A BUNDLE OF VERY LARGE, CULTIGEN AMARANTH (*AMARANTHUS* SP.) SEEDS FROM DYCK CLIFF DWELLING, ARIZONA

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Background

Two domesticated amaranths in the U.S. Southwest: *Amaranthus cruentus* L. and *A. hypochondriacus* L.

- Both domesticated in Mesoamerica ca. 4,000 – 6,000 years ago, as grain, greens, red dye
- Likely ancestor of both (per modern DNA) is *A. hybridus* L.
- Seed colors: pale (white or yellowish), red, black
- Seeds and flowering parts found in archaeological sites in the Greater Southwest
- Both grown today by traditional and indigenous farmers

Study Goals

- Analyze a bag of ancient amaranth seeds found at Dyck Cliff Dwelling
- Identify the seeds to species using multiple lines of evidence:
  - Ancient DNA
  - Seed measurement data
  - Seed coat thickness
  - Seed coat surface texture
  - Morphological traits of bracts, tepals, and utricle caps

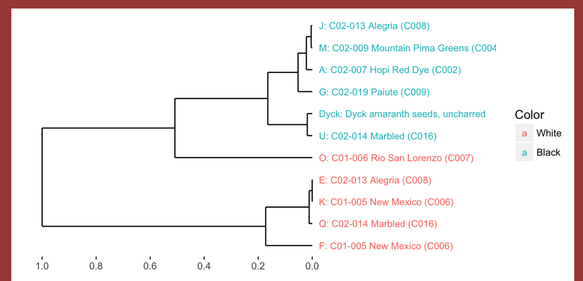
Figure 1. Subtilisin base-sequence (121-360, 360-600) differences between archaeological samples (X1/X2) and samples from Native Seeds/SEARCH (NS/S) and GenBank (GB) collections. Highlighted cells indicate no base pair differences.

	X1/X2 (Dyck)	A. cru (NS/S)	A. cru (GB)	A. hypo (NS/S)	A. cau (GB)	A. hyb (GB)	A. pow (GB)	A. retro (GB)	A. pal (GB)
X1/X2 (Dyck Cliff Dwelling)	/								
A. cruentus (NS/S)	0	/							
A. cruentus (GB)	0	0	/						
A. hypochondriacus (NS/S)	0	0	0	/					
A. caudatus (GB)	2	3	3	2	/				
A. hybridus (GB)	2	2	2	2	3	/			
A. powellii (GB)	2	2	2	2	1	2	/		
A. retroflexus (GB)	4	5	5	5	4	5	3	/	
A. palmeri (GB)	9-10 (ambiguity is a "Y")*	6	6	7	7	8	6	7	/

Amaranthus cruentus (NS/S), A, C, D, E, G, I, J, L, M, P, Q, R, U, V; *A. cruentus* (GB), KC747307.1 (Brazil); *A. hypochondriacus* (NS/S), F, K, O; *A. caudatus* (GB), KC747302.1 (Bolivia); *A. hybridus* (GB), KC747316.1 (US-Ohio); *A. powellii* (GB), KC747323.1 (France); *A. retroflexus* (GB), KC747329.1 (US-Iowa); *A. palmeri* (GB), KC47322.1 (Mexico-Puebla).

*Y: "pyrimidine" (C or T), possibly due to two seeds having different bases at that site.

Figure 2. Cluster Analysis (Hotelling's T^2) showing strong relationship between seed color and seed size. Dyck seeds cluster with dark-seeded *A. cruentus* (J, M, A, G, and U, in blue). White-colored accessions (shown in red) are either *A. hypochondriacus* (O, K and F) or *A. cruentus* (E, Q).



DNA Analysis (By Terence Murphy, UC Davis)

- Molecular base pair differences of three genes, amplified via PCR:
  - --Subtlisin
  - --ITS (chloroplast Internal Transcribed Spacer)
  - --Mat K (maturase K)
- Analyzed: 2 ancient samples of Dyck amaranth seeds (X1 and X2)
- 14 modern collections of *Amaranthus cruentus* (Native Seeds/SEARCH)
- 3 modern collections of *Amaranthus hypochondriacus* (Native Seeds/SEARCH)
- GENBANK data included for:
  - 4 wild amaranth species known in the Verde Valley (*A. hybridus, A. powellii, A. retroflexus, and A. palmeri*)
  - *Amaranthus caudatus*, an Andean domesticated amaranth
- For all three genes, subtilisin, ITS, and matK, all the sequences of *A. cruentus* and *A. hypochondriacus* from the Native Seeds/SEARCH collections were identical to each other **(Figure 1).**
- Sequences of the two samples of ancient Dyck Cliff Dwelling seeds (X1 and X2) were identical to those of the two modern domesticated amaranths, *A. cruentus* and *A. hypochondriacus*.
- The base sequences of Dyck Cliff Dwelling amaranth seeds are identical to those of *A. cruentus* and/or *A. hypochondriacus* for all three genes examined. However, it is not possible to declare which of the ancient domesticates these seeds represent. Further molecular analysis to segregate the two species of amaranth from each other is required.

