Dental and mtDNA Relatedness Among Thousand-Year-Old Remains from Huaca Loro, Peru

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ABSTRACT Within and between tombs at the site of Huaca Loro (ca. A.D. 1000) on north coastal Peru, biological relatedness based on 23 dental characters follows statistically significant patterns. mtDNA groupings that are based on lineages inferred from inherited derived D-loop bp sequences have also been traced among the individuals. The present study finds a significant, although rather poorly predictive, relation between mtDNA and dental interindividual linkages. When analyzing the individual dental traits for correspondence to mtDNA "lineages", several significant relations are found and one trait in particular, buccal expansion of the maxillary distal premolar, corresponds highly to mtDNA patterning.

Huaca Loro (ca. A.D. 1000) is a monumental adobe platform mound with a series of deep shaft tombs under and around its base. This mound with a temple at the top is situated in the Poma National Historical Sanctuary on the north coast of Peru. The Sicán Archaeological Project has conducted fieldwork in and around Poma for more than 2 decades (Shimada, 1981, 1990, 1995, 2000). Since 1990, excavations have included recovery of 34 individuals from the East Tomb, West Tomb and North Trench (Shimada et al., 1998, 2000; Shimada and Merkel, 1993).

Comparative odontological (Corruccini and Shimada, 2002) and other analyses (Shimada et al., 1998, 2001; Farnum et al., 1998) pertain to these remains. Corruccini (1998) cites other studies concurring that dental variables are informative for establishing genetic and familial affinities between samples. With the recent addition of mtDNA analysis of the teeth (Shimada et al., 2001), it is possible to examine the dental traits for overall (multivariate) and trait-specific (univariate) concordance with a known genetic variant.

Dental indications of biological relatedness were sought within and between the following nine partitions of the total sample: the Principal West Tomb burial at the center of the Central Chamber, a juvenile male "looking" at him from the Antechamber higher up, two accompanying (possibly sacrificial) females in south and north niches of the Central Chamber, eight scorable females to the south, eight scorable females from the grouping to the north, five inferred "commoners" from the North Trench, and the Principal (adult male) interment and three other individuals (two adult females and a juvenile) from the East Tomb. See Figure 1.

From silicon molds taken by R. Benfer and I. Shimada of maxillary and mandibular arches and dental stone casts made by W. Duncan, 23 dental traits scored by R. Corruccini yielded size-equalized Euclidian distance coefficients between those 29 adequately preserved individuals. The traits were scored for the most part according to Turner et al. (1991) and Corruccini and Potter (1981) on the best preserved side. Considerably more descriptive detail is in the publication by Corruccini and Shimada (2002):

1. Maxillary central incisor labial convexity.
2. Maxillary incisor shoveling.
3. Maxillary double shoveling.
4. Mandibular incisor shoveling.
5. Canine accessory ridge and basal tubercle.
6. Maxillary distal premolar buccal cusp (paracone) diameter.
7. Mandibular distal premolar lingual component mesiodistal diameter.
8-9. Hypocone development on maxillary M1 and M2.
10. Maxillary M3 metacone.
11-13. Cusp number for mandibular M1-3.
14-15. Chord from mesial fovea to central fovea for mandibular M1-2.
16-17. Chord from central fovea to distal fovea (or distal marginal ridge) for mandibular M1-2.
18-19. Chord from central to distal fovea on maxillary M1-2.
20. M_1 bilaterally lost in the presence of M_2 (not an appeal to congenital but rather pathological genetic tendencies).
21. M_2 bilaterally lost in the presence of M_1.
22. Central incisor winging.
23. Third cusp ("entoconid") lingual development on the distal mandibular premolar.

Random resampling of the resultant distances yielded p = 0.006 for the null hypothesis of random odontological intracemetery patterning. Among salient aspects of the statistically significant result (Corruccini and Shimada, 2002) were three particular patterns: the

morphological cohesiveness of the South females, the heterogeneity among North females, and high similarity among the inferred high-status males of East and West Tombs.

Teeth are useful for ancient mtDNA analysis, and an unrelated earlier study was successful in correlating biokinship measured from both mtDNA and odontometry (Shinoda et al., 1998). A well-preserved tooth was extracted by KS from each individual. Whole teeth were soaked in DNA contaminant removal solution, rinsed in distilled water and dried. mtDNA was extracted from ground tooth powder (Shimada et al., 2001) following a modified protocol (GENRCLEAN kit; BIO 101 Co.). Eluted DNA was amplified by PCR.

