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Provenance of African-Born Individuals from the Colonial Cemetery of Campeche (Mexico) by Means of Trace Elements Analysis

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ABSTRACT The present study investigates the issue of foreign provenance of African individuals buried in the early colonial cemetery of Campeche (Mexico) using multiple trace elements analysis from the first permanent molar. It rests on the assumption that, like for Strontium isotopes, the elemental pattern in the first molar reflects the hydro-geological environment the individual grew in. The individuals of African ethnicity were identified from their pattern of dental morphology. Twenty-eight individuals were analyzed in this context, eight of them supposedly Africans, while 15 were infants or early juveniles and five from the prehispanic site of Xcambó in northern Yucatán. The infants and juveniles were likely born in the area, thus serving as term of comparison for the |local" elemental pattern. The elements' ppm concentrations of the 28 individuals were elaborated using Principal Components Analysis. Results tend to cluster the infants and some of the African individuals together, though the majority of the Africans tend to group. One African individual in particular separates well from all the others. Assuming that dietary components might interfere with the individuals' distribution, only elements not related to diet were thus used, without different results from the previous analysis. Indeed, the elements correlating high with the first two components are non-dietary. Trace elements patterns indicate that some of the African individuals interred in the early colonial cemetery might have been born in other places, though we cannot infer on their place of origin, while others were probably born in the New World. *Dental Anthropology* 2004;17(3):65-69.

The presence of African individuals in the colonial town of Campeche goes back to the 16th century AD when African slaves were brought in by the Spanish colonizers as servants and workers (Mallafe, 1973). Their presence in the ancient colonial cemetery in the town's Main Plaza (Plaza Principal) was initially suggested by a series of dental non-metric traits and particular dental decoration patterns, that were unknown to native Mesoamericans (Scott and Turner, 1997; Tiesler, 2001; Tiesler and Zabala, 2001). The cemetery surrounded the ancient church that was in use from the mid 16th century AD to the end of the 17th century, when it was replaced by Campeche's cathedral. The graveyard's chronology was confirmed by maps of the colonial town and by a medallion, whose typology is known not to have updated the 1650s (Coronel et al., 2001; Deagan, 2002). The material evidence confirms the historical sources that report the presence of Africans along with the arrival of the Spaniards (Mallafe, 1973). Apparently, Africans were employed as servants in the Spanish households (Zabala *et al*., 2003), as the production economy in the town of Campeche had no need for hard-work slavery, as in plantations or mines.

This study, which presents the preliminary results of an ongoing project on provenance in the Maya area during precontact and contact times, addresses the issue of detecting the place of nativity of Campeche's ethnic Africans. It assumes that trace element pattern from the enamel of the first molar reflects the environment the individuals were born in. For this reason, the persons who had been "imported" or had migrated from another area should present a pattern that differs from that of the local population (Cucina et al., 2004). In this context, the African individuals' pattern is compared to the one established from homologous teeth of infants recovered from the same site, resting on the assumption that babies buried in the cemetery were likely born in town or in its neighboring areas.

MATERIALS AND METHODS

The sample analyzed in this context is part of the skeletal collection of 180 individuals unearthed during the 2000 archaeological salvage excavation in the Plaza Principal of the town of Campeche, which is located long the coast of the Gulf of Mexico, in the northern part of the Yucatan Peninsula (Fig. 1). At least 20 skeletons showed

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Fig. 1. Geographical location of the town of Campeche

a dental morphological pattern assignable to African ethnicity (Scott and Turner 1997; Irish 1997; 1998). Four of these individuals presented a pattern of dental mutilation unknown in the area during precolonial times (Tiesler, 2001) and similar to those introduced by African slaves throughout the Caribbean (Ortner, 1966; Handler *et al.*, 1982; Milner and Larsen, 1991; Crespo, 1992).

The analysis was conducted on the first permanent molar of 28 individuals, eight of them showing African dental morphological patterns, 15 being infants and early juveniles that should be representative of the area. Five additional specimens come from the precontact Maya site of Xcambó. The choice of the first permanent molar rests on its age of development and the likeliness that it reflects more than other teeth the hydro-geological environment in which the individuals grew up (Molleson, 1988; Burton *et al.*, 2003; Jones *et al.*, 2003). The Africans are listed in the
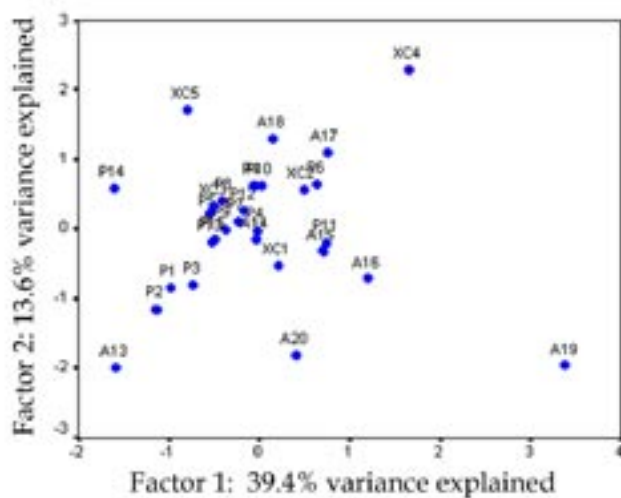
graphs as "A" and a serial number ranging from 13 to 20. The Campeche infants are labeled as "P" and a serial number from 1 to 15, while "XC" (from 1 to 5) represents the individuals from Xcambó.

Teeth were sectioned longitudinally, and the inner layers of the "hidden enamel" (Hillson, 1996) exposed and analyzed by means of laser-ablation using a New Wave 213-nm laser and elements read by an ICP Perking Elmer 6100 DRC mass spectrometer. The elements' intensity was then transformed into parts per million (ppm), and converted into its log values for multivariate analyses.

RESULTS

The elements' intensity that was high enough to be read by the mass spectrometer was converted from ppm into logarithmic ones. Excluding calcium, these elements have been employed to perform principal components statistical analyses. Table 1 lists the elements' components rotated matrix, while Table 3 shows the same elements' components for the non-dietary elements only. The elements with higher values for each component appear in bold. Figure 2 shows the individuals' bi-dimensional scatter plot for first versus second components using all the elements available, while Figure 3 shows the same scatter plot after dietary elements were removed.

A common distribution pattern emerges from all the graphs, despite the wide range of variability in their elemental composition. The individuals identified as Africans tend to be more widely spread in all the graphs than the site's infants and young adolescents (labeled as P). Within the African group, some (A19 above all) constantly separate from the rest. Interestingly, we obtained the same pattern when we used all the elements or only the non-dietary ones.

Fig. 2. Principal components analysis: distribution of the individuals along the first and second components using all the elements available (but Calcium) listed in Table 1. "A" labels the African individuals, "P" the autochthonous from Campeche, "X" the individuals from the precontact, Classic site of Xcambó.
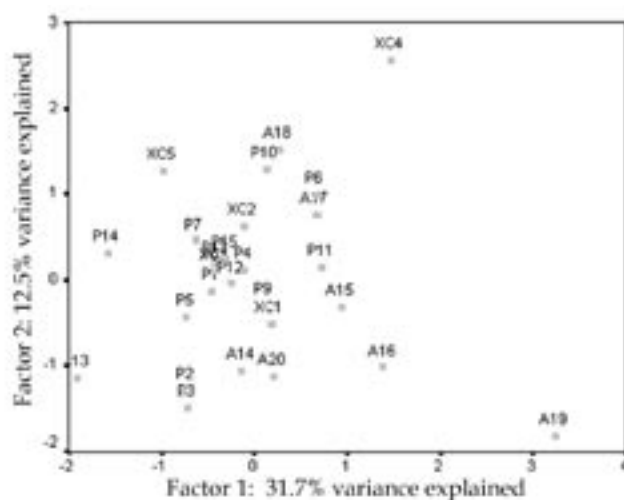

Fig. 3. Principal components analysis: distribution of the individuals along the first and second components using only non-dietary elements listed in Table 2.

Table 1. Matrix of rotated components of all the elements analyzed[1]

	1	2	3
NA23	0.089	0.501	-0.012
MG24	-0.222	0.756	-0.013
AL27	0.093	0.818	0.189
SI30	0.878	-0.159	0.074
K39	0.164	0.483	0.241
SC45	0.098	0.003	-0.032
TI47	0.101	0.448	0.364
V51	-0.255	-0.198	-0.100
CR52	0.820	-0.058	0.343
MN55	0.685	0.415	0.107
FE57	0.378	0.623	0.057
NI60	0.068	0.547	0.538
CU65	0.070	-0.017	0.868
ZN66	-0.455	0.091	0.009
AS75	-0.115	0.411	-0.011
RB85	0.571	0.261	0.583
SR88	0.000	0.102	0.217
ZR90	0.258	0.238	0.737
SB121	-0.050	-0.322	-0.073
CS133	0.297	0.126	0.005
BA138	0.021	0.086	0.094
LA139	0.153	0.657	0.279
CE140	0.568	0.023	0.398
GD158	0.765	0.284	0.323
DY164	0.186	0.026	-0.049
YB174	0.196	-0.027	0.061
LU175	0.571	0.623	0.071
HF180	-0.213	0.374	0.247
TA181	0.249	0.437	0.213
PB208	0.275	0.213	0.725
TH232	0.720	0.130	-0.072
U238	0.068	0.136	0.460

[1]Varimax rotation converged in 20 iterations. Values in bold indicate the elements.

Table 2. Component's rotated matrix of the non-dietary elements. Varimax rotation converged in 7 iterations[1]

	1	2	3
AL27	0.041	0.868	0.208
SI30	0.910	-0.117	0.007
SC45	0.133	0.045	-0.045
TI47	0.043	0.638	0.391
V51	-0.252	-0.047	-0.075
CR52	0.845	0.092	0.296
NI60	0.013	0.538	0.647
CU65	0.056	0.017	0.925
AS75	-0.084	0.603	-0.132
RB85	0.525	0.254	0.668
ZR90	0.311	0.309	0.597
SB121	-0.105	-0.057	-0.009
CS133	0.178	0.302	0.131
LA139	0.122	0.735	0.257
CE140	0.540	0.055	0.463
GD158	0.819	0.213	0.247
DY164	0.160	0.109	-0.001
YB174	0.195	0.045	0.083
LU175	0.532	0.672	0.042
HF180	-0.117	0.414	0.126
TA181	0.229	0.421	0.230
PB208	0.354	0.253	0.642
TH232	0.689	0.076	0.016
U238	0.046	0.195	0.486

[1]Values in bold indicate the elements.

DISCUSSION

Historical sources report the presence of African slaves in Campeche since the arrival of the Spanish (Scholes, 1936; Redondo, 1995). Their presence in the catholic cemetery is not unusual, as they were converted to the catholic religion upon arrival (Aguirre Beltrán, 1994). Therefore, we expect that the African population in the cemetery of the town's Plaza Principal may be representative of persons born both in the Old and in the New World.

Trace elements have been used to detect foreign provenance within archaeological samples (Burton *et al.*, 2003; Jones *et al.*, 2003), despite their broader range of variability and less stability when compared to stable isotopes. Trace elements are subject to dietary and physiological influence, as well as diagenetic changes (Sandford and Weaver, 2000), which may affect the hydro-geological marks left by the environment during growth (Molleson, 1988). Interestingly the results from this study bestow similar patterns regardless whether all the elements or only the non-dietary elements were used in teeth. Apparently, this indicates that neither diet nor diagenesis affect the elemental pattern from first molar's hidden enamel. The elements that show higher correlation values with each component are not the "dietary" ones, and if diagenesis had altered the chemical composition, results should have been flattened and more homogeneity should have been found.

The results indicate that some of the ethnic African individuals from Campeche's colonial cemetery may not have been born in the area, in particular the individual labeled as A19 and to a lesser extent A16, A20 and A15. Previous analyses on other batches of trace elements readings (Cucina, 2004; Cucina et al., 2004) still indicated an African individuals' distribution pattern almost independent from the native one. A16 and A15 showed a kind of dental decoration unknown in precolonial Mesoamerica (Tiesler, 2001) and apparently imported from the African continent. At this point of the analysis it appears that the decorated individuals were born outside Campeche, although we cannot make specific

inferences on whether these individuals had undergone the process when still in their country of origin or whether this practice was introduced and performed in Yucatán at least for a generation or two before disappearing. Further analyses and confirmation of nativity from stable isotopes on the same individuals will help us better understand this complex multiethnic society in colonial times.