Seed Coat Surface Texture

- Marginal zones of the Dyck Shelter amaranth seeds exhibit a clear reticulate pattern even at low (30 x) magnification.
- Similar sculpturing is barely discernable but far more subtle (visible only using electron microscopy) on the central portion of the seed coats . A zone of transition between the highly sculptured marginal zone and the smoother central zone is easy to demarcate.
- Previous researchers have noted that *A. cruentus* seeds display a sculpted surface texture, whereas the surface texture of *A. hypochondriacus* seeds is described as "inconspicuous" (Costea et al. 2001:947 and 957; see also McClung de Tapia et al. 1996:23). According to these descriptions and our personal observations, the Dyck Cliff Dwelling specimens correspond more closely to *A. cruentus*.

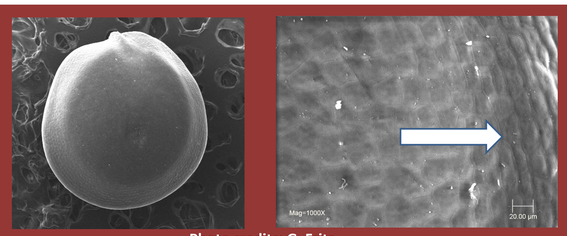


Photo credits: G. Fritz

Seed Measurement Data

Methods:

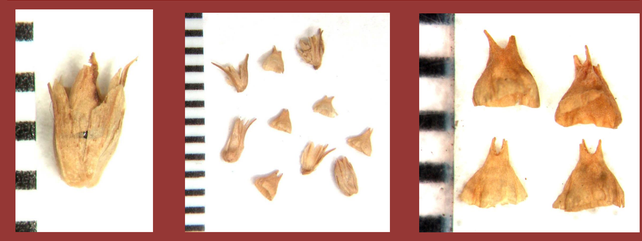
- 420 seeds measured for diameter and thickness (32 x mag., Zeiss binocular microscope with eyepiece micrometer):
  - 30 seeds measured from each of following groups:
    - 7 modern *Amaranthus cruentus* accessions (Native Seeds/SEARCH)
    - 6 modern *A. hypochondriacus* accessions " " "
    - Dyck Cliff Dwelling bag
- Outlier samples removed; Multivariate normality confirmed

Results:

- ***Distinctiveness***. The Dyck seeds are statistically distinct from all modern accessions, except accession U (black *A. cruentus* from Morelos). The Dyck seeds fall within the normal range of seed diameter (t=-0.94; df=28.26; p=3.55e-01) but are significantly less thick than the modern seeds (t=3.27; df=27.68; p=2.89e-03).
- ***Seed color and seed metrics.*** Multivariate analyses of dark- and pale-colored seeds from the same modern accessions show there are clear morphological differences between dark-colored and pale-colored seeds within domesticated species. Pale-colored seeds have greater thickness than dark-colored seeds.
- ***Seed metrics useful in identification.*** Multivariate and univariate analyses of modern *Amaranthus* seed samples show significant morphometric differences between *A. cruentus* and *A. hypochondriacus*. Species has significant effects on seed diameter and seed thickness. Species has a larger effect on seed thickness than on seed diameter.
- ***Meaningful clusters of amaranth accessions***. Several exploratory cluster analyses were performed by Kyle Bocinsky on the seed morphometric data **(Figure 2)**. In all cluster analyses, the Dyck samples form a rather discrete cluster with accession U from Morelos and are closely related to dark-seeded accessions from the Hopi and Paiute, and from Morelos. Additional morphometric data documenting the diversity of domesticated amaranths will further illuminate the relationships between species, phenotype, and environment, and will better allow us to establish connections between contemporary amaranth populations and their ancient progenitors.

Chaff

- All bracts, tepals, and utricle cap were examined for traits that might aid in species determination. The Dyck seed cache had clearly been carefully winnowed prior to storage, leaving behind little chaff.
- The few bracts varied from straight to outwardly curved at the tip, and extended beyond the height of the style branches on the utricle caps.
  - -- For morphological conformance with *A. cruentus* as described by Sauer (1950) and Adhikary and Pratt (2015), we expected to observe bracts that were straight, shorter than the utricle, and slightly longer than the tepals. This was not the case.
  - -- For conformance with *A. hypochondriacus*, we expected long, uniformly recurved bracts, which also did not hold true.
- Most Dyck utricle caps conform to *A. cruentus* in having short, erect style branches, but at least one of the style branches on one cap is more spreading than erect.
- None of the utricle caps displays enough apical narrowing to form a distinct "rostrum," which is notable because presence of a rostrum is characteristic of at least some regional variants of *A. cruentus* (Costea et al. 2001:947; Sauer 1950).
- Alternative explanations can be offered for these seemingly contradictory observations : (1) The Dyck amaranth might represent *A. cruentus* that crossed with one of the closely related local, weedy species, resulting in longer bracts and variable style branch posture. (2) Sinagua farmers or their trade partners grew a landrace of *Amaranthus* bred in the Greater Southwest that differs morphologically from previously described (modern) Mexican or Guatemalan varieties.