RESULTS

Several probably derived mtDNA sequences are identifiable among the interments. Most New World indigenous mtDNA polymorphisms are at the mitochondrial "D-loop" (Gonzalez-Oliver et al., 2001). A combination of RFLP haplotype and D-loop sequence analysis determined variants successfully for 18 West Tomb, 3 East Tomb and 2 North Trench individuals. Sequences encompassing 192 bp were determined in which mutations were found at 25 sites. At 24 sites transition mutations alone occurred, C to T transition observed 16 times and A to G 8 times. In studies involving DNA of ancient samples the original sequences may easily have changed. This potential limitation (implying differential degradation particularly involving A/G versus C/T transitions) may apply also to the celebrated Neandertal studies (Krings et al., 1997).

Among the individuals included in the dental analysis, the pertinent "lineages" implying shared maternal ancestry (of uncertain remoteness) are indicated in Table 1 and Figure 1. These include haplotypes uniting the North niche female and 3 other of the North females, and two distinct haplotypes occurring in 5 of the South females. Thus various women within but not between North and South groupings are related. In addition the East and West Tomb male principal interments (plus one other, North Trench burial) are more tenuously linked. Although the initial finding of relatedness between East and West Tomb principal individuals could not be replicated a second time owing to problems with extracting and reproducing the West individual's DNA, there was no negation of a genetic link between these two.

In comparing the dental distances within these genetically inferred matrilineages versus distance between them, an overall mean of 1.40 is found within the 13 interindividual d's (3+6+3+1) that are intralineage, and d=1.41 for all the remaining interlineage pairs admittedly not arguing for much difference. However, the time depth of the postulated shared maternal ancestry remains unknown, and could vary according to the different haplotypes, whereas the dental variants would be inherited through nuclear DNA recombined from both parents with a generational diluting effect on the matrilineage from ongoing male input.

Furthermore, the North females have an undue influence on results, constituting 46.2% of the intra-lineage d's in Table 1 (6/13) but only accounting for 7.6% of interlineage d's. Since these North females have consistently very large dental d's, they disproportionately inflate the intralineage distance. Accordingly a matched comparison can be designed as indicated in Table 1, contrasting the dental affinities of the 13 DNA-linked pairs with their spatially closest corresponding groups that are unlinked. Those individuals linked by mtDNA pattern are quite consistently (but very slightly) closer in dental pattern than the "unrelated" individuals (paired t = 3.12, 12 d.f., one-tailed p < 0.005). The p remains < 0.01 (11 df) when the one pair of distances involving East and West Tomb principal individuals, the most tenuous mtDNA linkage, is removed.

TABLE 1. Average linear Euclidian distances over 23 dental traits (converted to normalized shape variables) among individuals belonging to distinct mtDNA types compared to distances among the remainder of their archaeologically positioned group[1]

Lineage	d within	Compared to	d within
First* (3)	1.284	Other East Tomb, North Trench occupants	1.482
Second**(3)	1.566	North Niche to unrelated North females	1.694
Second**(3)	1.552	All other North females	1.573
Third***(3)	1.268	Other South females	1.250
Fourth****(1)	1.219	Other South females	1.250

[1]The number in parentheses is the number of comparisons within a mtDNA "lineage" that can be contrasted with matching average d within the appropriate comparison group.

*East Tomb principal interment, West Tomb principal, and burial 3 from North trench

**North Niche (sacrificial) Central Chamber female d to the other 3 North females of same mtDNA type, compared to her d from unrelated North females. Then the d within the seemingly related 3 North females is compared to the d among all other North females

