

EXCEPTIONAL GENETIC DIVERGENCE OF NORTHERN FLINT CORN¹

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ABSTRACT

Isozyme analysis of 18 Northern Flint populations supports the previously published hypothesis that Northern Flint was derived from maize races of the southwestern U.S. and northwestern Mexico. Isozyme data also show that during its short evolutionary history (approx. 1,000 yr) Northern Flint obtained a degree of genetic differentiation from other North American maize landraces more typical of a separate species than of landraces of the same cultigen. Genetic drift, changes in selection pressure associated with movement into a new environment, and reproductive isolation from other maize races may have contributed to the genetic dissimilarity of Northern Flint.

MAIZE OR CORN (*Zea mays* L. ssp. *mays*) is composed of approximately 250 land races which are native to the New World (Brown and Goodman, 1977). Most of these have restricted geographic distributions and are of little economic importance; only a few races are wide-spread, yield-well and account for most maize that enters into world commerce. Chief among the latter is Northern Flint Corn of eastern North America. This race and one other (Southern Dent) have contributed virtually all of the germplasm found in today's mid-western dent corns which account for about 50% of world corn production. In this paper, we examine the origin of Northern Flint by means of enzyme electrophoresis.

The origin of Northern Flint was a source of controversy during the 1950's and 60's, but the debate was never resolved. The problem has received little attention during the past 20 years and no new evidence bearing on the problem has been published. There are two hypotheses for the origin of Northern Flint. The first, articulated by Anderson and Brown, maintains that Northern Flint was derived from highland Guatemalan races (Brown and Anderson, 1947; Anderson and Brown, 1952). Anderson and Brown note that highland Gua-

temalan races morphologically resemble Northern Flint, but these authors are admittedly at a loss to explain how maize could have been transferred over such a tremendous distance without leaving a shred of evidence.

The second hypothesis, espoused by Galinat, maintains that the Northern Flint was derived from similar long-eared corns of northwestern Mexico and the southwestern U.S. (Galinat and Gunnerson, 1963; Galinat and Campbell, 1967). This provides a route of diffusion across the Great Plains, but it does not account for the fact that the long-eared southwestern races have many enlarged regions of heterochromatin (called knobs) on their chromosomes, while Northern Flint does not (Brown and Anderson, 1947). The highland Guatemalan races (and many other highland tropical corns), like Northern Flint, tend to lack chromosome knobs. Both Anderson and Brown (1952), on the one hand, and Galinat, on the other, have invoked the archaeological record to support their hypotheses.

MATERIAL AND METHODS—From 18 Northern Flint⁴ populations (Table 1), 12 plants each were examined for 13 enzyme systems encoded by 23 loci. Electrophoretic methods (Cardy, Stuber, and Goodman, 1983; Stuber and Goodman, 1983) and genetics of the loci examined (Goodman and Stuber, 1983) have been

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⁴ We refer to these 18 populations as Northern Flint, while, in fact, some have floury endosperm. A distinction is often made between the Great Plains Flints & Flours and the Northeastern Flints. At other times, both are subsumed under the name Northern Flint. We have followed the latter nomenclature.

TABLE 1. *List of groups analyzed, collections in each group and the principal component scores for Fig. 1*

