

Patterns of genetic diversity in sympatric and allopatric populations of maize and its wild relative teosinte in Mexico: evidence for hybridization

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Genetic diversity in maize and its wild relative teosinte (*Zea mays* ssp. *mexicana*) in Mexico is important for the sustainability and improvement of the third most important crop in the world. This study examines the genetic diversity of sympatric and allopatric populations of maize and teosinte and evidence for hybridization in Mexico using isozyme electrophoresis. We take a population level approach to the analysis of genetic diversity in standing Mexican populations. Our data provides evidence that hybridization contributes to the genetic similarity between sympatric pairs of maize and teosinte populations compared with allopatric populations of either subspecies. Introgressed populations of teosinte show an increase in diversity relative to isolated populations. To date, introgression from teosinte does not appear to play an important role in the genetic diversity of sympatric maize. Patterns of differentiation among sympatric and allopatric populations of maize and teosinte indicate that introgression occurs more often than previously thought. Additionally, recent introgression between maize and teosinte has resulted in morphological hybrids that often have genetically transgressed allele frequencies compared to adjacent maize and teosinte populations.

INTRODUCTION

In the Central Plateau and Valley of Mexico, maize (*Zea mays* L. ssp. *mays*) grows in sympatry with *Zea mays* ssp. *mexicana* (Schader) Iltis) one of the several teosinte taxa providing the opportunity for hybridization (Wilkes 1967). There is some evidence to suggest that *Z. mays* ssp. *mays* hybridizes more frequently with *Z. mays* ssp. *mexicana* than with other taxa of teosinte (Wilkes 1967; Doebley 1990). This gene flow between *Z. mays* ssp. *mays* and *Z. mays* ssp. *mexicana* could result in introgression, the incorporation of new genes from one taxon into the population of the other (Jarvis and Hodgkin 1999). Therefore hybridization between maize and teosinte is expected to alter the genetic diversity of sympatric populations. The impact of ongoing gene flow between these taxa on patterns of genetic diversity is of applied importance since collectively Mexican maize landraces and teosinte are potentially the most genetically diverse resource of *Zea* germplasm in the world (Senadhira 1976; Hancock 1992).

Evidence for hybridization and introgression in populations of maize and *Z. mays* ssp. *mexicana* in Mexico however remains inconclusive. There is no direct molecular evidence of hybridization at the population level by previous isozyme studies of *Z. mays* ssp. *mexicana* and maize. The purpose of this research was to seek genetic evidence for hybridization at the population level. We evaluate the current population genetic structure of maize (*Zea mays* ssp. *mays*) and its wild relative teosinte (*Z. mays* ssp. *mexicana*) from allopatric and sympatric populations in Mexico. We compared the population genetic structure of sympatric and allopatric populations of maize and *Z. mays* ssp. *mexicana* using isozyme electrophoresis. We considered evidence of hybridization and its effect in altering patterns of genetic variation in sympatric populations.

MATERIALS AND METHODS

Plant material, Allozyme electrophoresis, and Data analysis

Samples were collected from Mexican populations of teosinte (*Zea mays* ssp. *mexicana*) and cultivated maize (*Z. mays* ssp. *mays*) in the autumn of 1998 and 1999. Locations represent the geographical range of *Z. mays* ssp. *mexicana* in the Valley of Mexico and the Central Plateau and include moderate to large population sizes for both taxa. Allopatric populations of *Z. mays* ssp. *mexicana* are rare and presently appear limited to the state of Morelos. Each collected individual was assayed directly from fresh mature leaf material for twelve enzyme systems revealing eighteen loci. A full description of the plant material, electrophoresis protocol, and methods of analysis are published in Blancas (2001).

SUMMARY OF RESULTS

Genetic frequencies for each population are summarized for all polymorphic loci in Table 1 (Blancas 2001; Blancas et al. in review). Genetic diversity statistics are summarized in Table 2 (Blancas 2001; Blancas et al. in review). A hierarchical cluster dendrogram of Nei's genetic distances for the six major *Zea mays* L. groups is shown in Figure 1 (Blancas 2001, Blancas et al. in review).

DISCUSSION

Our data substantially support the long held, but controversial, supposition of hybridization between maize and teosinte. Our results show that allopatric and sympatric teosinte differ slightly in overall levels of diversity, measured in terms of polymorphic loci and estimated heterozygosity. Maize in populations sympatric with teosinte did not have higher levels of diversity than allopatric maize not currently associated with teosinte. Allozyme diversity, in terms of A and A_p , in morphological hybrids assayed from sympatric populations is equal to or greater than maize and teosinte from sympatric populations.

Typically, most population genetic studies comparing crops with their wild relatives contrast patterns of genetic diversity measures without addressing issues of introgression. Allozyme diversity within populations of maize is higher than within populations of teosinte when observed heterozygosity of all loci is examined. Allopatric teosinte populations, however, are small and isolated by comparison, and subject to loss of variation due to increased inbreeding and genetic drift (Ellstrand and Elam 1993). In this study, no two maize populations shared similar patterns of gene frequencies. In sympatric populations, the distribution of genetic variation in maize is different from teosinte. Allele frequencies at polymorphic loci can substantially vary between maize and teosinte from an associated sympatric population.

