Teosinte Inflorescence Phytolith Assemblages Mirror Zea Taxonomy

John P. Hart1, R. G. Matson2, Robert G. Thompson3, Michael Blake2*

1 New York State Museum, Albany, New York, United States of America, 2 Department of Anthropology, University of British Columbia, Vancouver, British Columbia, Canada, 3 Department of Anthropology, University of Minnesota, Minneapolis, Minnesota, United States of America

Abstract

Molecular DNA analyses of the New World grass (Poaceae) genus Zea, comprising five species, has resolved taxonomic issues including the most likely teosinte progenitor (Zea mays ssp. parviglumis) of maize (Zea mays ssp. mays). However, archaeologically, little is known about the use of teosinte by humans both prior to and after the domestication of maize. One potential line of evidence to explore these relationships is opaline phytoliths produced in teosinte fruit cases. Here we use multidimensional scaling and multiple discriminant analyses to determine if rondel phytolith assemblages from teosinte fruitcases reflect teosinte taxonomy. Our results indicate that rondel phytolith assemblages from the various taxa, including subspecies, can be statistically discriminated. This indicates that it will be possible to investigate the archaeological histories of teosinte use pending the recovery of appropriate samples.

Introduction

Teosinte consists of the undomesticated members of a genus of grasses (Zea) native to Mexico and Central America. Doebley and Iltis [1] and Iltis and Doebley [2] provided the current taxonomy of Zea, which they divide into two sections (also see [3]). Section Zea includes the annual teosintes Zea mays ssp. parviglumis Iltis and Doebley, ssp. mexicana (Schrader) Iltis, and ssp. huehuetenangensis (Iltis and Doebley) Doebley. Section Luxuriantes includes the annual teosintes Z. luxurians (Durieu and Ascherson) Bird and Z. nicaraguensis Iltis and Benz and the two perennial teosintes, Z. perennis (Hitchc.) Reeves and Mangelsdorf and Z. diploperennis Iltis, Doebley and Guzman.

During the last decade a series of Zea genetic studies have elucidated the phylogenetic relationships among the various species and subspecies of this genus (e.g., [3–5]). One of the most important outcomes of this research is the demonstration that all varieties of modern maize are genetically more closely related to each other and to Zea mays ssp. parviglumis than to any other subspecies or species of Zea. The phylogenetic proximity between all modern maize varieties and ssp. parviglumis supports the hypothesis that this subspecies is the progenitor of maize [4]. Matsuoka et al.’s [4] study examined 99 microsatellites (or SSRs) dispersed throughout the maize genome from 193 different maize plants, 33 ssp. mexicana plants, and 34 ssp. parviglumis plants, using 4 ssp. huehuetenangensis plants as an outgroup. This work indicated that it was most likely ssp. parviglumis that was domesticated beginning about 9000 years ago, giving rise to the earliest lineages of teosinte-like maize, which eventually evolved into the remarkable multi-rowed, large naked-kernelled, husk-covered ear of maize that is today one of the world’s most important crops.

More recently, Vigouroux et al. [5] extended this analysis by analyzing similar microsatellites from 771 additional maize and 5 ssp. parviglumis plants (as their outgroup). The combined data from these studies resulted in the broadest geographic coverage so far available, encompassing nearly all of the known races and varieties of maize from Canada to Chile. Vigouroux et al. found that allelic variation at 96 of the original 99 microsatellites allowed them to place all of the maize plants into four main clusters. These clusters (Highland Mexican, Tropical Lowland, Andean, and Northern US) correspond to the chronological spread of maize from central Mexico northwards into the southwestern US (and then subsequently into northeastern and eastern US and southeastern Canada) and southwards into the tropical lowlands above the equator (and then south into the Andean highlands and from there to the southern lowlands of South America).