Group or race (abbreviation)	Collections ^a	PC1	PC2
A. Northern Flints			
Assiniboine (ASB)	USDA: 213793	1.56	0.23
Blackfoot (BLK)	USDA: 213760, 213763	1.78	-0.23
Cheyenne (CHY)	USDA: 213748, 213749	2.34	-0.53
Gaspe and Canada Flint (GSP)	USDA: 214279, 401757; WCG: 83-304	1.69	-0.33
Longfellow and Smut Nose (LNG)	USDA: 214195, 217408, 222490	1.94	-0.57
Rhode Island and King Phillip (RHD)	WCG: 82-32, 83-307-2, 83-EB7A-3	2.28	-0.53
Sioux (SOX)	USDA: 213770, 401755	1.81	0.10
Winnebago (WIN)	USDA: 213771, 213774	1.44	-0.57
B. Northern and high elevation Mexico			
Apachito (APA)	b	-0.59	0.61
Azul (AZU)	b	-0.48	1.36
Blandito de Sonora (B-S)	CIMMYT: Son 32	-0.33	0.52
Celaya (CEL)	b	-0.60	-0.28
Chalqueno (CHL)	b	-0.60	-1.08
Chapalote (CHP)	CIMMYT: Sin 2, Son 55	-0.39	0.52
Conico (CON)	b	-0.40	1.30
Conico Norteno (C-N)	b	-0.42	0.26
Cristalino de Chihuahua (C-C)	CIMMYT: Son 21, 51	-0.21	-0.04
Gordo (GOR)	b	-0.70	0.68
Palomero Toluqueno (P-T)	b	-0.74	0.68
Tablilla de Ocho (T-O)	b	-0.54	0.01
C. Southern or low elevation Mexico.			
Bolita (BOL)	b	-0.73	0.44
Harinoso de Ocho (H-O)	b	-1.36	-2.45
Harinoso de Ocho Occidentales (HOO)	b	-0.53	-0.76
Jala (JAL)	b	-0.84	-0.76
Nal-Tel (N-T)	b	-0.37	-1.85
Olotillo (OLO)	b	-0.69	0.40
Reventador (REV)	b	-0.72	-1.69
Tabloncillo (TAB)	b	-0.70	-0.41
Tehua (TEH)	b	-0.84	-1.49
Tepecintle (TEP)	b	-0.70	-0.95
Tuxpeno (TUX)	b	-0.82	-1.90
Zapalote Chico (Z-C)	b	-0.87	0.23
Zapalote Grande (Z-G)	b	-0.98	-0.71
D. Southwestern U.S.			
Eastern Keres (EKS)	c	0.54	0.24
Havasupai (HAV)	c	0.76	0.43
Hopi (HOP)	c	0.25	1.47
Papago (PAP)	USDA: 213714, 217410, 218179, 218185	-0.43	0.31
Tewa (TEW)	c	0.02	0.04
Tiwa (TIW)	c	0.29	-0.16
Western Keres (WKS)	c	0.06	1.54
E. Miscellaneous			
Cherokee (CHK)	WLB: Hill-7, Smith-1, Wahnetah-1, 8-3	0.58	-1.06
<i>Z. mays</i> subsp. <i>mexicana</i> (MEX)	d	-0.59	2.57
<i>Z. mays</i> var. <i>parviglumis</i> (PAR)	d	-0.15	-0.23

^a Sources for kernels include: Plant Introduction Station, U.S. Department of Agriculture, Ames, Iowa; Walton C. Galinat (WCG), Univ. of Mass.; Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico City; and William L. Brown (WLB), Pioneer Hi-Bred International, Johnston, Iowa.

^b Collections listed in Doebley et al., 1985.

^c Collections listed in Doebley et al., 1983.

^d Collections listed in Doebley et al., 1984.

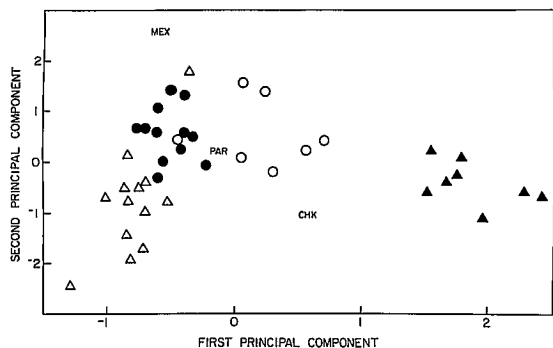


Fig. 1. Graph of the first two components from a principal component analysis of the groups listed in Table 1. The first component contains 38% of the total variation; the second component, 11%. Δ = southern Mexican races; \bullet = northern Mexican races; \circ = southwestern U.S. tribal groups; \blacktriangle = Northern Flint; CHK = Cherokee corn; MEX = *Z. mays* subsp. *mexicana*; and PAR = *Z. mays* var. *parviglumis*.

summarized elsewhere. For comparison, we include previously published isozyme data for 25 Mexican races (Doebley, Goodman, and Stuber, 1985), maize of 7 Indian tribes of the southwestern U.S. (Doebley et al., 1983) and two wild relatives of maize, *Zea mays* subsp. *mexicana* and subsp. *parviglumis* (Doebley et al., 1984) (Table 1).