In conclusion, trace elements provided promising results in detecting foreigners in archaeological samples. Differently from previous studies that focused on one or two elements, this analysis uses a multi-elements approach. The application of laser ablation permits to spot-read the hidden enamel, limiting the effects of diagenesis. Some individuals were not likely born in the area, although it is not yet possible to detect whether their place of origin was Africa or somewhere in the Caribbean or Mesoamerica, for an African comparison pattern in not available, the exact date of death is unknown (no tombstone or parish record) and the time span the cemetery was in use encompasses two centuries. Notwithstanding these limitations and the problems in obtaining consistent data due to calibration of the equipment, this analysis indicates that trace elements can find applications in studies of natality and migration patterns.

ACKNOWLEDGMENTS

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Dental Anthropology Association Celebrates Its 20th Meeting

In celebration of its 20th meeting, the Dental Anthropology Association is sponsoring a symposium at the 2005 American Association of Physical Anthropologists meetings to be held in Milwaukee, Wisconsin. Dental Anthropology 20 Years After: The State of the Science will consist of 14 papers covering a wide range of topics germane to the anthropological study of teeth. Organized and chaired by DAA Past President Joel Irish (Alaska, Fairbanks) and Greg Nelson (Oregon) the symposium brings together a myriad of researchers for a welcome look at the relevance and breadth of Dental Anthropology as it stands in the middle of the first decade of the 21st century. The Symposium is scheduled as Session 9 on Thursday afternoon April 7. This is fortuitous, as the symposium precedes the annual DAA business meeting scheduled for Thursday evening. We expect everyone to attend.

In addition to the symposium, dental anthropology will be well represented at the 2005 AAPA meetings with a podium session (session 3, Thursday morning) and a poster session (session 26, Saturday morning) scheduled. Following is the symposium abstract and a list of authors and paper titles:

Dental Anthropology 20 Years After: The State of the Science

Organizers and Chairs: Joel D. Irish, University of Alaska Fairbanks, and Greg C. Nelson, University of Oregon.

Commemorating the 20th anniversary meeting of the Dental Anthropology Association, this symposium highlights recent research in the subfield that is illuminating issues of fundamental anthropological importance. Using both established and innovative new methodological and technological approaches, scholars with interests ranging from the micro- to macroscopic levels of structure and expression present their latest findings on dental genetics, histology, growth and development, pathology, and morphometrics across a broad range of living and fossil human and non-human primate taxa. Thus, unlike many symposia that focus on specific topics and/or regions, the unifying theme here is diversity. The intent is to assess the current state of the subfield, emphasize its insights into diverse anthropological questions, and explore its potential future directions. Cosponsored by the Dental Anthropology Association.

Leslea Hlusko and Michael C. Mahaney. *Conceptualizing dental characters: Implications from baboon quantitative genetic analyses.*

Gary Schwartz. *The evolutionary history of growth and development: Sorting through the evidence with a fine tooth-comb.*

Helen Liversidge. *Dental age revisited.*

Debbie Guatelli-Steinberg. *Using perikymata to estimate the duration of growth disruptions in fossil hominin teeth.*

Louise Humphrey, Christopher Dean, and Teresa Jeffries. *Identification of the neonatal line using LA-ICP-MS.*

Mark Teaford. *Insights from life's little abrasions: Dental microwear at middle-age.*

Peter Ungar and Sarah Taylor. *Dental topographic analysis: Tooth wear and function.*

Simon Hillson. *The current state of dental decay.*

Brian Hemphill. *At what cost a full belly? An investigation of the seductive allure of sedentary horticulture in the Great Basin.*

Kalpana Agrawal and Peter Lucas. *Methods of ingestion and incisal designs.*

Shara Bailey. *The place of Neandertals in modern human evolution: Intra- and interspecific variation in occlusal dental morphology.*

Charles FitzGerald and Simon Hillson. *Dental reduction in late Pleistocene and early Holocene hominids: Alternative approaches to assessing tooth size.*

Roberto Macchiarelli and Luca Bondioli. *Virtual dentitions: Touching the hidden evidence.*

Discussants for the Symposium: John Luckas and Edward Harris.

Submitted by:
Greg C. Nelson

A System for the Acquisition and Analysis of Three-Dimensional Data Describing Dental Morphology

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ABSTRACT: Accurate, reproducible three-dimensional (3D) data provide an important contribution to our ability to describe, compare and understand dental morphology but the existing technology is often expensive or has technical limitations. Recently available, inexpensive 3D profilometers interfaced with standard personal computers offer the potential to overcome some of these problems. This technical note describes a system that uses a 3D profilometer and purpose written software to analyse changes in dental morphology resulting from tooth wear. The validity of the derived data was determined by comparing data derived from scans of objects of known dimensions with calculated volumes. These differences were less than 10% from objects that were difficult to scan because of their geometry and were commonly less than 5%. The reproductibility, expressed as intra- and inter-observer coefficients of variation, was less than 1%. The potential applications of systems of this type are outlined. *Dental Anthropology* 2004;17(3):70-74.

A number of systems for the acquisition and analysis of three-dimensional (3D) data have been developed (Roylet *et al.*, 1983; Delong *et al.*, 1985; McDowell *et al.*, 1988; Hewlett *et al.*, 1992) specifically for use in studies of dental morphology. In most cases the developers have recognised difficulties including hardware limitations, computational complexity or cost. In studies of dental morphology the practical problems are usually associated with the complex morphology of teeth that can make it impossible to scan parts of a surface from some directions or lead to with difficulties in defining appropriate reference points and planes. In some cases "internal" reference points (*i.e.*, anatomical features or prepared reference markers) are defined and scanning can extend either to a limit defined by another reference point or a predetermined distance. Alternatively, in some studies appropriate "external" reference points (eg points on the specimen mount) can be defined.

This paper describes the application of widely available hardware and software packages to provide an affordable system for acquiring 3D coordinates from the surface of a dental crown and subsequently for comparing three-dimensional data derived from these coordinates. As part of the development process, the system was validated by comparison of calculated data with the known dimensions and volumes of standard objects. The reproducibility of the derived data, both within and between observers, was also determined from repeated measurements.

To illustrate the application of the system, we have measured the loss of tooth occurring during simulated tooth wear. However, the software and hardware have the flexibility to provide valid, reproducible data in a broad range of studies of morphology.

MATERIALS AND METHODS

For data acquisition a 3D scanner (PIX-4, Roland DG, Tokyo, Japan) interfaced with a personal computer was used to record the heights (Z) of surface mesh points (X and Y). In this system an active piezo sensor detects contact between its stylus and the scanned surface (Fig. 1). The X and Y mesh steps can be set between 50μ and 5.00 mm in 50μ steps and the Z-axis direction has a resolution of 25μ. The "Dr.PICZA" software (Roland DG, Tokyo, Japan) provided with the scanner is a Windows or MAC OSX-based tool that allows the scan area to be defined to accommodate the dimensions of the specimen and the scanning resolution to be set according to the user's needs. This decision involves balancing the need for high resolution against the size of the resultant data set and the scanning duration, both of which are increased with increasing resolution. In addition, a lower limit and the approximate X and Y coordinates of the highest point of the specimen can be defined to further optimise the size of the data set and shorten the scanner's calibration and scanning times. The software allows basic manipulation and visualization of the data (Fig. 2) and has the facility to export data in a range of

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Fig. 1. Scanner with specimen mounted on scanning table.

formats for subsequent analysis.

In our example, we were aiming to measure the changes in dental crown volume resulting from simulated tooth wear. We therefore mounted our specimens (in this case either the buccal of lingual halves of human tooth crowns) with three reference markers (2 mm diameter titanium spheres) equally spaced around the specimen. After each period of simulated wear the specimen was re-scanned and the volume of the crown above the reference plane compared with previous volumes. Because the predicted changes were relatively small (expected to be of the order of 20 mm³) we chose the highest scanning resolution (*i.e.*, 50µ for the X and Y matrix and 25µ for the height (Z)). The derived data set was exported as a text file for detailed analysis.
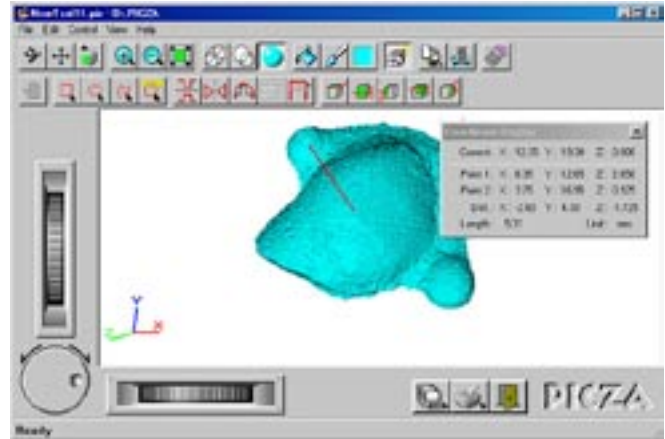

Fig. 2. Data visualization and co-ordinate display from "Dr Picza" software.

For data analysis, a purpose-written software package was developed using MATLAB (version 6, The Mathworks Inc, Natick MA, U.S.A.). The package accepts data from "Dr PICZA" in the form of (X, Y, Z) triples, where the X values are the west-east coordinates and the Y values the north-south coordinates. To make optimum use of MATLAB and its graphic facilities, we converted the data set to a regular mesh grid and saved the Z-values to a matrix (Z). The menu-driven software package then provides a series of options for defining the reference plane, graphing the data in 3D and deriving data describing the volume of the scanned object and the surface area and the height of the highest point on the object from the reference plane in cases where this is of interest.

In our example, we needed to find the volume bounded by two surfaces: the tooth surface and a planar surface defined by the three external reference points. The data transferred from Dr PICZA were plotted using the MATLAB routines and the maximum heights of

Table 1. Three-way analysis of variance comparing 10 repeated measures from two scans of a single specimen performed by three independent observers

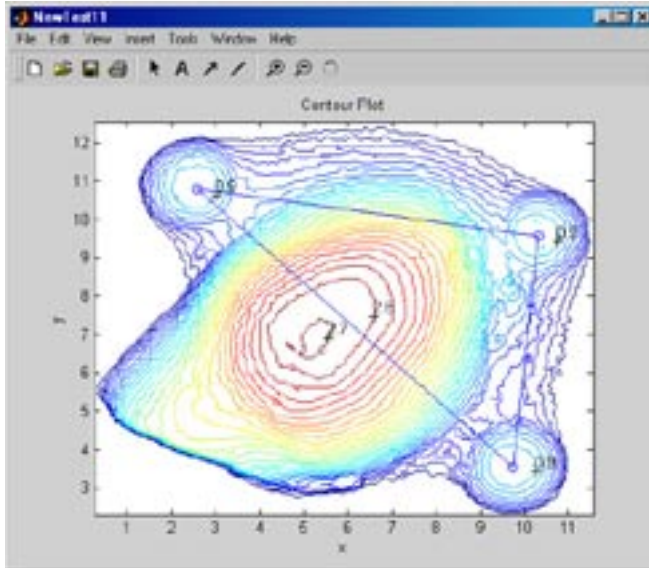| Source	Sum Squares	d.f.	Mean Square	F-Ratio	P-Value
Repeated measures	8.7303	9	0.97003	37.89	<0.001
Observers	0.0287	2	0.01437	0.56	0.5803
Scans	0.0003	1	0.00026	0.01	0.9214
Repeat-x-Observer	0.8173	18	0.04541	1.77	0.1169
Repeat-x-Scan	0.1028	9	0.01143	0.45	0.8914
Observer-x-Scan	0.1011	2	0.05054	1.97	0.1678
Error	0.4608	18	0.02560		
Total	10.2414	59			

Fig. 3. Data plot and reference plane identification using purpose-written software.

the three reference points were identified (Fig. 3). The volume of the part of the specimen above the reference plane was calculated.

Because 3D objects can on occasions include undercut areas, the package has an option to allow the reference plane to the re-aligned so that the calculations do not include parts of the specimen for which there are no data.

In our example this was undertaken by selecting the appropriate option from the menu, inspecting the graphical display and deciding on an appropriate realignment to avoid the undercut and recalculating the data (Fig. 4).

To establish the validity of the data obtained, objects of know dimensions were scanned and the calculated volumes compared with the volumes derived from the scanner data.

