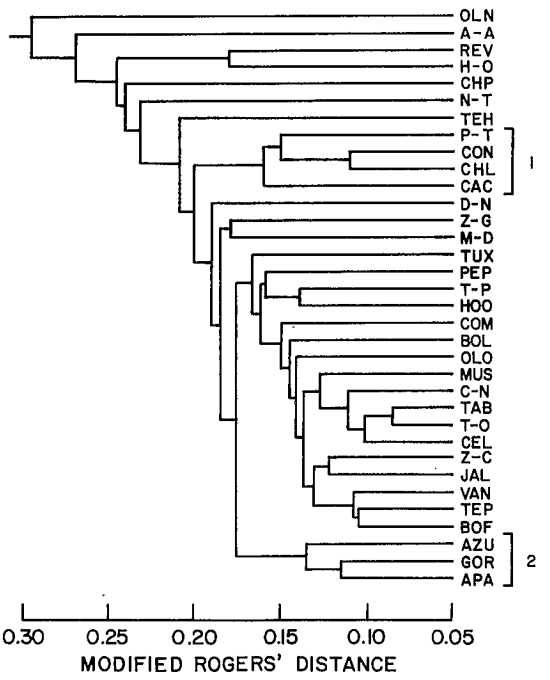

Fig. 3. Average linkage cluster analysis performed using modified Rogers' distance (Wright, 1978). 1 = races of Mexican pyramidal complex; 2 = races of Northwestern Mexico. For key to abbreviations, see Table 1.

than any other race analyzed. Morphology indicates that Chapalote (CHP), Harinoso de Ocho Occidentales (HOO), Tabloncillo (TAB), and Tabloncillo Perla (T-P) should be placed with Harinoso de Ocho and Reventador. Again, the isozyme data do not support segregating the former races (the Chapalote complex) from others such as Tuxpeño (TUX), Tehua (TEH), Pepitilla (PEP), and Comiteco (COM).

Another complex of ecologically and morphologically similar races includes Tuxpeño, Vandeño (VAN), Celaya (CEL), Zapalote Chico (Z-C), Zapalote Grande (Z-G), Bolita (BOL), and Tepecintle (TEP). This (the Tuxpeño complex) is a rather diverse group of dent corns, most with large plants and highly branched tassels. They grow at low to medium elevations. This group occupies the central portion of Fig. 2. They overlap with the Chapalote complex but are distinct from the northern and Mexican pyramidal complexes.

There are several races whose affinities to other races have been traditionally uncertain and deserve separate discussion. Olotillo (OLO) has been tentatively aligned with Harinoso de Ocho (Brown and Goodman, 1977) and hypothesized to be derived in part from South American races (Wellhausen et al., 1952). Isozyme data show it to be similar to other low-row-number, low-altitude, Mexican flour corns, including Tabloncillo and Harinoso de Ocho Occidentales.

Cacahuacintle (CAC) has usually been placed in the high-elevation Mexican pyramidal complex, but only with reservations (Brown and Goodman, 1977). Wellhausen et al. (1952) state that it was introduced from "the South." Isozyme data support its relationship to other high-elevation Mexican races.

As discussed above, Pepitilla has usually been placed in the high-elevation complex (Mexican pyramidal), although it grows at middle altitudes (Brown and Goodman, 1977). Our data do not support placing Pepitilla in the high-elevation complex. Rather, it appears associated with other middle-elevation flour corns, such as Tehua, Zapalote Grande, and Zapalote Chico.

Lastly, as a measure of similarity among the races, we calculated a matrix of genetic identities, I (Nei, 1972). Among all races, I is 0.948 (0.872 to 0.989). This value is comparable to I among populations of a single species (Gottlieb, 1981; Crawford, 1983).

Correlations between geography and isozymes—In the preceding discussion, we have mentioned the apparent correlation between isoenzymatic variation and altitude. In this section, we specifically examine this correlation as well as the correlation to latitude and longitude. Table 6 lists the significant correlations of individual allele frequencies with the altitude for the collections. The frequencies of

TABLE 6. *Significant Spearman correlation coefficients ($\hat{r}$) of allele frequency with altitude for Mexican maize collections. P = probability under the null hypothesis of r = 0. N = 64*