In 1984, Doebley et al. reported H_e of 0.234 and 0.249 and H_o of 0.231 and 0.215 obtained from bulk seed collections for Chalco and Central Plateau *Z. mays* ssp. *mexicana*, respectively. We obtained H_{ep} of 0.16 and 0.10 and H_{op} of 0.09 and 0.10, from our samples of sympatric and allopatric teosinte, respectively. The most polymorphic group was allopatric maize, with $H_{ep} = 0.22$ and $H_{op} = 0.05$. In general, genetic variation does not appear to be greater than the average outcrossing species as Doebley et al. (1987) suggested or according to the allozyme literature review by Hamrick and Godt (1989). Due to the nature of the local populations sown from an original seed source for consecutive years for many of the maize landraces, samples from a single population have a close genetic ancestry (i.e., correlated haplotypes) which contributes to a high F_p and an incongruity between H_{op} and H_{ep} .

A more detailed discussion of these findings can be found in Blancas (2001) and Blancas et al. (in review).

EVIDENCE FOR AND PATTERNS OF INTROGRESSION

Our data provide evidence for introgression of maize and teosinte in that sympatric teosinte populations are more genetically similar to maize than to allopatric teosinte. This observation seems to contradict evidence (Doebley et al. 1984) that sympatric teosinte maintains allozymic distinctness from maize (Doebley 1990). It seems instead that there is genetic exchange between maize and teosinte growing in close proximity or sympatrically. On the other hand, transgressed allozyme frequencies in hybrids indicate that hybrids are more distantly introgressed and maintain genetic differentiation thus behaving as a separate lineage. Additional evidence for introgression is observed in moderate to high genetic differentiation between hybrids of sympatric populations compared to allopatric populations of maize or allopatric teosinte. These data support previously obtained allozyme data (Doebley et al. 1984, 1987) that hybridization and introgression occurs between sympatrically located populations of maize and teosinte.

While our data confirm similar findings, our population level sampling suggests that hybridization events and introgression occur more often than previously estimated (Doebley et al. 1984, 1987). Extensive sampling of sympatric pairs and incorporation of morphologically intermediate hybrids with their segregation in their analysis shows a more complete picture of the genetic relationship between sympatric maize and teosinte. Doebley et al. (1984) hypothesized that continual introgression should result in a short genetic distance between sympatric pairs than non-sympatric pairs. However, the genetic distance between sympatric pairs can appear more distant if introgressed alleles of low or moderate frequency are not sampled. Small scale sampling of accessions and careful exclusion of hybrids from sympatric populations is likely to underestimate introgressed alleles, which explains why the results of Doebley et al. (1984) differ from ours.

In the absence of an appropriate diagnostic marker, accurate estimates of hybridization are difficult, especially in regions of Mexico where the maize seed is "recycled". However, we observed allele frequencies in the hybrids that transgress (frequencies that are not intermediate to the parents) those in the local parents at a number of loci. Gene flow (N_m) estimates based on F_{ST} would indicate that there are a higher number of migrants per generation among sympatric maize populations than among any other group. Continual migration among genetically similar (i.e., short genetic distance) populations reduces genetic divergence among populations (Slatkin 1985). Interestingly, allopatric teosinte populations have the second highest estimated gene flow rate of the groups we analyzed. This would suggest that the allopatric teosinte populations sampled here, although entirely allopatric with maize, are not isolated from one another.

Senadhira (1976) concluded that genetic variation of teosinte and its contribution to maize are small but represents a sizeable genetic resource based on her results. Fewer populations of teosinte both sympatric and allopatric to maize exist today (Wilkes 1967; Sanchez Gonzalez 1997; and pers. obs.). Although we can not determine the exact extent of hybridization, introgression in sympatric populations is evident, and hybridization is potentially more than the rare occurrence suggested by Doebley et al. (1987) contributing to the demise of teosinte populations. The role of introgression is significant in influencing the variation maintained in sympatric populations and in *Z. mays ssp. mexicana* as a whole. It is clear that there is a genetic difference between teosinte populations that are sympatric to those that have been historically isolated from maize. Although this first population genetic study of maize and teosinte in Mexico is based on populations that represent a small portion of the total number, our study shows that in addition to gene flow there are geographic genetic differences that contribute to differentiation at the population level.

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Table 1¹. Allele frequencies observed for allopatric maize and teosinte, and maize (M), putative hybrids (H), and teosinte (T) from sympatric populations (**AA**, **Pi**, **Pñ**, **SF**, and **TM**). *N* is the number of individuals per population group analyzed.