To establish the reproducibility of the method, intra-observer variation was assessed by repeated analysis by one observer (PZ) and inter-observer variation was determined by comparing data derived by different

Does the object have undercut areas (Y/N)? y
Please give lift value of the referenceplane, which you can
estimate from the contour diagram you have drawn.
Lift value = 1.012

Max distance of the remnant to reference plane is: 0.7130 mm
The convex surface area of the remnant is: 2.4011 mm^2
The volume of the remnant is: 7.6169 mm^3

If you want to see the 3-D picture of the original object and reference plane together,
please press 'Y', otherwise just press <Enter>.
Selection (Y/N): y
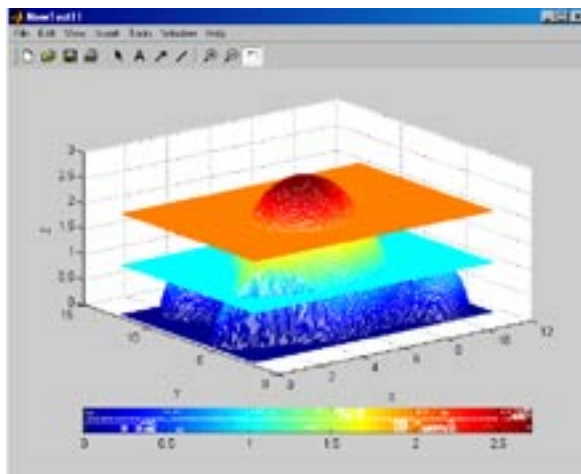
Congratulations! Your measurement of NewTest11 is done.

Matrix: Distance*, Surface*, Volume

B =   0.7130   2.4011   7.6169

Fig. 4. Example of menu-driven adjustment to reference plane height to avoid undercut areas on specimen.
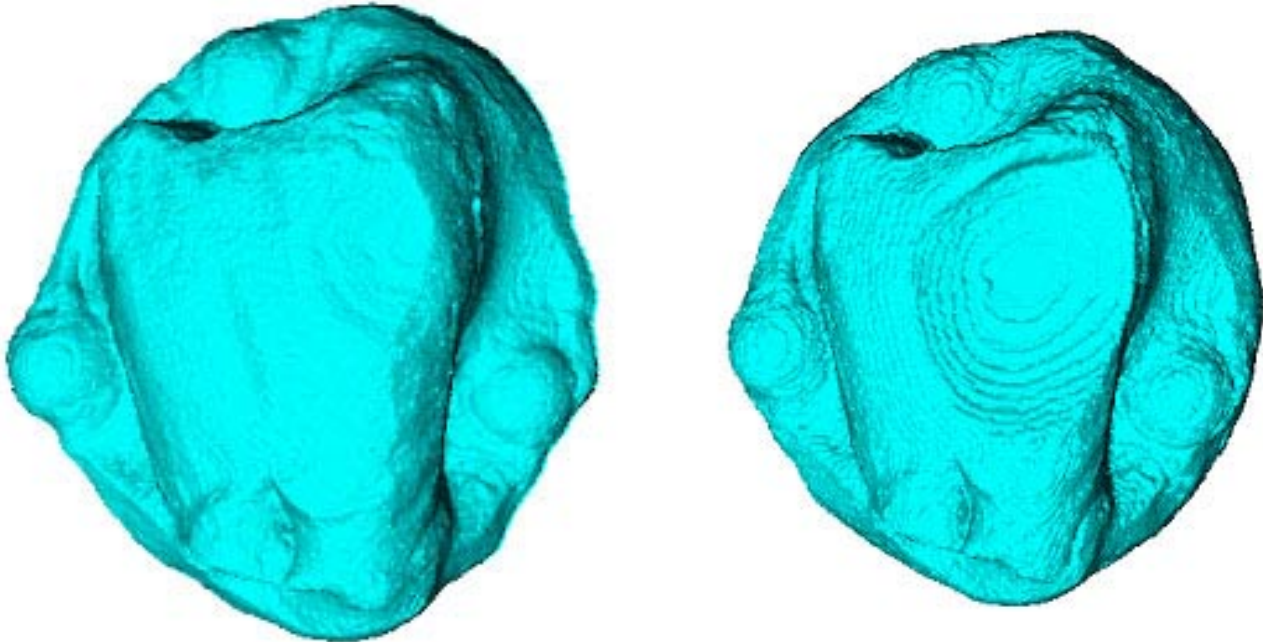
Fig. 5. (*left*) Data plot for specimen after 7,000 cycles of wear. (*right*) Data plot for specimen after 112,000 cycles of wear.

observers.

RESULTS

Scanning a relatively simple object (for example, a hemisphere of diameter 18.0 mm) gave a volume of 1583.27 mm^3 compared with a calculated volume of 1526.81 mm^3. The difference (56.45 mm^3) represents 3.7% of the true volume. A smaller, more complex object (the small cylindrical projection on a Lego® building block which has parallel sides and hence can have small undercut areas if the block is not mounted exactly horizontally) had a theoretical volume of 32.66 mm^3 and a volume derived from the scanning data of 35.45 mm^3 representing a difference of 8.5%.

The intra-observer variation in calculated volume was small with the coefficient of variation (100 x standard deviation/mean) being 0.90% for the most experienced observer and 0.91% for the least experienced observer.

To determine whether inter-observer variation or differences between repeated scans of the same object contributed significantly to the observed variation in repeated measures, one specimen was scanned on two occasions and each of these scans was analysed 10 times by three independent observers. A three-way analysis of variance (Table 1) revealed no significant variation between observers (p=0.58) or between repeated scans (P = 0.92) and no significant interaction between any of the considered factors suggesting that the performance of experienced and inexperienced observers was similar.

In our study of tooth wear the buccal surface of an extracted human tooth was subjected to 7000 cycles at the rate of 80 cycles per minute under a load of 3.2 kg with water at pH 7 used as a lubricant in an electro-mechanical tooth wear machine (Kaidonis *et al.*, 1998) to produce a wear facet (Fig. 5a). The specimen was scanned and the volume of the dental crown above the plane defined by the three 2mm diameter ball markers that were used as "external" reference points was calculated. This was compared with volume of the specimen after it had been subjected to a further 105,000 cycles of wear (Fig. 5b). The volume of enamel lost due to wear during this experiment (21.85 mm^3) was calculated by comparing the first volume (149.74 mm^3) with the final volume (127.89 mm^3).

DISCUSSION

Based on our assessment of the validity and reproducibility of the measurements derived using this system, we believe that it provides an affordable and reliable method for the acquisition of 3D data for the comparison of dental morphology. Like most systems it is limited in its ability to deal with undercut areas that makes it important to carefully select the initial orientation of the specimen and define an appropriate reference plane to avoid undercuts. Also, the acquisition of data from larger specimens at the highest resolution can be time consuming with high resolution scanning of a whole dental arch taking up to 30 or more hours.

The costs involved in setting up the system are relatively small compared with some other systems. If a suitable personal computer and a licensed copy of the MATLAB package are available then the total hardware

set up cost should be less than $US 1,200 compared with more than $US 100,000 for some commercial laser-based systems. The purpose-written MATLAB-based software package is available on request from the authors.

The validity of the data derived using the system was established by comparing volumes derived by scanning with the calculated volumes of objects of known dimensions. This indicated that calculated and scan-derived volumes differed by between 3.7 percent and 8.5 percent depending on the size and geometry of the specimen. Interpreting this information was complex for a number of reasons. In the case of the sphere (a computer-mouse ball), the difference between the scanned and calculated volumes was relatively small and challenged our ability to accurately measure the ball. A difference in radius of the ball of the order of 0.1 mm would result in a difference in volume of more than 3.4% and made it difficult to determine which of the calculated and scan-derived data was the more valid. In the case of the Lego® building block the differences were larger because the object presented some obvious and some hidden challenges and represented a "worst case" in terms of the ability of the system to derive valid data. The obvious challenge was the geometry of the object that, with its parallel sides, required precise orientation to avoid undercuts. In addition, the curve-fitting procedures that were used to define the surface were not ideally suited to objects of this type. The hidden challenge was the surface morphology of the object. The face of the projection on the block included the manufacturer's trademark etched into the surface. This was not obvious on observation and therefore not included in the calculations but was obvious on the enlarged scan and would contribute to the difference between the calculated and scan-derived volumes.

The reproducibility of the data was assessed by repeated measures of a test specimen by different observers. The intra- and inter-observer errors were all small with coefficients of variation for repeated measures being less than 1.0% for all scans and observers, and with no significant differences between observers or repeated scans.

Based on our experience, we believe that the system described is an affordable, valid and reliable method for obtaining 3D data for the description and comparison of dental morphology.

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Brief Report:

Maxillary Second Premolars with Paramolar Tubercles

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This communication describes the unusual morphology of maxillary second premolars (Fig. 1) that were encountered in an otherwise normal young adult. These teeth are distinctive because of the large accessory cusp that occurs bilaterally on the buccal surface of each maxillary second premolar. We present this case in hopes of stimulating discussion about similar teeth that other researchers have encountered and to solicit suggestions of likely causes of this variant.

The case is an 18-4 year old African American male who presented for routine orthodontic treatment. By our inspection (Figs. 1, 2) this young male is phenotypically normal aside from the uncommon premolars. All 32 permanent teeth are present, including the third molars where the maxillary teeth have initiated root formation and the mandibular teeth have their crowns mostly formed (Fig. 3). The orthodontic issues were (1) a palatally impacted right canine with just 2.5 mm of space between the lateral incisor and first premolar in this quadrant, (2) an anterior openbite with the right central and left lateral incisors in crossbite, and (3) tongue-thrust on swallowing. There is a Class I molar relationship (Angle) bilaterally. The young man was unaware of his unusual premolars. He did not have a regular dentist, though there are occlusal amalgams on his left and right mandibular first molars (all other teeth are noncarious).

These accessory cusps arise from the buccal cingulum. Apart from lingual cingula on the lingual aspects of incisors and canines, basal developments are uncommon in humans. The obvious exception is Carabelli's trait that occurs on the protocone of maxillary molars. Carabelli's complex has been amply described (*e.g.*, Kraus, 1959; Korenhof, 1960; Turner and Hawkey, 1998), and it is one of the few morphological variants commonly discussed in clinical dental texts (Zeisz and Nuckolls, 1949; Ash, 1993). Other cingular traits include (1) the paramolar tubercle of Bolk (Dahlberg, 1945) and (2) and talon cusps that arise from the lingual cingulum of incisors (*e.g.*, Harris and Owsley, 1991; Lorena *et al.*, 2003; Segura-Egea *et al.*, 2003; Dash *et al.* 2004). Dahlberg

(1950) suggests that paramolar cusp is a term applied to "any stylar or anomalous cusps, supernumerary inclusion or eminence occurring on the buccal surfaces of both upper and lower premolars and molars. Dahlberg used the term protostylid to distinguish just those cusp-like features occurring on the protoconid of lower molars near the buccal groove.

Various authors have commented on the association between Carabelli's cusp and size of the crown of the rest of the tooth (Garn, 1977; Hsu *et al.*, 1997). Scott has reported positive statistical associations between various cingular elements, notably (1) among the expressions of lingual tubercles on the maxillary incisors and canines

Fig. 1. Occlusal intraoral view of the young adult African-American described here. By our inspection, the accessory cusps on the second premolars are the only dental features outside normal limits.

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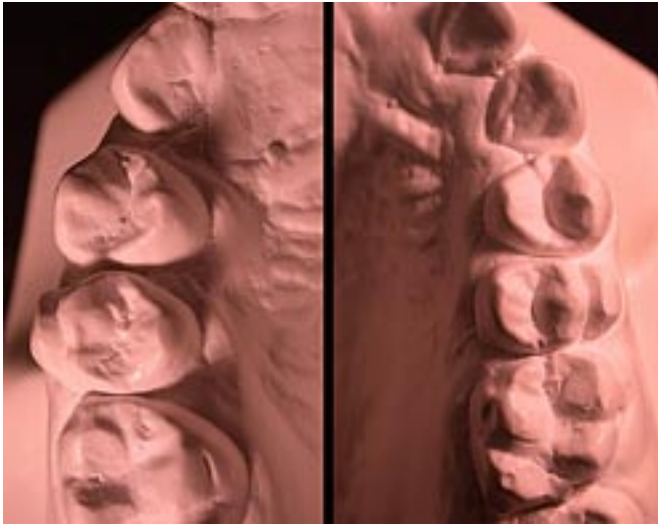
Fig. 2. Close-up occlusal views of the second premolars. Arch lengths are different in the two quadrants because of the unerupted (impacted) canine on the man's right.

Fig. 3. Panoramic radiograph. The palatally impacted right canine is noteworthy, but other features appear to be within normal limits.