RESULTS AND DISCUSSION—Isoenzymatic distinctiveness of Northern Flint—Figure 1 is a principal component analysis based on the among-group variance-covariance matrix of allele frequencies for the 42 groups listed in Table 1. A total of 206 alleles were involved in this analysis. Figure 1 shows a continuum from the southern and low elevation Mexican races (Δ), through the Northern and high elevation Mexican races (\bullet), to the southwestern U.S. Indian corns (\circ). One of the southern Mexican races, Nal-tel, appears closer to the northern Mexican races, and one of the southwestern Indian corns (Papago) also groups with the northern Mexican races. *Zea mays* var. *parviglumis* (PAR), race Balsas teosinte that has a very close genetic relationship to maize, falls in the midst of the continuum just described. *Z. mays* subsp. *mexicana* (MEX), a somewhat more distant relative of maize, segregates away from the maize races. The most interesting aspect of Fig. 1, however, is the distinctiveness of Northern Flint (\blacktriangle).

Figure 2 is an average linkage cluster analysis of the groups in Table 1. Again the segregation of Mexican races into Northern (NM) and Southern (SM) groups and the similarity of southwestern U.S. Indian corns (SW) to Mex-

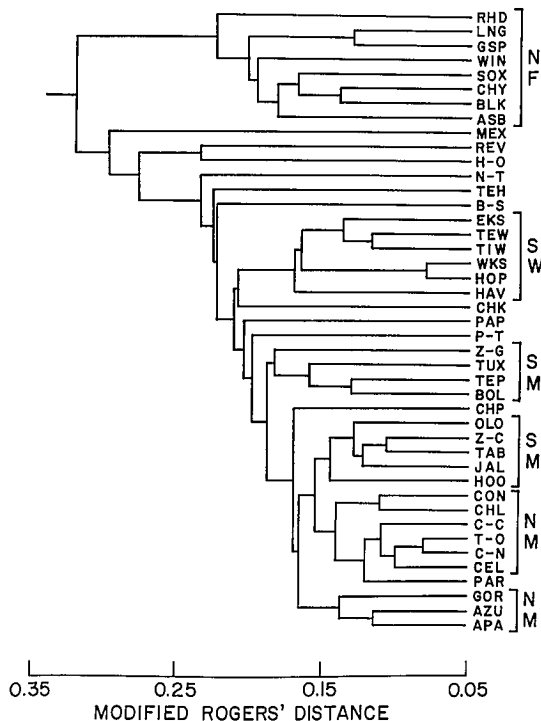


Fig. 2. Average linkage cluster analysis for isozyme allele frequencies of the groups listed in Table 1. NF = Northern Flint; SW = southwestern U.S.; NM = northern Mexico; and SM = southern Mexico. For key to abbreviations, see Table 1.

ican races are apparent. *Z. mays* subsp. *parviglumis* (PAR) shows a very close relationship to Mexican maize, and *Z. mays* subsp. *mexicana* (MEX) a much more distant relationship. Notably, Fig. 2 shows that Northern Flint (NF) displays considerable dissimilarity to other maize and even a greater distinctiveness than the teosinte, *Z. mays* subsp. *mexicana*.

The degree of dissimilarity between Northern Flint and other maize groups can be further assessed by use of Nei's (1972) genetic identity (I). An "I" of 1.0 signifies complete identity; "I" of 0.0, complete dissimilarity. In general, "I" among populations of a single species equals 0.90 to 1.0. "I" among congeneric species generally ranges from 0.60 to 0.90 (Gottlieb, 1981; Crawford, 1983). "I" between Northern Flints and Mexican Dents falls into the range typical of separate species. For example, "I" between race Tuxpeno and Rhode Island flint equals 0.803. This value is equivalent to the "I" between some species in *Zea* such as *Z. diploperennis* and *Z. luxurians*, for which "I" equals 0.812, and is smaller than the "I" of 0.842 between *Z. perennis* and *Z. mays* subsp. *mays* (Doebley et al., 1984).

