

# Influence of Cryptic Population Structure on Observed Mating Patterns in Teosinte

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## Introduction

The ecology and population biology of teosinte are surprisingly understudied given its role in the origin of maize. For example, knowledge of mating system parameters and patterns of pollen flow in natural teosinte populations is anecdotal and based largely upon assumptions about the breeding system of wind-pollinated grasses. Recent population-level studies of teosinte have revealed significant fine-scaled genetic structure (van Heerwaarden *et al.*, 2010). These data suggest variable patterns in mating system and pollen flow within populations and invite empirical investigation.

Quantitative measures of mating system are typically obtained through paternity analysis. Direct paternity analysis requires complete sampling of individuals in populations and is only feasible when population size is small. Indirect methods of pollen flow analysis allow for sub-sampling of populations and have been useful in large, dense plant populations (Smouse and Sork, 2004). However, these methods have yet to be applied to a grass species or in the context of subpopulation structure.

In this study, over a thousand geo-referenced plants from a single, large population of teosinte (*Zea mays* ssp. *parviglumis*) were genotyped and analyzed using an indirect two-generation method in order to assess:

1. Basic mating system parameters of teosinte (e.g., outcrossing, biparental inbreeding, correlated paternity, number of pollen donors per family)
2. Patterns of pollen flow such as average distance and the general shape of the dispersal curve;
3. The manner in which estimations are impacted by cryptic subpopulation structure.

## Materials and Methods

The Ejutla\_A population included in this study (Figs. 1 & 2) has been shown previously to have high levels of genetic diversity. It is a large and dense but somewhat patchily distributed population. Ejutla\_A was mapped in March of 2007 and progeny arrays were collected from maternal individuals at multiple sampling sites. Pooled seed samples were collected in a five-meter radius of each maternal individual for estimation of allele frequencies.

DNA was isolated from over 1000 individuals using a CTAB-based protocol. Each individual was genotyped at ten microsatellite loci. Cryptic population structure was assessed through spatially-explicit Bayesian assignment as implemented in the GENELAND R package. Mating system parameters and patterns of pollen flow were determined using the MLTR and KINDIST software packages respectively.

## Results and Discussion

### Cryptic Population Structure:

Bayesian assignment revealed two subpopulations in Ejutla\_A (Fig. 3). Subpopulation Ejutla\_A1 is located on south-facing slopes at higher elevation, is bisected by the El Grullo-Ejutla Highway and is lightly grazed. Ejutla\_A2 is situated on east-facing slopes at lower elevation and shows no clear signs of human impact. These areas also differed substantially in genetic diversity, inbreeding, canopy cover, slope and plant density.

### Mating System Parameters:

Teosinte showed high levels of outcrossing (Table 1), as would be expected of a wind-pollinated plant. Biparental inbreeding and correlated paternity were higher than reports from other species. Most similar studies have been conducted with tree species with high canopies and a prevalence of long-distance dispersal. These attributes are conducive to outcrossing among less-related individuals and may explain the differences observed in low-statured teosinte.



Figure 1: Map of study region in central Jalisco

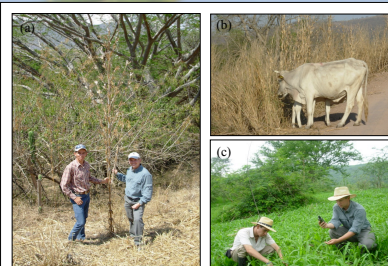


Figure 2: a) The typical pattern of lateral branching seen in teosinte; b) Teosinte is used as a dry-season forage in central Jalisco; c) The Ejutla\_A population consists of large, dense stands of teosinte.

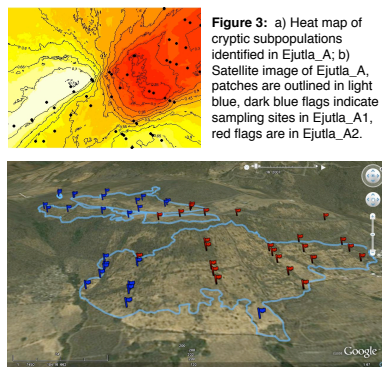


Figure 3: a) Heat map of cryptic subpopulations identified in Ejutla\_A; b) Satellite image of Ejutla\_A, patches are outlined in light blue, dark blue flags indicate sampling sites in Ejutla\_A1, red flags are in Ejutla\_A2.

Table 1: Mating system parameters in Ejutla\_A

	N	Genetic Diversity	Fixation Index	Out-crossing	Biparental Inbreeding	Correlated Paternity	Effective Pollen Donors
Ejutla_A Pop.	52	0.679	0.034	0.969	0.141	0.089	11.24
Ejutla_A1 Subpop.	26	0.662	0.135 <sup>A</sup>	0.967	0.127	0.075	13.33
Ejutla_A2 Subpop.	26	0.696	-0.067 <sup>B</sup>	0.970	0.135	0.079	12.65

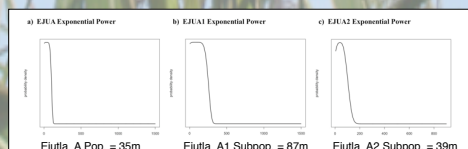


Figure 4: Pollen dispersal curves for the entire Ejutla\_A population (a) and the Ejutla\_A1 and Ejutla\_A2 subpopulations (b) and (c).

## Results and Discussion

### Mating System Parameters (continued):

Estimates of outcrossing were quite similar in both subpopulations and the entire population. Biparental inbreeding and correlated paternity were somewhat higher in Ejutla\_A2 than in the Ejutla\_A1, which could be a factor of its higher teosinte density and shorter average pollen flow distance. However, the most striking difference in mating system parameters was between the entire population and the subpopulation. As has been predicted by previous theory, subpopulation structure led to inflated estimates of biparental inbreeding and correlated paternity in the entire population when compared with cryptic subpopulations identified through Bayesian assignment.

### Pollen Flow:

Pollen flow in teosinte generally occurred over short distances (Fig. 4). Interestingly, estimates of the average distance of pollen flow were much lower in the entire population than in the two subpopulations. Moreover, pollen flow in the lightly grazed and less dense Ejutla\_A1 subpopulation occurred over twice the distance than that observed in Ejutla\_A2. These intrapopulation dynamics would have been overlooked had we failed to account for cryptic structure. Pollen dispersal distributions for the entire population and for subpopulations were thin-tailed, indicating a low level of long-distance pollen flow.

## Conclusions

Analysis of cryptic population structure was shown to be essential for estimating mating system parameters and pollen flow. Failure to account for substructure would have resulted in an overestimate of correlated paternity and biparental inbreeding and an underestimate of the average distance of pollen flow.

Conclusions can also be made regarding teosinte conservation. Thin-tailed teosinte pollen dispersal curves and inbreeding in low-density areas reveal a biological limitation in this species to outcross over long distances. Care should be taken to preserve connectivity and density in teosinte populations. Many teosinte populations are currently experiencing rapid habitat loss due to land use changes in Mexico (Wilkes, 2007). Our investigation has shown that teosinte, even though it is a wind-pollinated grass, may be acutely susceptible to habitat fragmentation.

## Literature Cited

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