N	Maize	AA			Pi			Pñ		SF			TM			Teosinte	222
		M	H	T	M	H	T	H	T	M	H	T	M	H	T		
GOT1	1	0.26	0.19	0.14	0.17	0.03	-			0.17	-	-	0.17				
	2	0.69	0.81	0.83	0.83	0.92	1.00	1.00	1.00	0.83			0.50	0.96	0.75	0.98	
	3	0.05		0.03		0.05							0.33	0.04	0.25	0.02	
GOT2	1	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	2	0.01															
GOT3	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	
	2											0.05					
LAP1	1	0.99	1.00	-	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	0.25	1.00	-	1.00
	2	0.01											0.75				
MDH1	1	0.58	1.00	1.00	0.02	0.58	-	0.17		0.38			0.21	0.31	0.78	0.95	
	2	0.41			0.98	0.42	1.00	0.83	1.00	0.62	1.00	1.00	0.79	0.69	0.22	0.05	
	3	0.01															
MDH2	1	0.79	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	0.97	
	2	0.21													0.07	0.03	
PGD1	1	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	2					0.02											
PGM1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
	2															0.16	
UDP1	1	0.10	0.07	0.02		0.48		0.47	0.32	-	0.47	0.81	0.57	1.00	1.00	1.00	0.87
	2	0.01			0.02			0.53	0.54								0.10
	3	0.71	0.88	0.55	0.84	0.21	0.91				0.47	0.19	0.43				0.03
	4	0.04		0.05	0.05	0.05			0.14		0.06						
	5	0.14	0.05	0.05	0.09	0.26	0.09										
	6			0.05													
UDP2	1	0.68	1.00	0.98	0.86	0.74	0.11	1.00	0.71	-	0.91	0.88	0.71	1.00	0.92	1.00	0.82
	2	0.32		0.02	0.14	0.26	0.89		0.29		0.09	0.12	0.29		0.08		0.18

= allele frequency not reported.

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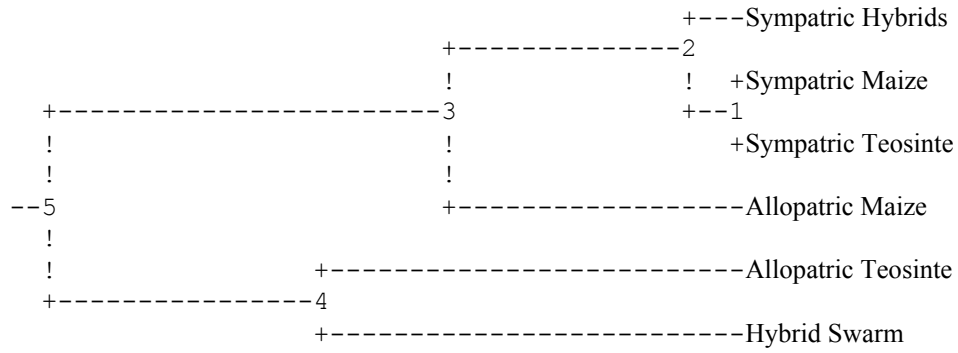
Table 2¹. Nei's (1987) genetic diversity statistics derived from allele frequency analysis at 18 allozyme loci for *Zea mays* L ssp. *mays* and *Z. mays* ssp. *mexicana* and putative hybrid populations in Mexico.

<i>Zea mays</i>	N	N_a	A	A_p	P	H	F_{ST}	N_m
<i>Z. mays</i> ssp. <i>mays</i> (allopatric)	6	50.8	1.22	2.63	0.44	0.12	0.505	0.245
<i>Z. mays</i> ssp. <i>mays</i> (sympatric)	4	17.75	1.17	2.60	0.28	0.07	0.290	0.611
<i>Z. mays</i> ssp. <i>mexicana</i> x <i>Z. mays</i> ssp. <i>mays</i> (sympatric)	5	22.8	1.16	3.00	0.28	0.04	0.497	0.253
<i>Z. mays</i> ssp. <i>mexicana</i> (sympatric)	5	19.6	1.17	2.67	0.33	0.04	0.751	0.083
<i>Z. mays</i> ssp. <i>mexicana</i> (allopatric)	4	55.5	1.07	2.20	0.28	0.05	0.382	0.403
<i>Z. mays</i> ssp. <i>mexicana</i> x <i>Z. mays</i> ssp. <i>mays</i> (swarm)	1	20	1.22	2.00	0.22	0.05	*	-

N = Number of populations sampled; N_a = Average number of plants sampled per population; A = Average number of alleles per locus; A_p = Mean number of alleles per polymorphic loci (total number of alleles from polymorphic loci divided by the total number of polymorphic loci); P = Proportion of polymorphic loci; H = Nei's estimated heterozygosity; F_{ST} = Nei's (1987) summary F statistics for population differentiation between populations within *Z. mays* ssp. *mays* and *Z. mays* ssp. *mexicana*; * F_{IT} = measure of individual diversity relative to the total population. Observed F_{IT} = 0.202; N_m = estimated genetic migration where N_m = [(1/ F_{ST}) - 1]/4 (Slatkin and Barton, 1989).

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Figure 1¹. Hierarchical cluster dendrogram based on Nei's genetic distances for six major *Zea mays* L. groups sampled.



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