

# Molecular characterization of MAIZE LANDRACES and various subspecies of TEOSINTE from Mexico and Central America

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#### **IIIII** Introduction

Teosinte (*Zea* sp.) are the closest wild related species to maize, (*Zea* mays mays) and contains the progenitor of domesticated maize, (Zea mays ssp. parviglumus) (Beadle, 1939). Previous characterization studies have generally compared subspecies of teosintes, or maize with few subspecies of teosinte. It is possible that post domestication gene flow between maize and different teosinte subspecies have influenced diversity within maize landraces from regions where teosinte grows (mainly, Mexico and Central America). This study was undertaken to compare the diversity of SSR markers in 20 teosinte subspecies with the diversity of 10 maize landraces from the same countries and 10 maize landraces from geographical regions who have been separated from the center of origin of maize for several centuries (landraces from Asia).

### **Materials and Methods**

Twenty five SSR markers were used to characterize 15 individuals per teosinte or maize population, using a bulking strategy to calculate allele frequencies in each population. Equal amounts of DNA from each individual were amplified with each SSR marker, run on an automatic DNA sequencer, and peak heights of each allele were used to calculate the frequency of that allele in the bulk according to Dubreuil et al., (2006). Genetic distances were calculated between each pair of populations in the study and a dendrogram generated using the software PowerMarker.

#### **IIIII** Results and Discussion

The relationships between populations in this study can be seen in Figure 1. The relationships between the teosinte populations in this study were in agreement with previously published results based on markers, morphology, and geography, as summarized in Buckler and Stevens (2006), with the addition of some subspecies not previously studied now located on the dendrogram (Figure 1a). The most distant and distinctive teosinte species are the three found in the first cluster at the top of Figure 1a (perennial taxa with elongated fruitcases, rhizomes and basal tillering). In the second cluster from the top are the annual diploid Z. luxurians from Central America which are closely related to the perennial taxa, with elongated fruitcases, unique tassel morphology and basal tillering. All other teosintes are annuals, have triangular fruitcases and a central culm lacking basal tillers but with branching at the upper nodes and a central spike in the tassel. These teosintes are fully fertile (95+%) with maize while elongated fruitcase types express impaired fertility(80-85%) in crosses with either maize or triangular seeded teosintes (G. Wilkes, pers. comm.).

The next two entries from Guatemala and Oaxaca, Mexico form two clusters independent from all other populations in the study; these are geographically isolated populations as well (at least a 200 kms from any other teosinte population). The next group contains 9 populations from Central Mexico that are geographically related and oriented on a North-South axis. The first two (Nobagame in Chihuahua and Durango), are more genetically and geographically distinct. Six of the other 7 populations are Z. parviglumus (Central Plateau or Chalco) and are genetically and geographically closely related; in fact, all were originally one panmictic group, although now are fractured. The surprise addition of one domesticated landrace (Conico) to this group, and clustering very closely with two Zea mays mexicana, has never been documented before. This may be the first evidence of geneflow between a non Balsas teosinte and maize. It probably occurred because this landrace has historically been grown in the same location. Central Plateau and Chalco teosinte are sympatric and form fertile hybrids with Conico. In addition, these three races (maize and teosinte) are all found at the highest extreme of where maize is typically grown in Mexico. It is interesting that the Chalco and Central Plateau teosintes are sources of the Ga1:ga1 incompatibility system of maize and the Teosinte Incompatility Complex caused by the *teosinte crossing barrier1* (*tcb1*) gene (respectively), which prevents maize pollen from growing on these teosinte races; however, the barrier is unidirectional and does not prevent teosinte from crossing onto maize (Kermicle and Allen, 1990; Evans and Kermicle 2001).

The final cluster in Figure 1b is a large and fairly diverse cluster containing the remaining 19 landraces and three teosintes from the Balsas and surrounding region. This population is the largest panmitic teosinte population (covering over 100,000 km), and because of it's size is probably holding more alleles than other teosinte populations. These three teosintes are of the subspecies that was the progenitor of domesticated maize. They are equally closely related to 9 Latin American landraces and 7 Asian landraces. The diversity within the cultivated maize landraces is extensive, even compared to the diversity in the wild species. Of a total of 386 alleles identified in the data set, 138 (36%) were unique to the teosinte species, and 38 (1%) were unique to the maize. Of these, 33 alleles were present only in the Asian maize, and only 5 were present in both Asian and Latin American maize landraces. The search for unique alleles in maize populations that are sympatric to teosinte accessions indicate the probability of diversity in maize being influenced by gene flow directly from wild maize subspecies.