While the Matsuoka et al. [4] and the Vigouroux et al. [5] phylogenies provide a plausible hypothesis on how maize evolved from an ancestral population of Zea mays ssp. parviglumis on the Pacific slope of west central Mexico, they did not elucidate the genetic relationships among the various species and subspecies of teosinte. Using a sample of 237 teosinte plants from all species and subspecies, and encompassing the geographic range from northern Mexico to Nicaragua, as well as two individual Tripsacum plants as their outgroup (one each of T. zopilotense and T. peruvianum), Fukunaga et al. [3] analyzed the allelic diversity in the same (or similar) set of microsatellites (SSRs) as used in the two maize studies. Their results confirm that across a broad set of SSRs, the teosintes can be divided into the two sections previously suggested by Doebley and Iltis [1]. They suggest that Zea luxurians is either ancestral to section Zea, or, more likely, the root lies somewhere between (i.e., ancestral to both) sections Zea and Luxuriantes (Figure 1).
These three studies suggest that the various teosinte species and subspecies should have predictable genetic and phenotypic similarities based on their ancestral proximity to one another. Dorweiler and Doebley [6] and Wang et al. [7] have demonstrated that the gene responsible for the development of glumes in \textit{Zea}, teosinte glume architecture1, \textit{tga1}, also controls the deposition of silica that produces opaline phytoliths in the cells of glumes. One of the most significant events in the domestication process was the change in the expression of \textit{tga1}, enabling the sealed, indurated fruitcase of teosinte to open up, allow a naked grain, and form a less lignified, softer glume. Work on phytolith assemblages from different taxa of \textit{Zea} has been progressing for many years. Although studies have focused on differentiating between the phytoliths produced in the glumes of maize and teosinte based on proportions of phytolith types (e.g., [8–10]), to date there has been no determination as to whether the glume (rondel) phytoliths from the various teosinte species and subspecies can be discriminated. The ability to make these identifications will be critical to elucidating human use of teosinte.
both prior to and following the evolution of maize. If it is possible to differentiate between the rondel phytoliths produced by the various teosinte taxa, reconstructing the histories of human-teosinte interactions both before and after the evolution of maize will be enhanced. Even though teosinte is assumed to have been an important resource as it evolved into maize, currently it is almost invisible in the archaeological record. Here we test two hypotheses:

H₀: Teosinte rondel phytolith “profiles” do not reflect teosinte phylogeny.
H₁: Teosinte rondel phytolith “profiles” reflect teosinte phylogeny.

We show that it is possible to discriminate between rondel phytolith assemblages of the various taxa using the proportions of as few as two morphological categories. The implication is that teosinte rondel phytolith assemblages are highly reflective of Zₚa phylogeny and that H₀ can be rejected. This demonstrates that the ability to differentiate maize from teosinte is not the only taxonomic utility of Zₚa phytolith assemblages.

Results

Metric Multidimensional Scaling (MDS) [11,12] using unstandardized Euclidean distances produced clear results (Figure 2) with plots identical (except for the scale) to those produced by Principal Coordinates Analysis [13–15] (not shown). MDS groups together known teosinte taxa assemblages, including those of Z. mays ssp. mexicana and Z. mays ssp. parviglumis, largely in accord with Zₚa taxonomy (Figures 2 and 3). A very similar pattern for the first two dimensions was found using chord distances (not shown) except that Z. mays ssp. mexicana–parviglumis separation was not perfect. However, the first four dimensions of the chord distance results could be rotated slightly, to achieve a perfect separation of these subspecies.

The third and fourth dimensions of the MDS results using Euclidean distances are shown in Figure 3, with most known teosinte samples grouped with other samples with the same taxon. These two dimensions account for only 13.62 percent of the total squared distance from the centroid (variance). No other dimension

Figure 3. Metric Multidimensional scaling, Dimensions 3 and 4. Coding as in Figure 2. Dimension 3 accounts for 9.1% of trace, dimension 4, 4.5% of trace.
doi:10.1371/journal.pone.0018349.g003
accounted for more than 4 percent of the variance. These results clearly demonstrate that variation in rondel morphological category abundance between teosinte taxa (and *Tripsacum*) assemblages corresponds very closely to the Fukunaga et al. [3] taxonomy. There is a high Spearman rank correlation of $+0.977$ between MDS dimension 1 and the abundance of morphological class A-1-B-3/3, the most common rondel category. Dimension 2 has a linear correlation of $+0.8598$ with category A-2-B-3/4, the second most abundant rondel form and dimension 3 has a linear correlation of $-0.5520$ with A-1-B-1/1. Two other correlations between dimensions and morphological categories were also noted, $+0.6841$ of A-2-B-1/4 with dimension 2 and $+0.4855$ of C-1-D-1-1/1 with dimension 3. Figure 4 shows the first two dimensions of the MDS with the intensity of the color indicating the abundance of A-1-B-3/3, a visual representation of its relationship with the first dimension. It is apparent, then, that three relatively abundant rondel categories can be used to distinguish between the various teosinte taxa. In fact, plotting the samples according to the abundance of two of the most highly correlated rondel categories, A-1-B-3/3 and A-1-B-1/1, results in a separation into the appropriate taxa (Figure 5). Eleven blind test (BT) phytolith assemblages were assigned to the class of their nearest neighbor in the Euclidean distance matrix using the full set of 45 morphological categories present in teosinte samples (Table 1). The assignments are in agreement with known taxa in all but one case for a 91% correct classification.