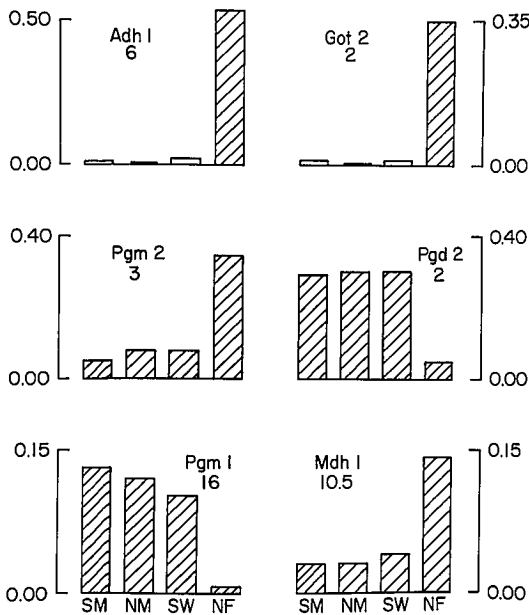


Fig. 3. Histograms showing changes in maize allele frequencies for six loci proceeding from southern Mexico (SM), to northern Mexico (NM), to southwestern U.S. (SW) and Northern Flint (NF). There is a strong discontinuity between Northern Flint and the others.

The principal component analysis, cluster analysis and genetic identities all show that Northern Flint is exceptionally distinct from other maize groups and shows a level of distinction characteristic of a separate species. This distinction does not result from differences at a few loci, but rather seems to involve most polymorphic loci under study. Because the isozyme loci analyzed resided on at least 7 of the maize chromosomes, we can conclude that the differences between Northern Flint and other maize races are spread throughout the genome. Figure 3 shows the changes in frequencies of alleles at six different loci as one proceeds from southern Mexico (SM) to northern Mexico (NM) to the southwestern U.S. (SW) to the Northern Flints (NF). Note that there is a dramatic discontinuity between Northern Flint and other maize groups. Figure 4 shows the differences in frequencies of alleles at six additional loci across the same geographic continuum. The latter alleles show a clinal pattern of variation.

Origin of Northern Flint—The isozyme data allow an examination of the proposed geographic origin of Northern Flint. Because maize is a cultigen with its origin in southern Mexico and because maize was unknown in the eastern U.S. prior to 500 B.C. (Ford, 1981), it is clear

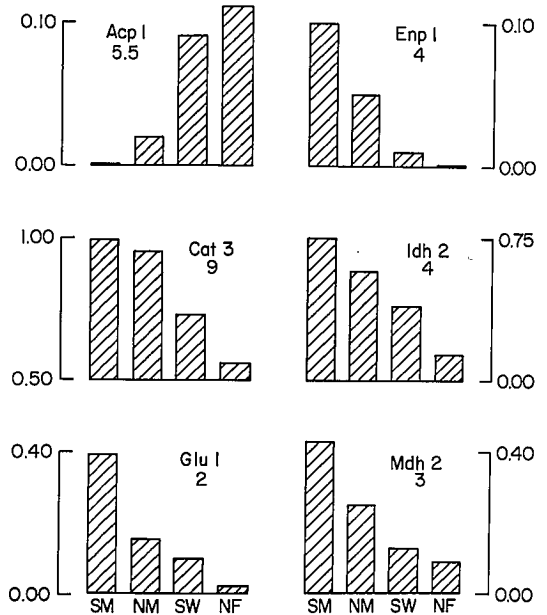


Fig. 4. Histograms showing clinal variation in maize allele frequencies for six loci proceeding from southern Mexico (SM) to northern Mexico (NM) to southwestern U.S. (SW) and Northern Flint (NF).

that Northern Flint must have its origin in some more southerly form. Our data show a relationship between the maize of the Pueblo Indians of northern New Mexico and Arizona and Northern Flint (Fig. 1, 4). Further, there are several rare or low frequency alleles which bind together Pueblo and Northern Flint maize, including *Cat3-6*, *Enp1-1*, *Enp1-10*, and *Cat3-12*. As part of an on-going survey of isozyme variation in maize races, Guatemalan and Caribbean maize has also been studied (Goodman and Stuber, unpublished data). Corn of these regions lacks the unusual isozyme features of Northern Flint and does not appear a likely source on the basis of isozymes. The two Guatemalan races, Serrano and San Marceno, implicated in the origin of Northern Flint (Brown and Goodman, 1977) do not possess the unusual isozyme characteristics. In this sense, our data seem most congruent with Galinat's hypothesis that Northern Flint was derived out of forms from northwestern Mexico which diffused through the Pueblo region of northern New Mexico. The isozyme data does not necessarily support Galinat's specific suggestion (Galinat and Campbell, 1967) that race *Harinoso de Ocho* was involved (Table 1, Fig. 1).