***South female burials 10, 13 and 14

****South female burials 6 and 8
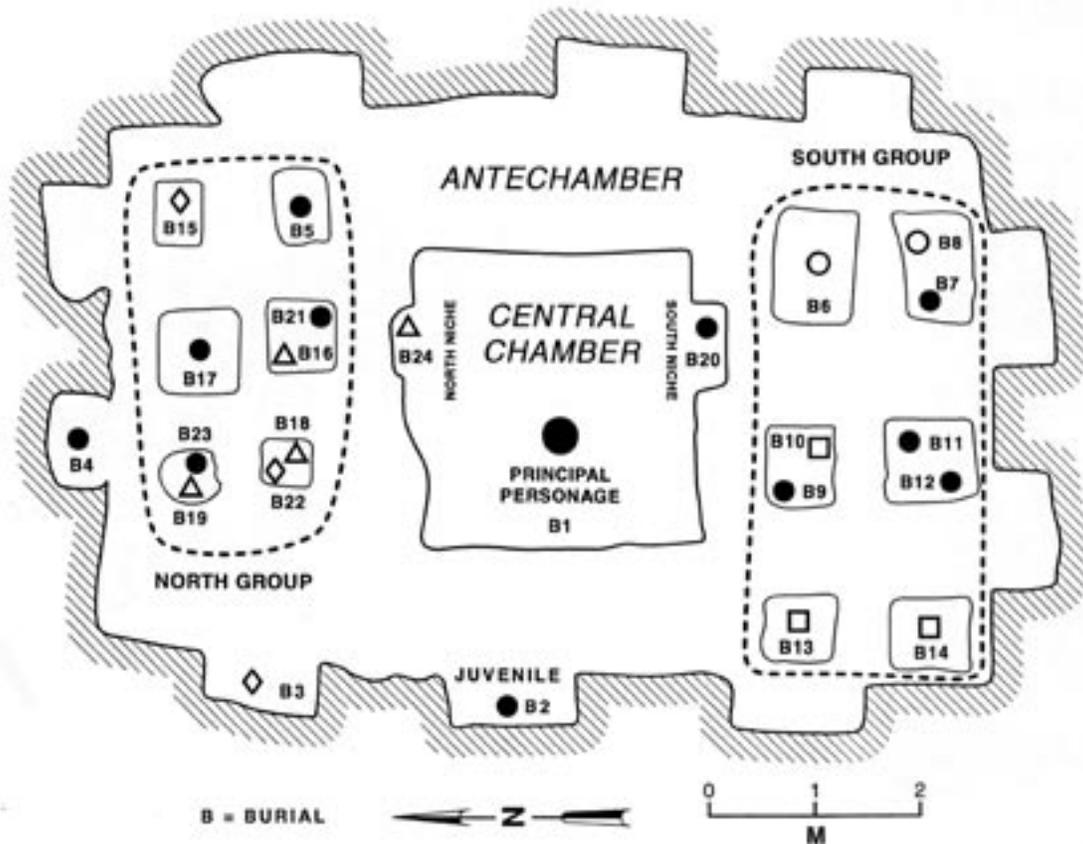
Fig. 1. Spatial distribution of individuals with matching haplotypes within the West Tomb indicated by outlined symbols (i.e., circle, triangle, square, and diamond). The solid black circles represent individuals who are not maternally related to anyone else within the tomb. Only one of the three females represented by diamonds preserved adequate teeth, so there is no intra-group contribution from that haplotype.

Having detected an admittedly subtle yet significant parallel between dental discordance and mtDNA unrelatedness, it is of interest to see which traits correspond most closely within these "matrilineages". This is attempted in Table 2, where the pairwise squared interindividual variance is contrasted within the linked groups and between remaining unlinked individuals. The F-ratio can be used to test the one-tailed proposition that variances shall be smaller among pairs of individuals within mtDNA types, but some consideration of the degrees of freedom is warranted. Only 29 total individuals have yielded (29 X 28)/2 = 406 pairwise differences. The F statistic will be subject to Type I error when read with an inflated 392 and 12 degrees of freedom (406-13-1 d's between and 13-1 d's within haplotypes). At the other extreme, reducing this to a minimalist 16 (17 unlinked individuals minus 1) and 11 (12 individuals involved in mtDNA matches minus 1) degrees of freedom will substantially overcorrect and bring about Type II error.

Furthermore, there is concern over the redundancy effect of testing multiple (23) separate null hypotheses using the same sample of individuals repeatedly. This is the Bonferroni effect (Sokal and Rohlf, 1987:17-18)

and can be corrected (probably too harshly, as there would only be partial redundancy) by adjusting the critical probability from the usual p = 0.05 to 0.05/23 = 0.0022. Table 2 shows the two extremes, i.e., maximized d.f. that will be very sensitive, and minimized d.f. with Bonferroni's correction included. The latter sets rather high standards for a significant result. The reality about the null probability is presumably somewhere between those estimates.