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Figure 1. UPGMA dendrogram based on Roger's genetic distances of 25 SSR markers between 20 teosinte populations and 20 maize landrace populations. Population name, species, race, and country and state of collection are all listed to the right of the dendrogram. Figure 1a contains only the 8 distinct teosinte species, and Figure 1b contains all 20 teosinte populations and all 20 maize landraces.

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SPECIES	RACE	COUNTRY	STATE/TYPE
Z_diploperennis	n/a	MEXICO	Jalisco
Z_diploperennis	n/a	MEXICO	Cuautitlan
Z_perennis	n/a	MEXICO	Jalisco
Z_perennis	n/a	MEXICO	Morelos
Z_guatemala	n/a	GUATEMALA	Chiquimula
Z_luxurias	n/a	NICARAGUA	Chinandega
Z_nicaraguensis	n/a	NICARAGUA	unknown
Z_mays_huehuetenangesis	Huehuetenango	GUATEMALA	Huehuetenango
_Z_mays_parviglumis_Balsas	Balsas	MEXICO	Oaxaca
Z_mays_mexicana_Nobogame	Nobogame	MEXICO	Chihuahua
Z_mays_mexicana_Durango	Durango	MEXICO	Durango
Z_mays_mexicana_CentPlateau	Central Plateau	MEXICO	Michoacan
Z_mays_mexicana_Chalco	Chalco	MEXICO	Mexico
Z_mays_mexicana_Chalco	Chalco	MEXICO	Distrito Federal
Z_mays_mexicana_Chalco	Chalco	MEXICO	Mexico
Z_mays_mexicana_Chalco	Chalco	MEXICO	Puebla
Z_mays_mays	Cónico	MEXICO	Mexico
Z_mays_mexicana_CentPlateau	Central Plateau	MEXICO	Guanajuato
Z_mays_mays	160178	CHINA	Henan
Z_mays_mays	yellow flint	VIETNAM	Gialai
Z_mays_mays	Perola	BOLIVIA	unknown
Z_mays_mays	250581	CHINA	Shanxi
Z_mays_parviglumis_Balsas	Balsas	MEXICO	Guerrero
Z_mays_parviglumis_Balsas	Balsas	MEXICO	Mexico
Z_mays_parviglumis_Balsas	Balsas	MEXICO	Michoacan
Z_mays_mays	Olotón	GUATEMALA	Huehuetenango
Z_mays_mays	San Geronimo	PERU	Ancash
Z_mays_mays	3300	INDONESIA	West Java
Z_mays_mays	Maiz Dulce	MEXICO	Jalisco
Z_mays_mays	Bolita	MEXICO	Oaxaca
Z_mays_mays	Chandelle	CUBA	Oriente
Z_mays_mays	IC 82097	INDIA	Rajasthan
Z_mays_mays	IC 251354	INDIA	Tripura
Z_mays_mays	Field corn	THAILAND	Farm Suwan
Z_mays_mays	Field corn	THAILAND	Farm Suwan
Z_mays_mays	3317	INDONESIA	Central Java
Z_mays_mays	Canguil	EQUADOR	unknown
Z_mays_mays	Purple Stem Waxy	VIETNAM	Daklak
Z_mays_mays	Nal Tel	MEXICO	Yucatan
Z_mays_mays	Тихреño	MEXICO	Veracruz



The summary phylogeny for the genus Zea, based on chromosomal number and morphology (Kato Y. 1976; Kato Y. and Lopez R., 1990), chloroplast (Doebley et al., 1987), ribosomal (Buckler and Holtsford, 1996), isozyme (Doebley et al., 1984), and simple sequence repeat (Matsuoka et al., 2002) data.

(Buckler and Stevens, 2005)



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## **IIII** Conclusions

- The allelic diversity within the Balsas teosinte should be explored more thoroughly.
- A large bulk collection from wild teosinte needs to be collected for gene bank preservation before this population fractionates, further or becomes locally or globally extract.
- There is a definite need to look at other cases where maize and teosinte hybridize such as Conico, Oloton, and Ancho with the Balsas or Huehuetenango teosinte, to find what has been the effect of introgression from several teosinte species in the evolution of maize landraces.

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