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DYCK CLIFF DWELLING SITE: Central Arizona, Verde Valley

- 9-room habitation site; Southern Sinagua Culture
- Occupied ~ A.D. 1000 – 1325
- Excavated 40 years ago by Charles Rozaire; materials in storage since then
- Cist 51 contained a bag of amaranth seeds; stored for future planting?
- Cist 51, which also contained ceremonial items, collapsed during occupation of Room 4
- Cist was then sealed with a mortared stone wall

The Bag of Amaranth Seeds

- A red, mineral-dyed, plain-weave cotton cloth bundle, tied up with yucca fiber cordage
- Opened cloth measures 30 cm x 18 cm
- Contained ~ 31,575 amaranth seeds, tepals, utricle caps and bracts
- C14 dates (AEON Laboratory) on two seeds: A.D. 1021 – 1165, and A.D. 1035 – 1186

Photo credit: K. Adams

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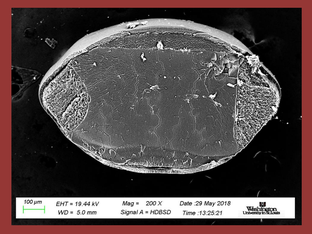
Seed Coat Thickness

- 10 seeds were dissected and mounted on SEM stubs:
  - EvoMA 10 (Environmental SEM at Washington U.- St. Louis, Dept. of Biology)
- Testa thickness range = 15 - 22 microns
- Up to 3-micron variation possible on an individual seed:
  - Minimum mean thickness = 17.4 µ; std. dev. 1.174
  - Maximum mean thickness = 18.9 µ; std. dev. 1.595
- Dyck amaranth seed coats are thicker than previously measured archaeological cultigens, as expected because:
  - Dyck seeds are black rather than pale
  - Dyck seeds are among the largest known ancient amaranths
- Dyck seeds fall within range of dark-seeded 'Mixteco' landrace of *A. hypochondriacus* grown in Mexico as leafy vegetable (McClung de Tapia et al. 1996).
- Testa measurements on dark-seeded Mexican landraces of *A. cruentus* are not available.
- We cannot conclude at this time that Dyck Cliff Dwelling seeds conform more closely to one species or other.



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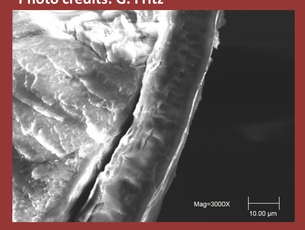

Conclusions

- Our molecular results based on base pair differences of three commonly studied genes confirm that the Dyck seeds represent a domesticate, either *A. cruentus* or *A. hypochondriacus*, but currently they do not allow us to definitively point to one species.
- On the basis of seed measurements and seed coat surface texture, the Dyke seeds conform most closely to dark-seeded accessions of *A. cruentus*. However, we currently lack metric data for dark-seeded *A. hypochondriacus*.
- Seed coat thickness falls within the range of the dark-seeded 'Mixteco' landrace of *A. hypochondriacus* grown in Mexico for use as a leafy vegetable, but corresponding evidence from black-seeded *A. cruentus* has yet to be acquired.
- Our study of chaffy parts is inconclusive due to the scarcity of tepals, bracts, and utricle caps, and the fragmentary condition of those that are preserved. The few chaffy parts that *are* present display variability precluding categorization to a single species.
- This is the first study of ancient amaranth DNA that we know of. Further ancient and modern DNA analysis focusing on the grain and dye amaranths from the southwestern United States and northwestern Mexico is needed in order to expand the existing body of data that, aside from our study, includes only modern accessions from central Mexico and Guatemala.
- Our team plans to pursue additional biomolecular research in continuing efforts to identify the Dyck Cliff Dwelling amaranth seeds and to better understand the history of cultigen amaranth in the U.S. Southwest.

Acknowledgements

We sincerely thank the dedicated volunteers of the Verde Valley Archaeological Center and the Paul Dyck family for making this project possible. Gratitude is also extended to Mana Hayashi Tang and Dianne Duncan for their expertise and assistance with Scanning Electron Microscopy at Washington University in St. Louis.