Using multiple discriminant analysis (MDA), the three rondel categories most highly correlated with MDS dimensions 1–3...
resulted in a perfect classification of the known assemblages (\(Z. mays\) ssp. \(parviglumis\), \(Z. mays\) ssp. \(mexicana\), Section Luxuriantes) and in the assignment of the BTs to taxa (not shown), excluding BT4 which did not fit into the categories (thereby reducing our sample of BTs from eleven to ten). The results of the MDA indicate that rondel category A-2-B-3/4 does not contribute significantly to the function. Eliminating that category resulted in a two-variable function that assigned all known and BT samples to their correct categories (Tables 2 and 3).

The correct assignment of BT samples to their known biological taxa supports the earlier results, confirming that the abundance of rondel categories matches very closely teosinte phylogeny based on microsatellite analysis [3]. Our collective results indicate, then, that inflorescence rondel phytoliths can be used to accurately discriminate between the various teosintes.

In an on-going investigation of Mexican maize phytolith assemblages, an MDA using only three rondel categories (A-2-B-3/3, A-1-B-4/4, and C-4-D-4-4/4) shows near perfect classification of maize and teosinte (Table 4). These results further demonstrate the ability of \(Zea\) taxa to be distinguished based on rondel phytolith assemblages using only a very few morphological categories.

**Discussion**

Our results clearly show that \(H_0\) can be rejected while \(H_1\) cannot; rondel phytolith profiles closely reflect current \(Zea\) phylogeny.

---

**Table 1.** Nearest neighbor assignment of blind test (BT) phytolith assemblages.

| No. | Classification | Nearest neighbor | Distance | correct? |
|-----|----------------|-----------------|----------|----------|
| BT1 | \(Z. luxurians/nicaraguensis\) (P1306615) | zmlx2 | 1.98 | Yes |
| BT4 | \(Z. mays\) ssp. \(huehuetenangensis\) (Ames 21889) | zmp10 | 1.55 | No** |
| BT5 | \(Z. mays\) ssp. \(parviglumis\) (Ames 21785) | zmp12 | 1.13 | Yes |
| BT7 | \(Z. mays\) ssp. \(parviglumis\) (PI 331785) | zmp14 | 1.18 | Yes |
| BT9 | \(Z. mays\) ssp. \(mexicana\) (Ames 8083) | zmm7 | 1.43 | Yes |
| BT10 | \(Z. mays\) ssp. \(parviglumis\) (PI 566689) | zmp11 | 1.14 | Yes |
| BT11 | \(Z. mays\) ssp. \(mexicana\) (PI 566674) | zmm7 | 1.17 | Yes |
| BT12 | \(Z. mays\) ssp. \(parviglumis\) (Ames 21890) | zmp5 | 1.24 | Yes² |
| BT13 | \(Z. mays\) ssp. \(parviglumis\) (PI 566688) | | | |
| BT14 | \(Z. mays\) ssp. \(parviglumis\) (PI 212889) | | | |
| BT17 | \(Z. mays\) ssp. \(parviglumis\) (PI 384066) | | | |

1BT3 was excluded because it had apparently been misidentified in its original repository prior to the present study.
2Iltis and Doebley [2] classify \(huehuetenangensis\) as a variety of \(parviglumis\).