TABLE 1. Crown dimensions[1]

Tooth	Mesiodistal		Buccolingual	
	Right	Left	Right	Left
Maxilla				
I1	9.4	9.4	6.7	6.6
I2	7.7	7.4	6.4	6.4
C	U	7.9	U	8.4
P1	8.5	8.2	10.7	10.7
P2	7.6	7.8	11.3	11.0
M1	11.1	11.1	12.4	12.5
M2	11.4	11.5	12.1	11.4
Mandible				
I1	6.0	6.0	4.5	4.8
I2	6.8	6.8	5.8	5.8
C	7.4	7.9	7.5	7.5
P1	8.4	8.1	8.3	8.6
P2	8.3	7.8	9.1	9.6
M1	13.5	13.0	12.2	12.3
M2	IE	12.4	11.6	11.8

[1]U = unerupted; IE = incompletely erupted.

(Scott, 1977), (2) between Carabelli's complex and size of the hypocone (Scott, 1979; also see review in Keene, 1968), and (3) between Carabelli's complex — on the lingual aspect of maxillary molars — and the protostylid — on the buccal aspect of mandibular molars (Scott, 1978).

Noteworthy features of the accessory cusps (Fig. 2) are their size and bilateral symmetry. There is no trace of this feature on the first premolar. Crown diameters (Table 1) were compared to a sample of American blacks (Richardson and Malhotra, 1975) but these standards are only available for mesiodistal diameters (Fig. 4). Comparisons also were made to the American white standards reported by Harris and Burris (2003). Our case possesses small mandibular incisors and large mandibular molars, but the UP2 with the accessory cusps is unremarkable in these comparisons. This suggests, along with the raw data, that the accessory
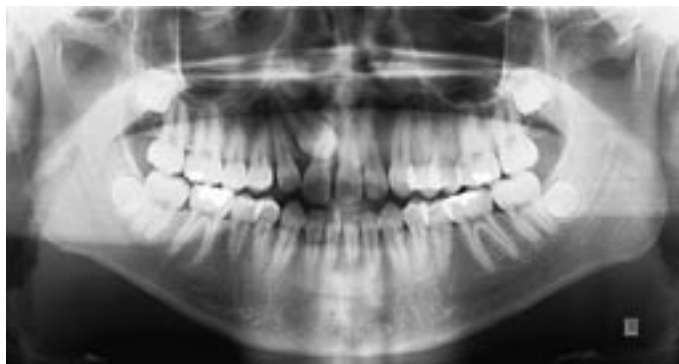
cusp constitutes part of the normal tooth's buccolingual width; the cusp is not simply added on to it. This agrees with the observation (Fig. 2) that the occlusal tables of the P2s are somewhat compressed buccolingually. Normally, the P2 dimension is at least as large as the
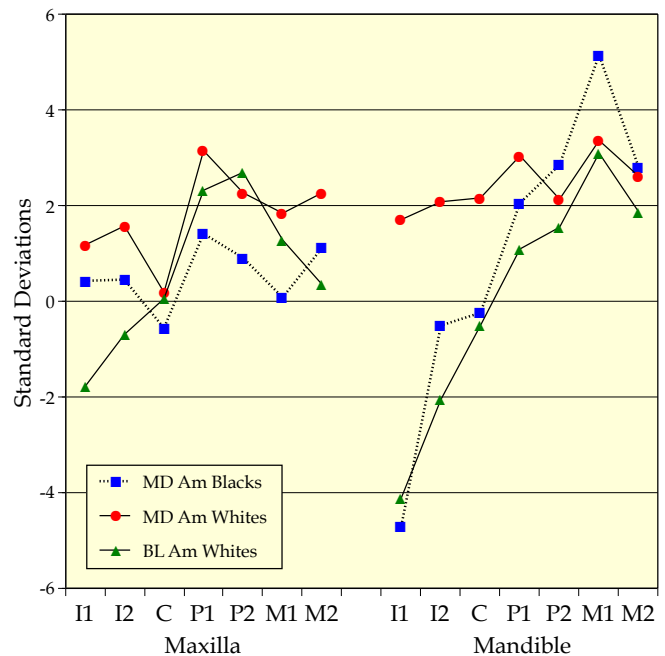

Fig. 4. Plots of z-scores for this case compared to mesiodistal diameters of American blacks (Richardson and Malhotra, 1975) and mesiodistal and buccolingual dimensions of American whites (Harris and Burris, 2003).

P1, but, on the left, the paracone-protocone distance is 6.0 mm on P1 and 5.9 on P2. On the right where the accessory cusp is slightly larger, the intercusp distance is 6.8 mm on P1 but just 5.8 on P2—again indicating that part of the normal tooth mass has been recruited for production of the extra cusp.

Periapical radiographs of these premolars (Fig. 5) are unremarkable. Both of these second premolars possess just one root, as is the norm (Ash, 1993), and there appears to be just a single, normal pulp chamber.

Crown morphology of the second premolars is not unusual. There are two main cusps separated by a central developmental groove. There are moderate-size mesial and distal ridges. The right UP2, that is slightly larger than its antimere, has a distal protocone ridge that is absent on the left tooth. The accessory cusp has a lunate (curvilinear) cross-section; it is so large mesiodistally that it essentially "wraps onto" the curvature of the buccal margin of the paracone (buccal cusp). The buccal aspect of the cusp itself is smooth and featureless, descending straight to the cementoenamel junction.

The accessory cusp has a single elevation (cusp) with the apex located exactly buccal of the paracone's apex. Distance between these cusp tips is 1.25 mm on both the right and left tooth.

Notably, these accessory cusps would never enter occlusion. That is, one might suppose that the cusp might be adaptive by contributing to the crown's overall occlusal area. As is normal, though, the paracone (buccal cusp) of the maxillary premolar overhangs the buccal cusp of the lower premolar and the maxillary premolar's lingual cusp (protocone) occludes into the lower premolar's central fossa. Consequently, regardless of how worn these premolars might become, the accessory cusp will always be buccal of the mandibular tooth—with nothing to occlude against. It seems doubtful, then, that this morphological variant has any adaptive significance, at least functionally.

Heights of the accessory cusps are about ½ mm short of the apices of the paracones, and these cusps have free apices that jut about 2 mm occlusal of where the accessory cusp melds into the paracone. This anatomy is relevant because it means that the cusp developed from its own enamel knot rather than proliferating at a later time from the paracone. Enamel knots are sites of nondividing cells that form during the bell stage of tooth formation. They occur in the stellate reticulum as projections from the inner enamel epithelium (Bhaskar, 1980). Enamel knots have been recognized for over a century (reviewed in Butler, 1956), though their function was unknown. Recent work by molecular biologists (Jernvall *et al.*, 1994; Thesleff *et al.*, 2001) has shown that knots produce
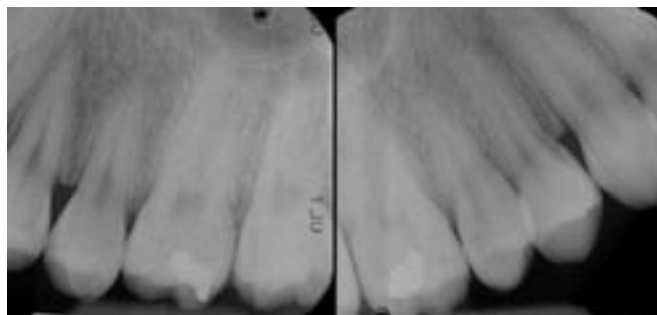

Fig. 5. Periapical radiographs of the maxillary second premolars.

substances that promote mitotic growth in the adjacent IEE. Since the knots themselves are nondividing, this creates irregularities in the IEE that become cusps (Jernvall *et al.*, 1994, 1998; Jernvall, 2000). It seems that the primary enamel knot, which is the most obvious on light microscopy and the earliest to form, determines the site of the tooth's occlusal table (or its counterpart in the simpler anterior teeth), while later-forming 'secondary' enamel knots coincide with formation of the individual cusps (Thesleff and Jernvall, 1997; Thesleff *et al.*, 2001; Thesleff, 2003). Separate enamel knots seem to coincide with separate centers of enamel formation since amelogenesis invariably progresses gingivally (Hillson and Bond, 1997). In the present case, it seems that an "accessory" enamel knot developed buccal to the presumptive paracone on the left and right second premolars, but not on the earlier-forming first premolars mesial to them.

We have contacted a few experts in the field concerning these teeth. Some have not encountered such a variant. Others stated that they have seen similar cases, but did not bother to record them. Certainly, the frequency of this variant is rare. Readers who have seen similar cases—or have comments on this report—are encouraged to contact the authors.

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Experimental Combat-Stress Model in Rats: Histological Examination of Effects on Amelogenesis— A Possible Measure of Diminished Vagal Tone Episodes

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ABSTRACT: Developmental defects of enamel-stress histomarker rings (accentuated striae) may be a potential measure of diminished vagal tone in research on extreme stress such as exposure to combat. To develop an animal model of this measure, we examined the enamel of rat incisors which erupt continuously. We examined incisors from 15 stressed-colony rats and 7 control-group rats for these histomarkers using the Visible Burrow System (VBS). VBS was developed to study combat stress in rats. No stress rings were found in any of the rat incisors examined. In contrast to humans, rats have likely evolved to prioritize incisor strength during combat stress. Studies of amelogenesis during combat stress in other rodents with continuouly growing incisors are warranted. Laboratory animals such as rabbits or marmosets may be especially suitable, since they less frequently use their incisors for self-defense. *Dental Anthropology* 2004;17(3):79-82.

There has been a rapidly growing interest in developing animal models resembling human situations of extreme life threat (e.g. military combat). For example, heart-rate variability (HRV) is now extensively studied in animals since HRV has been one of the more consistent physiological markers for research on combat-related posttraumatic stress disorder (CR-PTSD) and post-deployment syndromes of unclear etiology (Gorman and Sloan, 2000; Malaspina *et al*., 1997; Shalev, 2002). Porges has recently called attention to the vagal motor neurons originating in the nucleus ambiguus and their link to HRV (Porges, 1995). To our knowledge, dental anthropological techniques have not been previously used in research on combat-stress biology (Bracha *et al*., 2003).

In the anthropological literature, there has been little attention given to the fact that in addition to their role in HRV, the vagal motor neurons originating in the nucleus ambiguus also control the moment to moment fluctuations in the parasympathetic regulation of blood flow to the enamel secreting ameloblasts (as well as to the adjacent salivary glands). In humans, several tissues (*e.g.*, intestinal mucousae, other mucousae, skin, bone, teeth, hair, and nails) are of lower survival priority during life-threatening experiences such as war-zone exposure. These tissues grow predominantly during spans of high vagal tone such as rest and sleep (Appenzeller *et al*., 2002; Appenzeller, 1990; Bracha *et al*., 2003; Bracha *et al*., 2004; Bracha, 2004). Studying these anatomical structures of lowest survival priority may

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be a novel research approach to examine the negative effects of combat-related stress.

While little research has been done on the topic, amelogenesis of the still erupting teeth is one parasympathetic trophic "luxury" function likely to be among the lowest priorities during extreme stress and thus provides a sensitive stress indicator in humans (Yui *et al*., 2002; Bracha *et al*., 2002). Unlike nails, and most of the human bones, skin and mucousae, the parasympathetic innervation of the ameloblast layer and the nearby salivary gland and larynx originates not in the dorsal motor nucleus of the vagus, but in the more rostral and more limbic-connected nucleus ambiguus of the vagus. This neural circuit is known to be involved in the human fear response (Porges, 2001; Porges, 1995; Bracha *et al*., 2003).

The amelogenesis defects seen in human tooth enamel have been reproduced using laboratory-induced stress in large herbivores such as sheep, pigs, and deer (Guatelli-Steinberg, 2001; Guatelli-Steinberg and Lukacs, 1999; Suckling *et al*., 1986; Dean *et al*., 2001; Dirks *et al*., 2002). For our line of clinical research, which focuses on the effect of combat stress on mineralized tissues such as bones and teeth, rodent incisors are an especially attractive tissue in which to examine histological biomarkers of extreme stress. The constant gnawing motion of the rat's jaw rapidly wears the incisors. Therefore, new enamel is formed in the ameloblast layer to replace the worn incisor enamel throughout the lifetime of the rat. While enamel research in dental anthropology has focused on nutritional or chronic stress, this is to our knowledge, the first controlled study attempting to use dental anthropological techniques to understand the effects of combat stress.

To study the effects of combat-like stress on mineralized dental tissue, we used the Visible Burrow System (VBS) developed by Blanchard et al. (Blanchard *et al*., 1995). The VBS is an important novel system to study combat stress among rats (Monder *et al*., 1994). Using the VBS, acute episodes of combat stress can be experimentally induced at known intervals. Previous studies have shown that behavior highly reminiscent of human combat ensues among male rats in the VBS. For a review of the VBS, see Blanchard et al. (Blanchard et al., 1995) and Monder et al. (Monder et al., 1994).