Locus-allele	$\hat{r}$	P
Idh2-6	0.507	0.000
Idh2-4	−0.488	0.000
*Glu1-13**	0.417	0.001
Acp1-2	0.410	0.001
Phi1-2	0.363	0.003
*Pgd1-1.8**	0.362	0.003
*Glu1-8**	0.356	0.004
Mdh5-15	0.345	0.005
Mdh2-6	0.341	0.006
Glu1-n	0.337	0.007
*Got3-6**	0.334	0.007
Mdh5-12	−0.319	0.010
Glu1-2	−0.313	0.012
Phi1-4	−0.313	0.012
*Mdh1-0.05**	−0.313	0.012
Acp1-3	−0.302	0.015
*Glu1-3.5**	0.283	0.024
*Got3-3**	0.282	0.024
Glu1-7	−0.265	0.034
E8-6	0.258	0.040
*Got3-2**	−0.253	0.044
Glu1-10	0.251	0.045

* Frequency of allele <0.05.

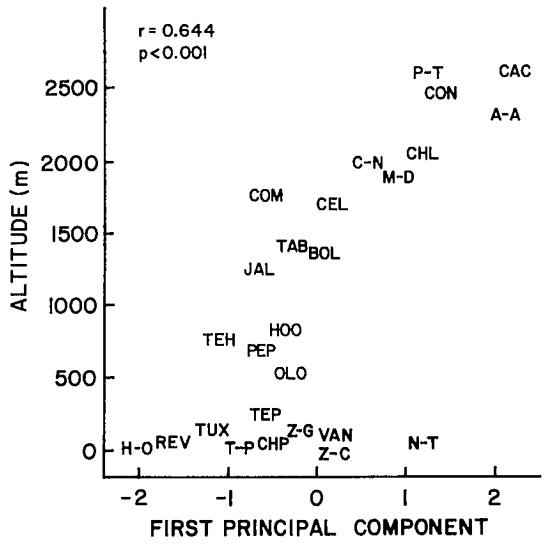

Fig. 4. Graph of the first principal component from Fig. 2 against altitude for 26 of the races analyzed.

22 alleles are significantly correlated with altitude. For Bolivian maize, Goodman and Stuber (1983b) report 12 alleles significantly correlated with altitude. Only 2 alleles, *Acp1-3* and *Glu1-2,* show significant correlations in both studies. For both alleles, the correlation is negative in Bolivia and in Mexico. As more data are gathered and analyzed, it will be possible to test whether the same alleles show the same correlations with altitude in different geographic regions.

The general correlation of isozyme data with altitude is graphically portrayed in Fig. 4. Here, a strong correlation ($\hat{r} = 0.644$) is shown between the first principal component of Fig. 2 and altitude. Nal-Tel is an extreme outlier. Figure 4, along with Table 6, reveals that isozyme variation for Mexican maize can be largely accounted for by altitude. In Mexico, altitude is predominately associated with changes in precipitation, length of growing season, and temperature. In general, the low altitudes are hot and moist; the high altitudes are drier and relatively cooler with a shorter growing season (see Doebley, 1984).

The frequencies of some alleles also show significant correlations with latitude and longitude (Tables 7, 8). However, neither the first nor the second principal component of Fig. 2 showed a significant correlation with either latitude or longitude. For latitude vs. PCA1, $\hat{r} = -0.12$ ($P[r = 0] \geq 0.56$); for latitude vs. PCA2, $\hat{r} = -0.13$ ($P[r = 0] \geq 0.51$); for longitude vs. PCA1, $\hat{r} = -0.17$ ($P[r = 0] \geq 0.40$); for longitude vs. PCA2, $\hat{r} = -0.25$ ($P[r = 0] \geq 0.21$). This can be explained because latitude and longitude in Mexico are only weakly associated with environment and the associations that exist tend not to be strictly linear.

CONCLUDING REMARKS—Our analysis of isozyme variation within and among the races of maize of Mexico provides a view of Mexican maize hitherto unavailable. The maize of Mexico, like maize elsewhere, is rich in allelic diversity (Doebley, Goodman and Stuber, 1983; Goodman and Stuber, 1983b). This diversity exceeds that found in the vast majority of plant species that have been surveyed isoenzymatically. Similarly, the level of heterozygosity in maize is over twice that of the average outcrossing species and is roughly equivalent to that amount found in the highly heterozygous conifers (Yeh, 1979; Guries and Ledig, 1982). The long-lived perennial growth form of conifers has been suggested as contributing to their high degree of heterozygosity (Hamrick, Mitton and Linhart, 1979). Obviously, this is not the case with maize, an annual herb, and the factors contributing to the exceptional genetic variation of maize are not known. Its monoecy and nearly complete outcrossing are probably contributing factors.