---

**Table 2.** Two-variable (A-1-B-3/3, A-1-B-1/1) MDA classification matrix of known assemblages (direct method).

| Observed | Predicted | Percent |
|----------|-----------|---------|
| Lux | Zmm | Zmp |
| Section Luxuriantes (Lux) | 6 | 0 | 0 | 100 |
| \(Z. mays\) ssp. \(mexicana\) (Zmm) | 0 | 9 | 0 | 100 |
| \(Z. mays\) ssp. \(parviglumis\) (Zmp) | 0 | 0 | 15 | 100 |
| Total | 6 | 9 | 15 | 100 |

doi:10.1371/journal.pone.0018349.t002

---

**Table 3.** Two-variable (A-1-B-3/3, A-1-B-1/1) MDA classification matrix of BT assemblages (direct method).

| Case Actual | Predicted¹ |
|-------------|------------|
| | 1 | 2 | 3 |
| BT1 | \(Z. mays\) luxurians/Z. nicaraguensis (P1306615) | Lux | Zmm | Zmp |
| BT5 | \(Z. mays\) ssp. \(parviglumis\) (Ames 21785) | Zmp | Zmm | Lux |
| BT7 | \(Z. mays\) ssp. \(parviglumis\) (PI 331785) | Zmp | Zmm | Lux |
| BT9 | \(Z. mays\) ssp. \(mexicana\) (Ames 8083) | Zmm | Zmp | Lux |
| BT10 | \(Z. mays\) ssp. \(parviglumis\) (PI 566689) | Zmp | Zmm | Lux |
| BT11 | \(Z. mays\) ssp. \(mexicana\) (PI 566674) | Zmm | Zmp | Lux |
| BT12 | \(Z. mays\) ssp. \(parviglumis\) (Ames 21890) | Zmp | Zmm | Lux |
| BT13 | \(Z. mays\) ssp. \(parviglumis\) (PI 566688) | Zmp | Zmm | Lux |
| BT14 | \(Z. mays\) ssp. \(parviglumis\) (PI 212889) | Zmp | Zmm | Lux |
| BT17 | \(Z. mays\) ssp. \(parviglumis\) (PI 384066) | Zmp | Zmm | Lux |

¹1 = highest predicted group, 2 = second highest predicted group, etc.

doi:10.1371/journal.pone.0018349.t003

---

**Table 4.** Three-variable (A-2-B-3/3, A-1-B-4/4, C-4-D-4-4/4) MDA classification matrix of teosinte and Mexican maize samples (direct method).

| Phytolith Assemblages | Predicted Group Membership |
|-----------------------|---------------------------|
|                       | Teosinte | Maize | Total |
| Teosinte Samples      | 30 | 0 | 30 |
| Maize Samples         | 1 | 28 | 29 |
| "Unknown" Samples (Teosinte)¹ | 14 | 1 | 15 |

¹MDA is sensitive to unequal sample sizes, so 30 of the teosinte samples were used as "Knowns" and the remaining 15 were used as "unknowns", a recommended procedure for cases like this [16 p51].

doi:10.1371/journal.pone.0018349.t004

---

References:

1. Doebley JF, Iltis JA (2011) Teosinte Inflorescence Phytoliths. PLoS ONE 6(3): e18349.
2. Iltis JA, Doebley JF (2011) Teosinte Inflorescence Phytoliths. PLoS ONE 6(3): e18349.
phylogeny based on microsatellite DNA. The results of both scaling and discriminant analyses led to the identification of the same rondel morphological categories as being the important discriminators. Plots using the frequencies of only two rondel categories result in the same pattern without multivariate manipulation. Our analysis demonstrates that there are consistent proportions of these rondel categories within each teosinte taxon and that there are consistent differences in proportions between the various taxa. Expanding on an idea originally suggested by Piperno and Pearsall [10], we hope that our analysis will eventually help in identifying specific changes in inflorescence phytolith assemblages during the long course of maize’s evolution. This in turn will help elucidate human-teosinte interactions both before and after the evolution of maize. For example, one hypothesis for the relative absence of teosinte in the archaeological record is that teosinte (and early maize) was used primarily as a source of sugar rather than as grains [17,18]. Another hypothesis is that teosinte and very early maize inflorescences were consumed as raw greens while still immature (Pearsall cited in [19]). The most likely source of rondel phytolith assemblages for analysis are quids and coprolites recovered from dry caves in central Mexico such as the Téhuacán Valley caves.