METHODS

Using the VBS, we controlled the timing of experimental combat stress in male rats and subsequently studied its effects on mineralized dental tissue formation. We examined 22 male rats that were subjected alternately to stressed and unstressed periods over several months. During the three-week baseline (no-stress) period the male rats were kept in individual cages. During the second three-week (low

stress) period, rats from the control group (n=7) were each placed in cages with a single female rat. During the same three-week (combat-stress) period, the test rats (n = 15) were placed in colonies of three male rats to one female rat. During this combat-stress period, a behavior highly reminiscent of human combat ensued among the male rats (Blanchard *et al*., 1995). After this period, the rats were returned to their individual cages for another three-week (no-stress) period. This cycle was repeated three times for all of the rats in the study.

After the three combat-stress cycles, the two upper and two lower incisors from each rat were removed. The incisors from a total of 15 stressed-colony rats and 7 control-group rats were examined for "Developmental Defects of Enamel-Stress Histomarker Rings" (DDE-SH Rings; also known in dental anthropology as "accentuated striae"). The rat teeth were examined at 10X, 100X, and 400X by a trained dental anthropologist (JLLJ) who was blind to group assignment.

RESULTS

At least 3 teeth were available from each of the 22 rats. Because of the curvature of the rat incisors in the sagittal plane, one of the two lower incisors from each of four rats were not suitable for sectioning leaving us with 84 incisors out of a possible 88.

Unlike human teeth, the rat teeth showed markedly more decussation of the enamel rods giving them a twisted rope-like appearance. No DDE-SH Rings (accentuated striae) were found in any of the 84 incisors examined regardless of group assignment.

DISCUSSION

These negative results replicate and extend earlier research demonstrating the unusually high stress-resilience of rat amelogenesis. Fejerskov, using earlier stress-inducing methods reported similar negative results (Fejerskov, 1979). We propose that the explanation for this inter-species difference in the response to acute combat stress involves inter-species evolutionary differences in stress-response adaptation. It is likely that the rat genome has evolved to place high priority on incisor strength during life threatening experiences. Unlike humans and herbivores (such as sheep and deer), incisor strength is unlikely to be a luxury function for rats involved in combat. From an evolutionary point of view, short-term survival of the rat is more dependent on the stress-resilience of their incisors. Therefore, rat incisors may have evolved to achieve a greater degree of stress-resilience compared with sheep, deer, or human incisors.

Our finding that rat incisors show dramatically more enamel rod decussation is consistent with the above speculation. Enamel rod decussation is a histological feature known to increase the strength of

enamel (Fejerskov, 1979).

Preliminary data using enamel stress rings to chronicle episodes of diminished vagal tone in human teeth are promising (Bracha et al. unpublished). Therefore, it may be premature to abandon all laboratory animals as experimental models of acute episodes of extreme stress. For example, small herbivores, which in the wild only infrequently use their incisors for combat, may be a better choice than rats. Marmosets and rabbits, like rats, have constantly growing incisors and are as easy to study. However, marmosets and rabbits may resemble humans in stress prioritization with regard to the ameloblast tissue layer. Therefore their incisor enamel may be a promising model for research on combat stress.

Additionally, the newly developed animal research designs which induce extremely stressful but non-lethal exposure to larger predators (Cohen et al., 2003) may be especially useful for this line of research on the effects of acute combat stress on calcified tissue. The latest National Institute of Mental Health (NIMH) recommendations for future research directions on fear-circuitry disorders emphasize the "… need [for] research designed to develop better measures of the environment..." and the need to have "stress conceptualized broadly" (Davidson *et al.*, 2002). Similar conclusions were drawn by Charney (Charney, 2004). Developing an experimental rodent model of dental biomarkers of acute stress is also consistent with the conclusions of the NIMH workshop on developing newer animal models of anxiety disorders (Shekhar *et al.*, 2001). The line of research described here is well suited to address the above recommendations. A new technique for estimating vagal tone chronology may be a useful complement to the important research on HRV in laboratory animals and humans (Porges, 1995; Cohen *et al.*, 2003).

In summary, laboratory animals that infrequently use their constantly growing incisors for combat may be a better choice than rats for this line of combat stress research. Research designs that provide extreme but non-lethal exposure to larger predators are especially recommended for this line of research.

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Calculation of Smith's Mean Measure of Divergence for Intergroup Comparisons Using Nonmetric Data

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ABSTRACT: The Mean Measure of Divergence (MMD) is a formula that converts a battery of trait frequencies into a numerical value such that the more dissimilar two samples are, the greater the value. This measure of phenetic distance was developed by the statistician Cedric A. B. Smith and has become popular among dental anthropologists and osteologists for estimating the dissimilarity among groups in order to help reconstruct populations' movements and structure over time and space. The purpose of the present study is to present the correct formulae and procedures for the MMD given that (1) numerous errors have entered into the literature concerning the formulae themselves, (2) improvements have been described that should be incorporated, and (3) various misunderstandings and misinterpretations have developed that need clarification. *Dental Anthropology* 2004;17(3):83-93.

Dental anthropologists commonly use morphological data to estimate the degree of dissimilarity among samples—so-called biological distance or phenetic distance. It is supposed that the greater the dissimilarity between two samples, the less the genetic contact between the groups due to separation by time and/or space.

An issue of some interest is how, statistically, to quantify the degree of dissimilarity among groups in an objective manner. Despite the numerous methods suggested in the literature (reviewed in Constandse-Westermann, 1972), dental anthropologists have focused almost exclusively on the use of Cedric A. B. Smith's mean measure of divergence (MMD). Our experience is, however, that there are misunderstandings about the MMD. There seems to be no commercially available computer program to calculate MMD, which would promote consistency, and the purported formula for MMD (if reported at all) differs among authors (including the repeated publication of statistical errors). The purpose of this note is to clarify the calculation of MMD in a simple, intentionally nontechnical manner.

Overview

Constandse-Westermann (1972) and, in particular, Sjøvold (1973, 1977) provide detailed descriptions of the development and use of the MMD. In brief, the British statistician Cedric A. B. Smith devised this statistic for M. S. Grewal (1962) who used it to estimate the biological divergences that had developed across generations in sublines of the common C57BL strain of laboratory mice. Grewal calculated trait frequencies for 27 cranioskeletal bony variants such as the occurrence of foramina, accessory sutures, and bony processes (traits primarily described by Grüneberg, 1950 and by Deol, 1955). It was supposed that the sublines diverged with time due to latent heterozygosity in the inbred line but, primarily, from the accumulation of mutations distinct to each subline—which is why the term *divergence* is used for this phenetic measure rather than *distance*. The MMD subsequently was popularized in anthropological circles by A. C. Berry and R. J. Berry, notably in their nonmetic skeletal comparisons among human groups (*e.g.*, Berry and Berry, 1967; Berry, 1968; Berry, 1974, 1976; and elsewhere). This pair of authors promoted the use of "minor skeletal variants" as epigenetic features that, from their analyses, had a genetic basis but were essentially independent of age and sex and size of the individual.

These minor skeletodental variants, such as the presence of nutrient foramina and accessory molar cusps, can each be viewed as a dichotomous feature, so summary of a sample is easily expressed as a trait frequency—and Smith's MMD provides a method of estimating the phenetic distances among samples arrayed through space and/or time.

Smith's original formula as described by Grewal (1962) is

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$$\text{MMD} = \frac{\sum\limits_{k=1}^{r} \left(\theta_{ik} - \theta_{jk}\right)^2}{r} - \left(\frac{1}{n_i} + \frac{1}{n_j}\right)$$

[Eq. 1]

That is, the difference between samples *i* and *j* for the frequencies of trait *k* is calculated and then this difference is squared so that positive and negative differences do not cancel one another. The sum of the differences is divided by r, the number of traits used in the equation, in order to generate the "mean" or average difference between samples *i* and *j*. The correction term $1/n_i + 1/n_j$ then is subtracted from this average to correct for sampling fluctuations. Grewal (1962:229-230) actually described the MMD in the text of his paper rather than presenting Eq. 1, which led to misinterpretations by other researchers.

It follows from this equation that the "size" of a MMD depends on the battery of traits used, and MMDs generated from different sets of variables are not comparable, even for the same pair of groups. These conditions hold for all measures of "biological distance" (Sokal and Sneath, 1963; Constandse-Westermann, 1972; Reyment, 1991). While it is not our purpose to critique the merits of the MMD, one noteworthy issue is that it does not account for intertrait correlations, which commonly is viewed as a shortcoming. Intertrait associations ("correlated traits") inflate the MMD because correlated traits share some of the same informational content, and this shared (redundant) information increases with the strength of the correlation. For example, the occurrence of incisor lingual shoveling (Hrdlička, 1920, 1922) is strongly intercorrelated on the maxillary central and lateral incisors (and between homologous teeth in the two quadrants), so including trait frequencies of shoveling on both UI1 and UI2 carries a lot of statistically redundant information. Studies have disclosed that nonmetric intertrait associations are more common than expected by chance (*e.g.*, Corruccini, 1974; Scott, 1977, 1978, 1979). On the other hand, Constandse-Westermann (1972) points out that, within an analysis, the same suite of traits is used for all of the pairwise comparisons so that, insofar as intertrait associations are a species-wide phenomenon, the effect of statistical redundancies can be viewed as a constant across the study.

Statistically significant intertrait correlations may also occur by chance. At the conventional alpha level of 0.05, one expects to make a Type I error (*i.e.*, reject a true null hypothesis) 5% of the time. Suppose that a battery of 30 morphological traits is scored (Table 1). One would expect that 21 of the matrix of 435 pairwise correlations would be statistically significant due to chance alone. An associated issue is that the ability to detect statistically significant differences depends on the available sample size (degrees of freedom)

available (*e.g.*, Fisher and Van Belle, 1993). Biologically real but weak correlations generally cannot be detected with small sample sizes. Statistical textbooks deal with the subject in much more detail, but guidelines for detecting biologically real intertrait correlations are (1) comparable correlations should appear in the analyses of multiple samples and (2) correlations found in larger samples, where effects of sampling fluctuations are dampened, generally are more reliable. Weak associations, particularly with the sample sizes normally encountered in anthropological studies, will not seriously distort MMD results.

Frequency transformations

The MMD was devised to deal with percentages of dichotomous data (also termed nonmetric or, occasionally, discontinuous traits). This is in contrast to quantitative (interval and ratio scale) data where more common statistical methods can be employed, such as Pearson's (1926) virtually-defunct coefficient of racial likeness, Penrose's formulae (1953) for distance, size and shape (where distance = size + shape), and the current gold-standard, Mahalanobis' D^2 (Mahalanobis, 1936).

Qualitative data, like the frequency of the Dryopithecus Y-5 pattern on a lower molar (Hellman, 1928), generally are converted to percentages, commonly termed trait frequencies. Such data either are scored as dichotomous traits or a "cut-point" is decided upon along an ordinal grading scheme to create dichotomous traits. Formally, the sample frequency of a trait can be expressed as *p* (and the frequency of absence as *q*) such that $p + q = 1$ and $p = 1 - q$. This simply relates to the binomial distribution. The sample variance of this distribution is pq/n (*e.g.*, Sokal and Rohlf, 1995: 419), where *p* and *q* are the frequencies of trait presence and trait absence, respectively, and n is the sample size. For a given sample size, the sample variance is tied to the frequencies of *p* and *q*. The degree of distortion (that is, the changing value of the variance through the frequency distribution from zero to one) increases as the sample size decreases (Fig. 1). This nonlinear association between the variance and the trait frequency

TABLE 1. Number of statistically significant pairwise associations expected in variously-sized batteries of traits

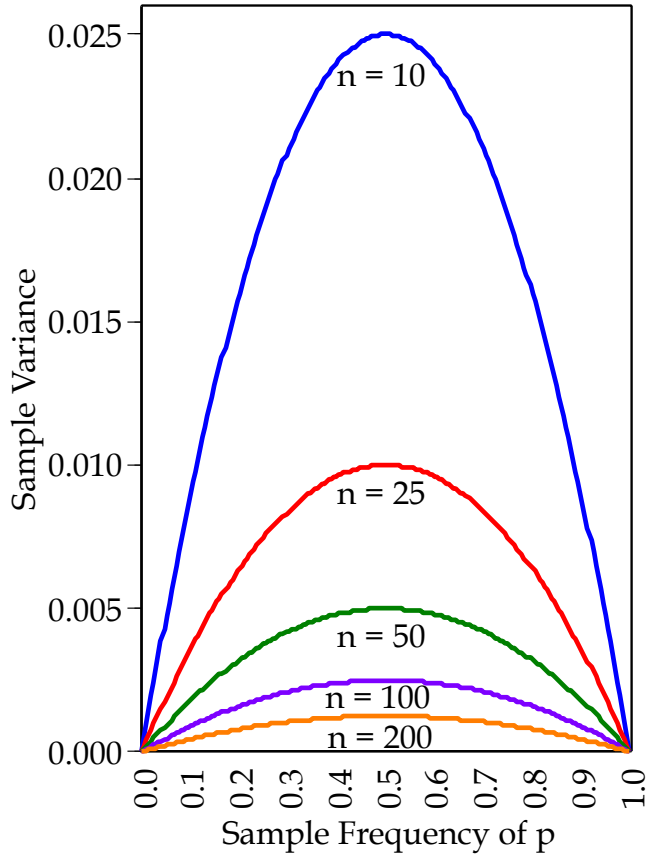| Number of Traits	Number of Correlations	Number Expected from Chance
5	10	1
10	45	2
20	190	9
30	435	21
40	780	39
50	1,225	61

Fig. 1. Examples of how the variance of the trait frequency p changes depending on sample size. In all cases, sample variance is at its maximum when $p = q = 0.5$, but the range of values diminishes as sample size increases.