Johns, Strommer and Freeling (1983) demonstrated that the degree of variation in restriction sites near *Adh1* among maize lines

TABLE 7. *Significant Spearman correlation coefficients ($\hat{r}$) of allele frequencies to latitude for Mexican maize collections. P = probability under the null hypothesis of r = 0. N = 66*

Locus-allele	$\hat{r}$	P
*E8-5**	−0.478	0.000
Mdh2-3	−0.381	0.002
*Mdh1-0.05**	0.324	0.008
Pgd1-2	−0.318	0.009
Pgd1-3.8	0.317	0.010
Mmm-m	0.291	0.018
*Idh2-4.2**	0.286	0.020
*Cat3-7**	−0.266	0.031
*Pgm1-7**	−0.264	0.032
*mmm-m1**	−0.261	0.034
Glu1-6	−0.257	0.037
Glu-2	0.251	0.043
Got2-4	0.244	0.048

* Frequency of allele <0.05.

TABLE 8. *Significant Spearman correlation coefficients ($\hat{r}$) of allele frequencies with longitude for Mexican maize collections. P = probability under the null hypothesis of r = 0. N = 66*

Locus-allele	$\hat{r}$	P
*E8-5**	−0.508	0.000
Mdh2-3	−0.388	0.001
*Mdh1-0.05**	0.328	0.007
*Got3-2**	−0.314	0.010
*Idh2-4.2**	0.290	0.018
*Cat3-7**	−0.289	0.019
Enp1-4	−0.276	0.025
*Pgm1-7**	−0.266	0.031
*Mdh1-10.5**	0.262	0.034
E8-6	0.253	0.041
Mdh2-3.5	0.251	0.041
*Mdh1-6.4**	−0.248	0.045
Phi1-5	−0.244	0.048

* Frequency of allele <0.05.

equalled that found among well-diverged *Drosophila* species. Similar results were obtained for sucrose synthetase by Burr et al. (1983). These studies concur with our isozyme work and together establish maize as one of the genetically most variable plant species.

Variation in Mexican maize is not equitably distributed among the loci studied. *Got2, Mmm, Me1,* and *Adh1* are the least variable loci. Each is essentially monomorphic in Mexico, though each has two or three rare allelic variants. *Glu1,* with 18 alleles in Mexico alone, is the most variable locus. This locus also has an unusual system of null alleles that is not yet fully understood. One is left to wonder whether the unusually high degree of polymorphism at this locus can all be attributed to amino acid substitutions or if other mechanisms such as transposition may be involved.

Mexican maize is similar to conifers in possessing a high degree of isozyme variation, but the variation is structured differently in these two taxa. In most wide-ranging species of conifers, populations of a single species are relatively poorly differentiated isoenzymatically, with F_{st} or G_{st} generally less than 0.05 (Guries and Ledig, 1982). For Mexican maize, differentiation among collections is far greater. *Camellia japonica* seems to resemble maize more closely in the genetic structuring of its populations (Wendel and Parks, 1985). *Coreopsis grandiflora* resembles maize in that G_{st} is large in both; however, it differs from maize by its lower level of heterozygosity (Crawford and Smith, 1984).

It has been suggested that the domestication process or the switch from natural to artificial selection in maize could contribute to its exceptional genetic diversity (Johns et al., 1983). For the isozyme data this does not appear to be the case, since levels of variation in maize do not exceed those found in teosinte (Doebley et al., 1984). Rather, it seems that a high level of genetic diversity is characteristic of most taxa of *Zea.*

Isozyme data allow an examination of the amount of variation within and among races and collections. As expected (based on studies of other plant species), most variation resides within collections. Surprisingly, however, substantial variation exists among the collections of any one race. This indicates that a single collection may not adequately represent its race for isozyme variation. Our data also indicated that germplasm bank collections may be somewhat less variable than maize in the farmers' fields in Mexico; however, this question needs to be examined with a larger sample of field collections.

The isozyme data also prove useful in the understanding of systematic relationships among the races. The data show that divergence among the races is equivalent to that normally found among populations of a single species. This is expected given that maize probably was domesticated between 7,000 and 10,000 years ago, and thus, the races have rather short evolutionary histories. Nevertheless, three complexes of morphologically similar races could be defined on the basis of isozymes: 1) the high-elevation, Mexican pyramidal complex; 2) the northern complex, including Azul, Apachito, and others; and 3) the remaining bulk of races, including the southern and western lowland dents and flours, such as Tuxpeño and Tabloncillo.

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