Table 5. Descriptions of important discriminating rondel phytolith categories mentioned in text.

| Code       | Phytolith Category Attributes and Corresponding Figure Number |
|------------|---------------------------------------------------------------|
| A-1-B-1/1  | A–thin face is a complete circle or oval outline, without decorations. 1–thin face is approximately the same size as the thick face. B–thick face is a complete circular or oval outline, with decorations present 1/1–both faces are circular | Figure 6a |
| A-1-B-3/3  | A–thin face is an complete circle or oval outline, without decorations 1–thin face is approximately the same size as the thick face B–thick face is a complete circular or oval outline, with decorations present 3/3–both faces are oval | Figure 6b |
| A-1-B-4/4  | A–thin face is an complete circle or oval outline, without decorations 1–thin face is approximately the same size as the thick face B–thick face is a complete circular or oval outline, with decorations present 4/4–both faces are oval with squared corners on the ends | Figure 6c |
| A-2-B-1/4  | A–thin face is an complete circle or oval outline, without decorations 2–thin face is substantially different in size from the thick face B–thick face is a complete circular or oval outline, with decorations present 1–thin face is circular 4–thick face is oval with squared corners on ends | Figure 6d |
| A-2-B-3/3  | A–thin face is an complete circle or oval outline, without decorations. 2–thin face is substantially different in size from the thick face B–thick face is a complete circular or oval outline, with decorations present 3/3–both faces are oval | Figure 6e |
| A-2-B-3/4  | A–thin face is an complete circle or oval outline, without decorations. 2–thin face is substantially different in size from the thick face B–thick face is a complete circular or oval outline, with decorations present 3–thin face is oval 4–thick face is oval with squared corners on ends | Figure 6f |
| C-1-D-1-1/1| C-1–thin face has one indentation D-1–thick face has one indentation 1/1–both faces are circular | Figure 6g |
| C-4-D-4-4/4| C-4–thin face has three indentations D-4–thick face has four indentations 4/4–both faces are oval with squared corners on the ends | Figure 6h |

doi:10.1371/journal.pone.0018349.t005
Figure 6. Photographs of representative of the important discriminating rondel phytolith categories listed in Table 5. Original magnification 1000× except D at 400×. Arrows in C and F indicate the phytolith belonging to the relevant category. doi:10.1371/journal.pone.0018349.g006
MATERIALS AND METHODS

Fruitcases from teosinte of known genetic background were analyzed, and a database reflecting their phytolith assemblage phenotypes was developed. Germplasm from each of *Zea* taxa is archived at the North Central Plant Introduction Station (NCPI). These samples are of known genetic background, and the populations from which the kernels were collected are known. This is the source from which the plants used in this study were grown, augmented by samples obtained from Mary Eubanks. Teosinte fruitcases were recovered from known samples by randomly removing three fruitcases from each plant. The samples obtained from NCPI totaled from 25 to 100 seeds (the amount of seed in each sample was determined based on availability by NCPI). Samples obtained from Mary Eubanks contained five seeds.

The harvested fruitcases were treated with heated nitric acid, which dissolved the organic matter, leaving the opal phytoliths. Following nitric acid removal of organics, the solutions were placed in centrifuge tubes and centrifuged at 3000 rpm for 15 minutes at a time to concentrate the phytoliths in the bottom of the test tubes. The supernatant nitric acid was then pipetted off and replaced with distilled water. After five repetitions of centrifuging, pipetting off the supernatant liquid, and replacing with distilled water, the procedure was duplicated, replacing the distilled water with ethanol.

Phytoliths were then pipetted onto slides and after the alcohol evaporated the phytoliths were sealed under a cover slip with permount. Each of 100 rondel phytoliths from every sample was assigned to a morphological category using a morphological taxonomy originally developed by Mulholland and Rapp [24] for Poaceae and subsequently expanded and modified by Thompson based on his experience with *Zea* [25].