242-244) illustrates, the results are mathematically identical. Radians are a trigonometric device that simplify many calculations. Several deterministic equalities between degrees and radians can be noted, such as

$$\pi \text{ radians} = 180°$$

$$2\pi \text{ radians} = 360°$$

$$1 \text{ radian} = \frac{180°}{\pi} \approx \frac{180°}{3.14159} \approx 57°17.75'$$

For present purposes, radians are desirable because the transformed frequencies of $\sin^{-1}\sqrt{p}$ have the simple-to-compute variance of *about* $1/4n$, where n is the sample size. The point needs to be emphasized that radians rather than degrees are to be used unless one also incorporates the appropriate variance correction into the MMD equation.

Grewal's (1962) transformation of p is $\sin^{-1}(1-2p)$, and its variance is 4 times as large as that for Fisher's transformation, namely $1/n$ (because 4 times $1/4n = 1/n$), when both are expressed in the same units, either degrees or radians.

Green and Suchey (1976) compared some published frequency transformations and concluded that the formula suggested by Freeman and Tukey (1950) did a decidedly better job of stabilizing the variance than Grewal's $\sin^{-1}(1-2p)$ transformation. The Freeman-Tukey transformation is

$$\theta = \frac{1}{2}\arcsin(1 - \frac{2m}{n+1}) + \frac{1}{2}\arcsin\left(1\text{-}2\left(\frac{m+1}{n+1}\right)\right)$$
[Eq. 2]

where m is the number of occurrences of the trait in the sample and n is the number of scorable specimens in the sample so the trait frequency is $p = m/n$. θ is computed for the kth trait in sample i and likewise for sample j, then these two values are entered into Eq. 1. This means that the raw counts (m and n) are needed to calculate the MMD, not the trait frequencies. Graphs of these three arcsine transformations of the trait frequency are shown in Figure 2.

In practice, there is very little improvement with the Freeman-Tukey transformation compared to another transformation proposed by Anscombe (1948), namely

$$\theta = \sin^{-1}\left(1\text{-}2\left(\frac{m+\frac{3}{8}}{n+\frac{3}{4}}\right)\right)$$
[Eq. 3]

Indeed, according to the graphical comparisons in Green and Suchey (1976:63), Anscombe's transformation is slightly better than the Freeman-Tukey formula at asymptotically stabilizing sampling variance. Both

is obvious in the range of most anthropological samples—either of living or skeletal specimens.

An appropriate transformation of the percentages diminishes this association between a trait frequency and its variance, making the variance more stable. Historically, the transformation can be as simple as $\sin^{-1}\sqrt{p}$ (Fisher, 1958), but other choices work much better. The arcsine (or "arcsin" or "inverse sine") transformation is a trigonometric function, generally coded as $\sin^{-1}$, and it can be expressed either in degrees or radians. (The arcsine function $\sin^{-1}(x)$ is in no way related to $1/\sin(x)$ as might be guessed.)

Transforming trait frequencies introduces an issue that has not been appreciated universally when calculating MMDs. If a researcher uses a familiar transformation—such as $\sin^{-1}\sqrt{p}$ (Fisher, 1958)—with the units in degrees, then the sampling variance of this value is $820.7/n$ (Constandse-Westermann, 1972:118; Sjøvold, 1973:208). Historically, this value was cumbersome when manually calculating the MMD. Instead, the convention has been to express the transformation in radians rather than percentages, but, as Smith (1972:
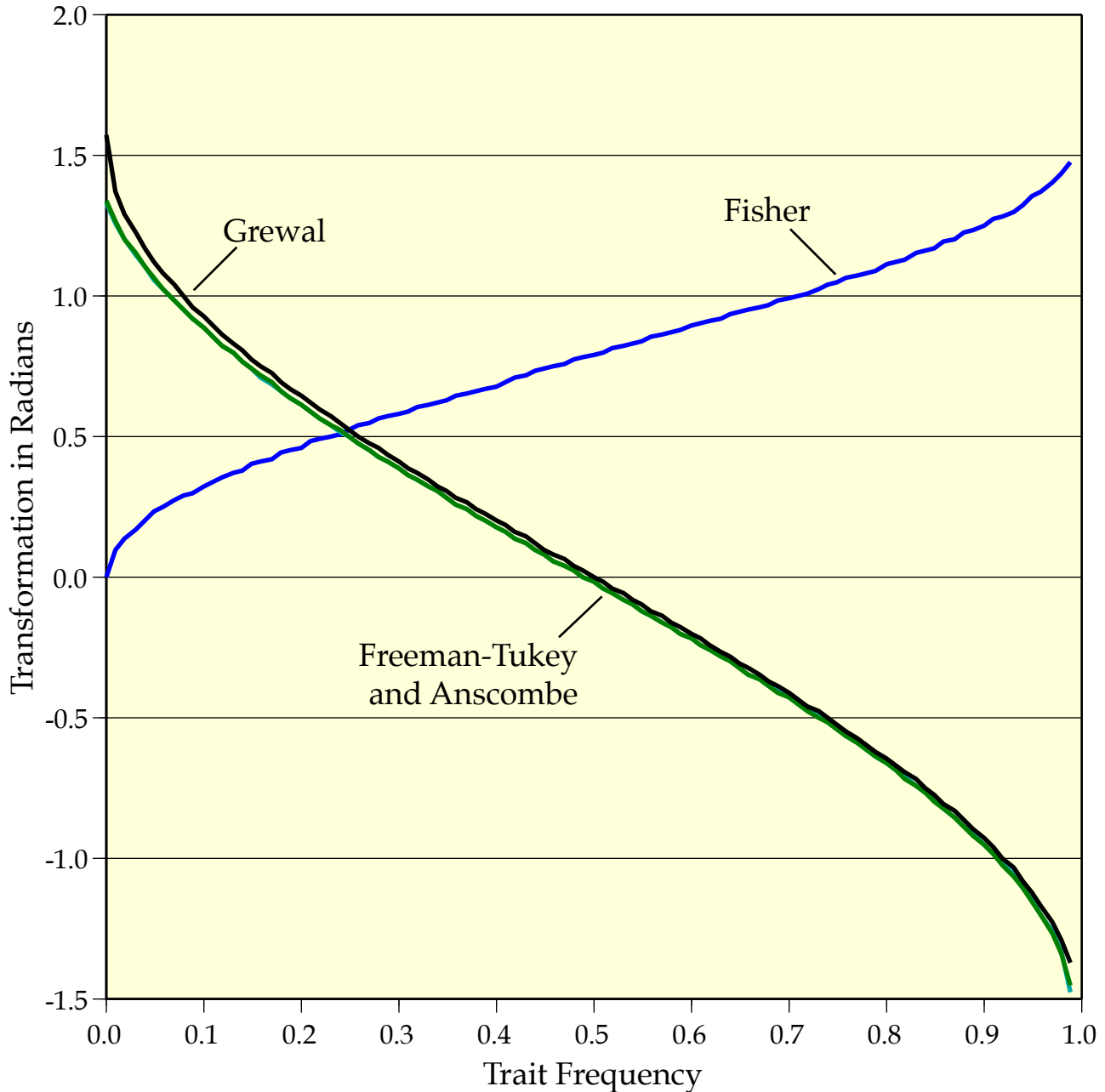
Fig. 2. Graphs of the arcsine transformations of trait frequency p discussed here, namely (1) Fisher's $\sin^{-1}\sqrt{p}$, (2) Grewal's $\sin^{-1}(1-2p)$, (3) the Freeman-Tukey transformation shown in Eq. 2, and (4) the Anscombe's transformation shown in Eq. 3. Sjøvold (1977) has shown that these latter two transformations are mathematically very similar — which is why they are superimposed here throughout their ranges.

are clear-cut improvements over Grewal's transformation in terms of stabilizing the variances of the binomial variable. We suggest that Anscombe transformation is preferrable for a couple of reasons. Historically, Rao (1952) recommended Anscombe's transformation when sample sizes are moderately large. This transformation also has the advantage that it can be rewritten as a single arcsine:

or, equivalently,

$$\sin^{-1}\left(\frac{n}{n+\frac{3}{4}}\right)(1\text{-}2p)$$

[Eq. 4]

$$\sin^{-1}\left(\frac{1}{1+\left(\frac{3}{4}n\right)}\right)(1\text{-}2p)$$

[Eq. 5]

The Freeman-Tukey transformation is quite complicated by comparison. Moreover, Anscombe's formula can be extended to multistate traits (in contrast to dichotomous traits)—though we do not discuss that option in this paper—and this is not true of the Freeman-Tukey formula.

Adjusting for variances

Smith's MMD originally was published without explicit directions (Grewal, 1962), then ambiguously by Berry and Berry (1967:370), and then incorrectly by Berry (1968:115). These shortcomings created a rocky start for the MMD, generating errors that occasionally reappear. Constandse-Westermann (1972:119) was the first to explicitly publicize this formula:

$$MMD = \frac{\sum_{k=1}^{r}\left(\theta_{ik}-\theta_{jk}\right)^2 - \left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)}{r}$$

[Eq. 6]

though knowing what the equation should be makes the description by Grewal (1962:229-230) clear. Notice that in Eq. 6 the correction term applies to each variable, not just the summary value as indicated in Eq. 1. The quantity $(1/n_{ik}+1/n_{jk})$ is subtracted from the squared difference of trait frequencies to adjust for the mathematical properties of the squared differences between the theta values (θ) that overestimate the divergence between the corresponding populations. That is, $1/n_{ik}+1/n_{jk}$ is the variance of the two angular values. These theoretical and observed distributions coincide more closely as n increases. n_{ik} and n_{jk} are the sample sizes for the kth trait so that, depending on how fragmentary the dental or skeletal data are, the usable (scorable) sample sizes will vary from trait to trait.

Notice too that the correction term in Eq. 6 has the subscript k that was absent in Eq. 1. Equation 1 assumes that the data are complete, so sample sizes are identical across the whole suite of traits. This commonly is not the case because of damaged skeletal elements or attrition, caries, or loss of teeth. If there are missing data, sample size needs to be subscripted so it can vary by trait.

Green and Suchey (1976) and Green *et al.* (1979) note that this conventional correction formula overestimates the true variance and that, instead, the correction term (attributable to Freeman and Tukey, 1950) should be

$$\frac{1}{n_{ik}+\dfrac{1}{2}}+\frac{1}{n_{jk}+\dfrac{1}{2}}$$

[Eq. 7]

Square-root transformation

If one reviews the various publications using the MMD, it will be seen that a square-root transformation crept into the formula with time. For example, A. C. Berry (1974:348) reports the formula to be:

$$MMD=\sqrt{\frac{\sum_{k=1}^{r}\left(\theta_{ik}-\theta_{jk}\right)^2 - \left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)}{r}}$$

[Eq. 8]

This square-root modification is due to R. J. Berry (1969), and we suggest a couple of reasons for this addition. The square-root modification may be supposed to be an improvement towards the goal of "triangular equality" among the MMDs. Given three groups, say A, B and C, the squared distance between two groups (say A and B) could be greater than the sum of the squared distances between the other two pairs, so AB > (BC + AC). This actually is not true. The actual effect of the square-root transformation is to change the reference space from Cartesian space to a sphere, which creates mathematical problems (Sjøvold, 1977). Even though the square root modification (Eq. 8) is commonly encountered, it stems from a misunderstanding, and we strongly suggest that it not be used.