We have mentioned that the lack of chromosome knobs in Northern Flint would seem to preclude their derivation from the knobbed forms of the southwestern U.S. However, the

knobless state seems to exist wherever maize is found in cool, short-seasoned environments (McClintock, Kato Y., and Blumenschein, 1981). This is similar to the situation that occurs in *Triticale* (Bennett, 1976, 1977). Thus, one may hypothesize that the knobless state found in Northern Flint and the highland tropical races resulted from the convergent loss of knobs. The loss of knobs in Northern Flint seems to be part of a general reduction in their genome size as has been demonstrated by Rayburn (1984).

Uniqueness of Northern Flint—While the isozyme data are congruent with an origin of Northern Flint from the maize of the southwestern United States, these data also demonstrate its isoenzymatic uniqueness. What is most startling about this observation is that the earliest archaeological evidence for maize in the eastern U.S. is 500 B.C. and maize was not an important staple there until 800 A.D. (Ford, 1981). Historically, it is known that varieties resembling Northern Flint were present when the Pilgrims landed in Massachusetts (Galinat, 1967). Thus, in a relatively short time (1,000 years), Northern Flint achieved a level of isozyme distinctiveness typical of a separate species. Genetic drift may have been a contributing factor if very few ears (or kernels) formed the founder populations that entered the eastern U.S. The relatively low levels of isozyme variation in the Northern Flints provide some support for this view. Comparing Mexican maize vs. Northern Flint, one sees a reduction in variation as measured by expected heterozygosity (0.182 vs. 0.136), alleles per collection (39 vs. 34) and proportion of polymorphic loci per population (0.48 vs. 0.38). Stringent selection may also have played a role in the differentiation of Northern Flint. Maize probably originated in the seasonally moist tropics of southern Mexico, characterized by a long growing season and warm temperatures. The environment of the temperate eastern U.S. is considerably different, and few introduced maize plants may have survived there, further altering gene frequencies. Once established, the uniqueness of Northern Flint was probably maintained by isolation because on the Great Plains there was little maize agriculture, creating a barrier to gene flow between the southwestern U.S. and the eastern woodlands (Weatherwax, 1954).

CONCLUDING REMARKS—The midwestern hybrid corns form the genetic base of what is probably the world's most productive agricultural system. These corns are largely derived

from two land races, Southern Dent and Northern Flint. The latter race appears on the basis of isozyme analysis to have been derived from the maize of northwestern Mexico and the southwestern U.S. The isozyme data also suggest that Northern Flint underwent an extreme degree of differentiation during its rather short (1,000 yr) evolutionary history. Genetic drift, stringent selection and isolation may have contributed prominently to the differentiation of Northern Flint and resulted in their obtaining a level of isozyme distinctiveness typical of a separate species.

Finally, the results demonstrate that, under unusual circumstances, populations can diverge rapidly for biochemical markers that are generally assumed to be neutral. Part of this divergence may be due to linkage of neutral marker (isozyme) loci to other loci which are under strong selection. Regardless of the mechanism, the isozyme distinctiveness of Northern Flint demonstrates that the "molecular clock" may on occasion skip a beat. Further, the situation with Northern Flint offers an interesting counter-example to recent examples of rapid morphological divergence with little or no biochemical divergence (Doebley et al., 1984; Ganders and Nagata, 1984; Lowrey and Crawford, 1985). For Northern Flint and Mexican maize, we have an example of taxa which have retained the same basic morphological features so that their taxonomic unity is unquestioned, yet their biochemical (isozyme) constitution could be used to justify separate species status.

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