Each rondel phytolith was examined in planar (upright) view for coding. The taxonomy produces an alpha-numeric code for each rondel phytolith based on the shapes of the thin (larger) and thick (smaller) faces. For example, code A-1-B-1/1 represents a rondel phytolith with a thin face that is a complete circle without decorations and is approximately the same size as the circular thick face that has decorations [25,26]. Listings of attributes for various rondel phytolith categories identified above as important discriminators between the *Zea* taxa are presented in Table 5. Images of representative phytoliths in these categories are shown in Figure 6.

Following [25,27–29] we used a quantitative approach by measuring similarity on the basis of the abundance of the rondel morphological categories. Previous studies have demonstrated that rondel phytolith assemblages can be used to distinguish maize from non-maize grasses from both modern and archaeological samples. Hart and Matson [29] were able to replicate the results in Hart et al. [28] with multivariate discriminant analysis (MDA) using a substantially reduced number of rondel morphological categories. The present sample consists of rondel assemblages from 43 teosinte and two Tripsacum plants, with each having from 99 to 101 rondels (mode = 100) assigned to 45 morphological categories (see supplemental data). Unlike much previous work using morphological rondel categories (e.g., [27]) no size information is used in the present analysis.

Previous analyses of rondel assemblages used squared chord distances [30,31] as a measure of similarity. In the current analyses we found that unstandardized Euclidean distances [11,32] result in clearer patterns with the teosinte dataset. We analyzed the resulting 45×45 distance matrix with a number of statistical techniques, including metric Multidimensional Scaling (MDS) [12].

Blind tests were conducted to confirm the ability of rondel phytolith assemblages to reflect the taxa from which they were recovered. Blind test samples were prepared by Mary Eubanks. These tests were doubly blind in that the analyst classifying the sample did not know to which taxa the sample belonged and the analyzers of the data initially had no knowledge of the meaning of “BT” in the dataset. We chose to initially use a procedure to classify the blind test (BT) phytolith assemblages similar to that developed by Hart et al. [28] to assign archaeologically derived phytolith assemblages to either maize or indigenous grass categories.

Following Hart and Matson [29] we subsequently used multivariate discriminant analysis (MDA) as a further test of the hypotheses. For this analysis we assigned all of the known phytolith assemblages to one of three categories: *Zea mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, and section Luxuriantes including *Z. luxurians*, *Z. diploperennis*, *Z. perennis*, and *Z. nicaraguensis* [3].

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: RGM JPH RGT. Performed the experiments: RGM JPH MB. Analyzed the data: RGM JPH MB. Wrote the paper: JPH RGM RGT. Coded data: RGT.