Alternatively, the square-root modification may have been perceived as a "correction" for estimating a squared divergence, so taking the square root would estimate the unsquared (linear) divergence. Analogously, other researchers have used the square-root of Mahalanobis' D^2, supposing that D is a more relevant measure of intergroup distance than D^2. The modification is unwarranted, though, because the MMD (Eq. 6) is an unbiased estimated of the squared divergence between the populations from which the samples were drawn, but $\sqrt{MMD}$ is *not* an unbiased estimated of the unsquared divergence (Sjøvold, 1977:46).

Consider too that the MMD commonly is less than 1.0, so $\sqrt{MMD}$ will be *larger* than MMD. Artificially increasing MMD by using the square-root transformations makes the test of significance (discussed below) inappropriate because the $\sqrt{MMD}$ are inflated values, so it is (falsely) harder to achieve statistical significance if it is not understood that the MMD and not the $\sqrt{MMD}$ needs to be tested.

Sample size

A tangential issue is how to score fragmentary data, particularly dental traits that typically occur bilaterally (*e.g.*, Turner *et al.*, 1991). Incompletely preserved skeletodental data, where the left and right occurrences of a trait cannot always be determined, is a common problem in archeological samples, but the same issue arises with living specimens when the dentition is compromised by caries, attrition, dental restorations, extractions and other causes of tooth loss. Green *et al.* (1979) reviewed the options for scoring incomplete data, concluding that the least biased method is to consider both left and right sides and calculate the trait frequency as the number of times the feature occurs on either side divided by the number of scorable sides. This maximizes the amount of usable information without artificially inflating sample sizes by using sides instead of individuals as the unit of study. It does assume that there is no systematic side preference in trait frequencies, which seems to be the case in the main.

A related issue of sample size becomes obvious from inspection of Eq. 6. If the sample size for a trait is small in one or both samples being compared, then the adjustment factor can be as large or larger than the phenetic difference that is measured as $(\theta_{ik}-\theta_{jk})^2$. This leads to a MMD that is zero or negative, but not because of the similarity in trait frequencies but because of small sample sizes. That is, the adjustment—which is wholly a function of sample sizes—can readily overwhelm the biological measure of difference ($\theta_{ik}-\theta_{jk}$)², so MMD may well be "controlled" by inadequate sample sizes when dealing with samples in the range typically encountered in anthropological collections.

This artifactual effect of diminutive sample sizes can easily pervade an analysis for several reasons. One, the MMD almost invariably has been applied within a species, so the range of trait frequencies (and, thereby, differences between groups) is not great. Berry and Berry (*e.g.*, 1967) argued that discrete skeletodental traits exhibit considerable differences in frequencies among groups, but this has not been substantiated in the dental anthropological literature (*e.g.*, Lasker and Lee, 1957; Scott and Turner, 1997). Bigger between-group differences in trait frequencies obviously can "offset" the reductionist effect of small sample sizes. Two, sample sizes generally are comparable for the whole suite of traits in a sample; there is little chance of small sample sizes for some traits being offset by substantially larger samples of other traits. Three, when sample sizes are small vis-à-vis the phenetic difference $(\theta_{ik}-\theta_{jk})^2$, the adjustment produces a negative distance for that trait, but it seems that researchers have simply averaged this negative value into the MMD. In fact, a negative value for a trait has no biological meaning; it is wholly an artifact of the

Table 2. Representative sample sizes and associated correction term[1]

Sample size	Correction term
10	0.040
15	0.018
20	0.010
25	0.006
30	0.004
40	0.003
50	0.002
75	7×10^{-4}
100	4×10^{-4}

[1]Sample size is the scorable number of individuals per group and assumes $n_i = n_j$.

frequencies being too similar and/or the samples sizes being too small.

Negative distances

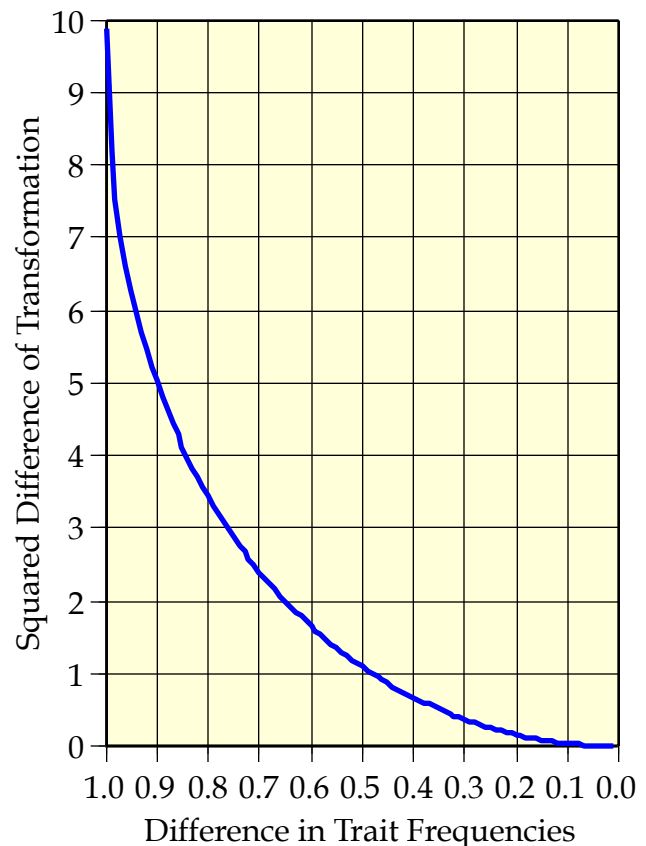Consider the largest possible difference between a pair of trait frequencies. Suppose, hypothetically,

Fig. 3. Graph of the correspondence between the difference in trait frequencies in a pair of samples and the squared difference $(\theta_{ik}-\theta_{jk})^2$ using Grewal's transformation.
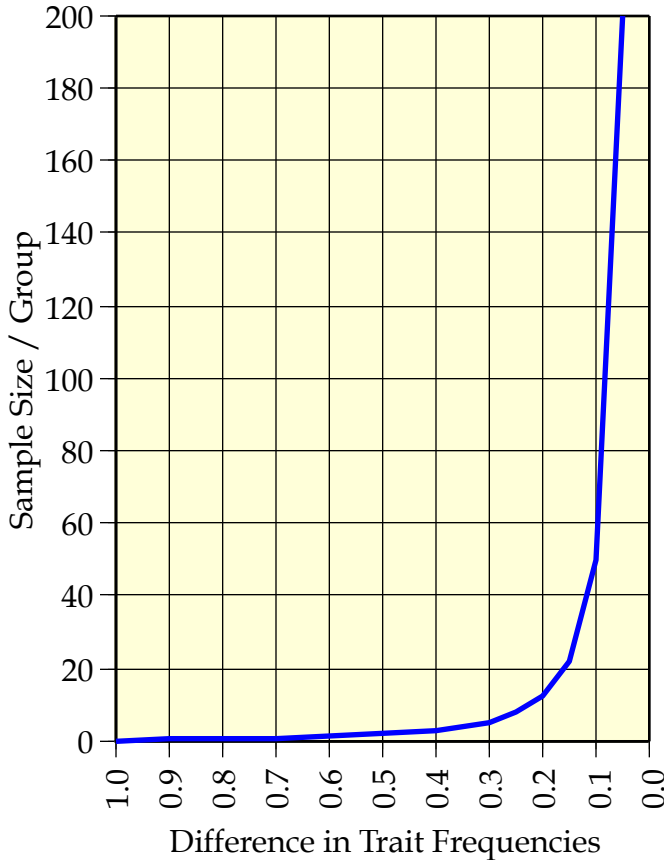
Fig. 4. Graph showing where the difference in trait frequencies (X axis) equals the correction term (Y axis) that is a function of sample sizes.

that a trait like the three-rooted mandibular first molar (Tratman, 1938) is virtually fixed at 99.99% in Group *i* but is quite rare, 0.01%, in Group *j*. This squared difference $(\theta_{ik}-\theta_{jk})^2$ using Grewal's arcsine transformation is 9.62. All other between-group comparisons other than this extreme will be less than 9.62. Obviously, too, as trait frequencies approach each another in two samples—as occurs when groups are genetically and phenotypically more similar—the smaller the $(\theta_{ik}-\theta_{jk})^2$ difference will be and the greater the relative influence of the correction term.

We can look at some simple examples to guage the influence of the correction term (Table 2). The relationship is linear. When sample sizes are less than about 20 (per sample, assuming $n_i = n_j$), the term is fairly large, in excess of 0.10. If sample sizes are 50, the term is 0.002, and if sample sizes are 100, the term is just 0.0004.

These values can be compared to those generated by the squared differences of the transformed frequencies $(\theta_{ik}-\theta_{jk})^2$ as shown in Figure 3. There is a negative hyperbolic relationship here. As examples, when the difference in trait frequency is 0.85, the contribution to the MMD will be 4; when the difference is 0.65, the

contribution will be 2; and when the difference is 0.48 the difference will be 1.

Figure 4 graphs these two opposing values, namely the squared difference in trait frequencies $(\theta_{ik}-\theta_{jk})^2$ on the X axis and the sample size (per group) at which this difference is nullified by the correction factor. We see that sample size (per group) can be less than 20 and there will still be a positive contribution to the MMD so long as trait frequencies differ by at least 15 percentage points. If the difference in frequencies is just 10 points, then sample sizes less than 40 will generate a negative MD for that trait. If the difference is just 5 percentage points, sample sizes need to be at least 200 per group. This graph should provide some helpful guidelines when the researcher is deciding which skeletodental traits possess enough intergroup variation to generate meaningful MMDs.

One can see that the potential magnitude of an MMD is limited; the lower limit is zero and the upper limit is less than about 9.6. This upper limit assumes that the sample sizes of the two groups are very large (so the correction factor is effectively nil) and that the trait frequencies between groups are as different as possible for all traits considered. In practice, actual values for the MMD will be far smaller than this. Because the obtained MMD values are small (generally below 0.50), some researchers have multiplied them by 100 or 1,000 for presentation, and this has led to misunderstanding when the research report was not adequately scrutinized by subsequent investigators.

Test of significance

Two groups can have a nonzero MMD simply due to chance deviations because we are dealing with finite *samples* of specimens, not statistical populations. This might make a test of statistical significance useful. The smaller a group's sample sizes, the more the MMD can differ from zero due to sampling fluctuations that do not represent a "true" biological difference.

C. A. B. Smith developed a test of statistical significance for the MMD based on its variance, though, like the distance formula itself, several early publications contain errors. Constandse-Westermann (1972:120) lists the correct formulation of the variance of MMD:

$$4\frac{\sum_{k=1}^{r}\frac{\left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)}{r}\left[\left(\theta_{ik}-\theta_{jk}\right)^2-\left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)\right]}{r^2} \qquad \text{[Eq. 9]}$$

To be clear, the standard deviation of this variance is the square root of Eq. 9, namely

$$\sqrt{4\dfrac{\displaystyle\sum_{k=1}^{r}\dfrac{\left(\dfrac{1}{n_{ik}}+\dfrac{1}{n_{jk}}\right)}{r}\left[\left(\theta_{ik}-\theta_{jk}\right)^2-\left(\dfrac{1}{n_{ik}}+\dfrac{1}{n_{jk}}\right)\right]}{r^2}}$$

[Eq. 10]

Sjøvold (1973:210; 1977:30; also see Green and Suchey, 1976:67) notes that, under the null hypothesis, the variance simplifies to

$$\frac{2}{r^2}\sum_{k=1}^{r}\left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)^2$$

[Eq. 11]

so the square root of Eq. 11 is the standard deviation of MMD

$$\sqrt{\frac{2}{r^2}\sum_{k=1}^{r}\left(\frac{1}{n_{ik}}+\frac{1}{n_{jk}}\right)^2}$$

[Eq. 12]

Standard statistical theory indicates that two samples will differ significantly at alpha = 0.05 when their means differ by at least 1.96 their standard deviation. This value of 1.96 rounds to 2, which is where the statements come from (*e.g.*, Sjøvold, 1973:216; Green and Suchey, 1976:67) that the null hypothesis of "no difference" can be rejected when the MMD is more than twice its standard deviation (Eq. 12). This rule of thumb is, however, a rough estimate, particularly if the usable sample sizes vary much among the traits used.

There are, however, at least three considerations that detract from the value of testing the statistical significance of MMD: One, the meaning of a "significant" difference is quite vague biologically. This relates to group selection; if two samples are sufficiently different on the basis of geography, anthropology (*i.e.*, race, language, and culture), or distance, then they already characterize separate populations, and no test is required. If, as occurs too frequently in the anthropological literature, samples differ in time, then of course they constitute samples of different populations because a biological population (Mayr, 1963:136) is a

> community of potentially interbreeding individuals at a given locality. All members of a local population share in a single gene pool, and such a population may be defined also as a group of individuals so situated that any two of them have equal probability of mating with each other and producing offspring….