Six of the 23 traits indicate significant partition of variance according to mtDNA homogeneity, quite a bit more than the random expectation of 23 X 0.05 = 1.15 results expected to be due to Type I error when the critical p is 0.05. Thus there does seem to be familial resemblance affecting the teeth, although this might be thought unlikely to be detectable unless the shared maternal ancestry is fairly recent or the trait is sex-linked. Looked at another way, the directional F-ratios have a geometric mean well over 1.0, again suggesting overall segregation of dental variance according to mtDNA affinity. That one of the traits, the distal maxillary premolar paracone inflation, clears the Bonferroni hurdle suggests not only that this particular variable is confidently rejecting the null hypothesis,

TABLE 2. mtDNA "lineage" segregation (variance among non-lineage individual pairs divided by variance within mtDNA lineage pairs) over the 23 dental traits for 29 individuals

Trait Number	Among	Within	F-ratio
1	0.349	0.174	2.09
2	0.954	0.634	1.51
3	0.752	1.406	0.53
4	0.848	0.350	2.42*
5	0.585	0.814	0.72
6	72.022	8.094	8.90**
7	54.925	16.000	3.43*
8	2.303	4.111	0.56
9	21.258	14.778	1.44
10	0.188	0.136	1.38
11	1.559	4.400	0.35
12	11.824	26.818	0.44
13	35.154	48.714	0.72
14	17.962	26.400	0.68
15	23.747	19.900	1.19
16	27.527	8.050	3.42*
17	29.201	16.500	1.77
18	16.109	28.889	0.56
19	36.314	48.875	0.74
20	0.118	0.231	0.51
21	0.221	0.154	1.43
22	0.168	0.038	4.37*
23	0.355	0.066	5.36*

*Broadly significant, F for 392 and 12 d.f. yields $p < 0.05$
**Narrowly significant, F for 16 and 11 d.f. yields $p < 0.05/23 = 0.0022$

but clears the way for the conclusion of a significant "treatment effect" among the variables in general.

In addition to the strongly significant paracone diameter, two other of the traits indicating significant similarity among relatives (according to the broader interpretation of d.f.) are metric, the second mandibular molar's mesial occlusal diameter (trait 15) and the mandibular distal premolar's lingual mesiodistal diameter; the latter (trait 7) measures somewhat the same thing as P4 third cusp presence (trait 23) which is also significant. The other non-mensurational traits with significant F in Table 2 are mandibular incisor shoveling and incisor winging.

DISCUSSION

Molar traits figure less than premolar traits in the list of significant results in Table 2, but this may signify little for genetic interpretations of odontological variants. Correspondence to mtDNA affinity may be haphazard for dental traits, although of some interest in analyses of prehistoric samples in regard to the matrilocal/patrilocal question (Corruccini, 1998).

Comparison to Nichol (1989:Table 4) yields perspective from a vaguely related (Amerind: Pima) sample, for which segregation analysis of individual dental variables estimates the heritable tendencies among families. Nichol does not detect unusually strong genetic segregation for winging or shoveling, although both probably have significant transmissibility (the former fitting a polygenic and the latter a dominant or major gene model best). The distal mandibular premolar lingual extra cusp may fit a dominant or polygenic model with higher transmissibility than winging and shoveling, but not higher than other traits.

The possibility of sex-linked heritability is particularly interesting here due to maternal mode of transmission of mtDNA. However Nichol does not examine sex-linked tendencies. Other studies send mixed signals (Garn et al., 1965; Townsend and Brown, 1978) regarding sex-linkage of overall tooth size. One crown variant, Carabelli's cusp, has been examined thoroughly and does not appear sex-linked in its heritability (Townsend and Martin, 1992; Garn et al., 1966).

Some confidence is gained here for the widely accepted procedure of treating dental variables as genetic indicators. Speculation regarding specific family affinity of individuals, and sex-linked inheritance of variables that correspond to mtDNA "lineages", is just that. One outstanding biological dilemma is provided by the relatively widespread mtDNA connection among North females who are relatively dentally disparate.

Models incorporating incremental generational change in polygenic dental variables, contrasting with the unadmixed maternal mtDNA transmission, could be contemplated. Perhaps the South females, dentally similar, are closely related by way of males such as would come about through, say, a sororal polygynous background, while the North females (who are also distinct in terms of archaeological indicators) might be distantly related through female ancestors such as could result from matrilocal background.

Thus familial resemblance affecting the highly heritable dental traits reverberates somewhat through the mtDNA linkages, but, as is quite expectable, the correspondence is imperfect and susceptible to speculations about matrilocal versus patrilocal biological transmission.

ACKNOWLEDGMENTS

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