References

1. Doebley JF (1986) Taxonomy of *Zea* I: subgeneric classification with key to taxa. Am J Bot 67: 986–993.
2. Doebley JF, Iltis HH (1989) Taxonomy of *Zea* (Gramineae). II: Subspecific categories in the *Zea mays* complex and a generic synopsis. Am J Bot 67: 1004–1014.
3. Fukamizu K, Hill J, Vigouroux Y, Matsuoka Y, Sanchez GJ, et al. (2005) Genetic diversity and population structure of teosinte. Gen 105: 2241–2254.
4. Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez GJ, Bucker E, et al. (2002) A single domestication for maize shown by multilocus microsatellite genotyping. Proc Nat Acad Sci U S A 99: 6080–6084.
5. Vigouroux Y, Glaubitz JC, Matsuoka Y, Goodman MM, Sanchez GJ, et al. (2008) Population structure and genetic diversity of New World maize races assessed by DNA microsatellites. Am J Bot 95: 1290–1293.
6. Donweiler JE, Doebley JF (1997) Developmental analysis of Teosinte glume architecture: a key locus in the evolution of maize (*Zea*). Am J Bot 84: 1313–1322.
7. Wang H, Nusbaum-Wagler T, Li B, Zhao Q, Vigouroux Y, et al. (2005) The origin of the naked grains of maize. Nat 436: 714–719.
8. Pearsall DM, Chandler-Ezell K, Chandler-Ezell A (2000) Identifying maize in neotropical sediments and soils using cob phytoliths. J Arch Sci 30: 611–627.
9. Piperno DR (2006) Phytoliths: a comprehensive guide for archaeologists and paleoecologists. New York: Altamira Press. 238 p.
10. Piperno DR, Pearsall DM (1993) Phytoliths in the reproductive structures of maize and teosinte: implications for the study of maize evolution. J Arch Sci 20: 337–362.
11. Matson RG, True DL (1974) Site relationships at Quebrada Tarapaca, Chile: A new look at an old problem. Econ Bot 54: 7–42.
12. Klecka WR (1980) Discriminant Analysis. Beverly Hills: Sage Publications Inc.
13. Iltis HH, Doebley JF (1980) Taxonomy of *Zea* (Gramineae). Proc Nat Acad Sci U S A 77: 5011–5015.
14. Iltis HH (2000) Homeotic sexual translocations and the origin of maize (*Zea mays*). Cur Anth 44: 675–703.
15. Iltis HH (2008) Population structure and genetic diversity of New World maize races and teosinte: implications for the study of maize evolution. J Arch Sci 20: 337–362.
16. Klecka WR (1980) Discriminant Analysis. Beverly Hills: Sage Publications Inc.
17. Beltrán L, Huhndorf S, Merli S (2003) Some distance properties of latent root and vector methods used in multivariate analysis. Biomet 53: 325–338.
18. Gower JC (1967) Multivariate analysis and multidimensional geometry. The Stat 17, 13–28.
19. Floyd, W. 1966. Metric Multidimensional Scaling (MDS) [12].
20. Osker MK, Robertson LT (1998) Origins of Agriculture in the Lowland Neotropics. San Diego: Academic Press. 161 p.
20. Callen EO (1967) Analysis of Tehuacan coprolites. In Byers DS, ed. Prehistory of the Tehuacan Valley: Vol. 1, Environment and Subsistence. Austin: University of Texas Press. pp 261–289.
21. Mangelsdorf PC, MacNeish RS, Galinat WC (1967) Prehistoric wild and cultivated maize. In Byers DS, ed. Prehistory of the Tehuacan Valley: Vol. 1, Environment and Subsistence. Austin: Univ. of Texas Press. pp 178–200.
22. Flannery KF (1986) Güila Naquitz: Archaic foraging and early agriculture in Oaxaca, Mexico. Orlando: Academic Press. 538 p.
23. MacNeish RS (1958) Preliminary archaeological investigations in the Sierra Tamaulipas, Mexico. Trans of the Amer Phil Soc No 48(6). Philadelphia.
24. Mulholland SC, Rapp G, Jr. (1992) A morphological classification of grass silica bodies. In Rapp G, Jr., Mulholland SC, eds. Phytolith systematics: emerging issues. New York: Plenum Press. pp 63–89.
25. Thompson RG (2006) Documenting the presence of maize in Central and South America through phytolith analysis of food residues. Zeder MA, Bradley DG, Emshwiller E, Smith BD, eds. Documenting domestication: new genetic and archaeological paradigms. Berkeley: University of California Press. pp 82–98.
26. Hart JP, Thompson RG, Brumbach HJ (2003) Phytolith evidence for early maize (Zea mays) in the northern Finger Lakes region of New York. Am Antiq 68: 619–646.
27. Chavez SJ, Thompson RG (2006) Early maize on the Copacabana Peninsula: implications for the archaeology of the Lake Titicaca basin. Staller JE, Tykot RH, Benz BF, eds. Histories of Maize: Multidisciplinary Approaches to the Prehistory, Linguistics, Biogeography, Domestication, and Evolution of Maize. . BurlingtonMassachusetts: Academic Press. pp 415–428.
28. Hart JP, Brumbach HJ, Lusteck R (2007) Extending the phytolith evidence for early maize (Zea mays ssp. mays) and squash (Cucurbita sp.) in central New York. Am Antiq 72: 563–583.
29. Hart JP, Matson RG (2009) The use of multiple discriminant analysis in classifying prehistoric phytolith assemblages recovered from cooking residues. J Arch Sci 36: 74–83.
30. Hammer Ø, Harper DAT (2006) Paleontological data analysis. MaldenMassachusetts: Blackwell Publishing. 351 p.
31. Overpeck JT, Webb T, III, Prentice IC (1985) Quantitative interpretation of fossil pollen spectra: dissimilarity coefficients and the method of modern analogues. Quat Res 23: 87–108.
32. Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. San Francisco: W. H. Freeman. 573 p.