This is where the oxymoron of a "skeletal population" is seen to be absurd (Cadien *et al.*, 1974).

It might be countered that the aim is to see whether two samples are so similar that they can be considered to be drawn fro the same statistical population. Smith

(1972:243) notes that, "Alas, this seems to confuse the ideas and uses of a 'distance' and a 'test of significance'. Also, it is usually a nonsensical question, for two distinct populations are distinct, and are not in any reasonable way samples from a single population." Moreover, there are more appropriate and more efficient statistical methods for testing the differences in trait frequencies than the averaged result given by the MMD (see, *e.g.*, Fleiss, 1981; Sokal and Rohlf, 1995).

Two, "The crucial point in every problem concerning biological divergence or distance—and in fact for the study of biological distance in general as well—is the choice of variables of a given set to use" (Sjøvold, 1977:31). The issue here is that the size of the MMD between pairs of samples can be increased or diminished simply by varying the traits used. This issue has been reviewed in depth in books on numerical taxonomy because trait selection—which traits and how many traits—is so central to the results obtained (*e.g.*, Sokal and Sneath, 1963; Reyment, 1991). The issue revolves on two considerations (see Sjøvold, 1977:31), one is whether the chosen trait frequencies are sufficiently different among the groups while still being representative of the groups and, two, whether intergroup divergence is diminished or accentuated by the traits selected in the prior consideration. Those familiar with population differences in dental trait frequencies (reviewed in Turner *et al.*, 1991; Hillson, 1996; Scott and Turner, 1997) will appreciate that different traits discriminate between different groups; important discriminators for one comparison are noncontributory in other comparisons. The "best" discriminators depend wholly on the groups being compared. Put simply, the quantitative results from the MMD (and other distance statistics) are prone to researchers' biases in trait selection. A test of statistical significance is, then, of little practical use.

The researcher needs to be aware of the influence of trait selection and be prepared to defend the suite of traits used for an analysis. The simple inclusion of "lots" of dental traits actually is counterproductive because most do not differ sufficiently among groups or, like the paramolar tubercle of Bolk (Dahlberg, 1945) or the Uto-Aztecan premolar (Morris *et al.*, 1978), occur too infrequently to contribute numerically to a MMD. Sjøvold (1973:211) also makes the point that "dummy" variables are not to be used; these are traits that are fixed across all of the samples studied (either always present or always absent).

Sjøvold recommends the use of Bartlett's adjustment (Bartlett, 1936) when the trait frequency is fixed in a given sample: If the trait does not occur in a sample ($p = 0$) then it should be replaced by $p = 1/4n$. If the trait always occurs ($p = 1$) then it should be replaced by $p = 1 - (1/4n)$. Green and Suchey (1976) also promote the use of Bartlett's adjustment to help correct for ex-

treme trait frequencies.

Trait selection

The MMD necessitates some care in trait selection in order to preserve its statistical properties. This can present a perceptual conflict with the goals of numerical taxonomy. On the one hand, long-held goals in numerical taxonomy are *repeatability* and *objectivity*. A matrix of MMDs should not depend on the traits selected; instead, a goal is that different researchers, using different sets of traits should arrive at a comparable set of intergroup relationships. An obvious and attractive way of seeking this goal is to use *many* variables, without selection, so the resulting MMDs will constitute a broad, comprehensive consensus of how the groups are related phenetically. Sokal and Sneath put forth the seldom-achieved suggestion that, "At least sixty [traits] seem desirable, and less than forty should never be used" (1963:51). The idea is that *many* traits will more-thoroughly sample the battery of available or possible traits, thus diminishing the influence of any one or a few traits, and similarly will guard against biases in trait selection, thus making the phenetic distances more *objective*.

The statistical problem with this approach is that some—perhaps several or, even, most—traits will be nondiscriminatory among the groups. As seen from Eq. 6, when there is little or no difference in trait frequency among the groups, the contribution of that trait to the MMD will not be zero. Instead, because of the correction factor, the trait's contribution will be negative, which has no biological meaning. And, obviously, intergroup differences in trait frequencies need to be larger to be contributory when sample sizes are smaller.

An obvious solution to the accumulation of negative values in the calculation of an MMD would simply be to set the negative values to zero on a trait-by-trait basis. This, however, creates another problem, so it is not recommended. When MMDs are calculated as in Eq. 6, they are *unbiased* estimates of the underlying population differences. This feature is lost—and with it several statistical properties—if negative contributions are set to zero. If negative values are set to zero, the MMDs will over-estimate the population differences. Instead, we recommend the following two-step approach:

One, *a priori* a scientist should propose to use as large a battery of traits as feasible, thereby seeking the goals of repeatability and objectivity set forth by Sokal and Sneath (1963). This initial list needs to be made explicit in the publication; it may well supply important information for other researchers following up with later studies. However, these proposed traits need to be tested to see which ones contain contributory information, which we define as a trait showing a statistically significant difference between at least one pair of the groups being evaluated. These intergroup differences can be evaluated by any of a number of statistical tests appropriate for rates and proportions (*e.g.*, Fleiss, 1981; Siegel and Castellan, 1988).

This winnowing process (1) removes those traits that will generate negatives values across all pairs of groups during calculation of the MMDs, but (2) does not bias the MMDs' estimates. Again, we contend that it is important to provide the full list of traits (and their trait frequencies) prior to the omission of noncontributory traits.

As an optional third step, those MMDs that are negative can be set to zero, both conceptually and practically, if subsequent use is to be made of them (such as input for cluster analysis or phenograms or other graphical representations). Indeed, it is permissible to set all MMDs that are less than twice their standard deviations to zero since, statistically, these estimates of the underlying population differences are nonsignificant. Such values are simply within the range of random sampling fluctuations, so their expected values are zero.

The error of "standardization"

Sofaer and colleagues (1986) introduced quite a different approach to calculating the MMD that they term "standardized MMD." They developed their method to try and resolve a serious shortcoming of their data, namely: What if you want to develop a matrix of MMDs for a set of samples, but you did not score the same suite of morphological traits for all of the groups? Sofaer's solution was creative, but wrong.

In concept, one suite of traits ought to produce roughly the same phenetic relationships as another (*e.g.*, Sokal and Sneath, 1963). If enough traits are used, and all of them possess the same inter-group relationships, and each trait produces the same magnitude of inter-group "distances," then this would be approximately true. In actuality, of course, different sets of traits seldom produce comparable phenetic results.

Sofaer's solution was to use MMDs generated between pairs of groups—where different groups were represented by different traits and different numbers of traits. The authors then "standardized" the MMDs by dividing each MMD by its standard deviation (using a formula similar to Eq. 12). This was claimed to be analogous to the conventional z-score standardization,

$$z = \frac{(X - \mu)}{\sigma}$$ [Eq. 13]

(*e.g.*, Sokal and Rohlf, 1995:101-111) but the analogy quickly breaks down.

Recall that standardizing a normally distributed sample yields z-scores with a mean of zero and a stan-

dard deviation of one (often termed "unit variance" since $\sigma^2 = 1 = \sigma$). Such a distribution occasionally is coded as N(0, 1). This standardization cannot be properly applied to a series of MMDs—unless all of the MMDs are zero (so $\mu = 0$), in which case the operation is pointless. The whole purpose of calculating MMDs among groups is that the groups differ according to some set of trait frequencies. More precisely, one supposes that the *populations* from which the samples are drawn possess meaningfully different trait frequencies; indeed, the degree of phenetic distance (MMD) is expected to differ among groups on a pair-by-pair basis—some groups being more similar and others more different than others for a given set of traits. For a given set of comparisons, some, most, or all of the MMDs will be different from zero. Regardless of particulars, the MMDs will not be zero, nor will the mean of the MMDs be zero.

Moreover, the standard deviation (Eq. 12) is going to suffer from random variations in sample size from trait to trait. Given that (1) most anthropological samples are modest in size, (2) they are samples of convenience (so sample size seldom can be controlled), (3) sample sizes differ among traits, sometimes dramatically, due to unscorable specimens, and (4) trait frequencies seldom vary much among groups, especially after sampling fluctuations are accounted for, "standardization" of MMDs effectively is an exercise in introducing random errors of unknown magnitude that differ in unknown but differing ways from comparison to comparison depending on sample sizes and other random errors, also of unknown and differing magnitudes.

There is, in fact, no analogy between the conventional z-score and Sofaer's treatment of the MMDs. With a set of MMDs, the population mean is not zero, and there is a different standard deviation for every MMD (Eq. 12). Since these standard deviations are primarily tied to the sample sizes of the traits available in the study, "standardization" as described by Sofaer *et al.* divides each MMD by a different and biologically meaningless value. We obviously see no merit—and several problems—with this attempt at "standardization."

Problems with "standardization" seem obvious to us, but the method was applied uncritically by Sutter and Mertz (2004)—evidently with the passive assent of the reviewers as well. What strikes us as particularly unfortunate is that (1) the proper source of the "standardization" method (*i.e.*, Sofaer *et al.*, 1986) does not even appear in the literature cited and (2) the method is wrongly-attributed (on their page 136) to Sjøvold (1973), who decidedly did not mention or advocate any such approach. This error is yet another example of where hasty scholarship has created impediments to the correct calculation of MMDs.

Summary

The purpose of this note is to publicize the correct calculation of Cedric A. B. Smith's MMD. This can be summarized in four steps: (1) Eq. 6 is the correct formula for the MMD as devised by Smith and modified by Berry (1969); (2) Smith's arcsine transformation of trait frequencies should be replaced by Anscombe's transformation (Eq. 3) and expressed in radians, not degrees; (3) the sampling correction in Eq. 6 should be replaced by the more accurate term in Eq. 7; and (4) the preliminary battery of traits should be tested univariately for among-group differences and those traits without statistically significant differences in frequencies across all samples should be omitted. Additionally, Bartlett's adjustment should be applied when the sample trait frequency is fixed at 0 or 1. Statistical significance between a pair of populations occurs when the MMD exceeds twice its standard deviation (Eq. 12). The lack of statistical significance does not mean that the samples can be supposed to derive from the same population, but that it is not possible to distinguish the populations they come from by means of the data and/or the sample sizes available.

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Occlusal Morphology of the Mandibular First and Second Premolars in Iranian Adolescents

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ABSTRACT: In dental textbooks, the mandibular premolar occlusal morphology has been described as having a predominantly "U-shaped" central groove on the first premolar and a "Y-shaped" central groove on the second premolar. In this study, we examined students (n = 400) of Isfahan high schools (Iran) and first and second premolars were examined bilaterally. Morphological features of the crown, number, height and position of cusps, central grooves shape and sex of the teethwere recorded. For the mandibular first premolars, 21.5% of students exhibited H-shape grooves bilaterally; 70.0% had bilateral U-shape grooves; and 8.5% were mixed. For the mandibular second premolars, 73.0% exhibited 2-cusp forms bilaterally; 15.8% had 3-cusp forms bilaterally; and 11.3% were mixed. In the 2-cusp forms, the predominant occlusal pattern was U-shaped (44.0%). In this Iranian sample, the predominant occlusal pattern was U-shaped in both the first premolar and second premolar, which contrasts with conventional textbook descriptions. *Dental Anthropology* 2004;17(3):94-96.

The mandibular first premolar (LP1) is the smallest premolar in the human dentition and typically has two cusps. Its buccal cusp is much larger than the lingual cusp, causing the central groove to be U-shaped, with the bottom of the "U" directed lingually. But, in some instances, the lingual cusp is wider buccolingually and the central groove becomes H-shaped. In these latter cases, the coronal morphology of this tooth is more similar to maxillary premolars (Van Beek, 1983; Ash, 1993).

The occlusal morphology of the mandibular second premolar (LP2) is variable with two or more cusps. The variation occurs in the lingual portion of the crown that may present as a single cusp or may be divided into two or three cusps giving a more angular and square outline (Loh, 1993). Van Beek (1983) stated that, like first premolars, various occlusal patterns are seen in the 2-cusp forms with the predominant pattern an H-shaped central groove. In the multiple cusp forms, the LP2 crown appears to have a more-square outline (Loh, 1993), with the buccal cusp much broader than either of the lingual cusps. A "Y-shaped" form occurs when there is a central pit with three grooves (mesial, distal and lingual developmental grooves) radiating from it (Van Beek, 1983).

In dental textbooks, the occlusal morphology of LP1 and LP2 are described as usually having a "U-shaped" central groove on the first premolars and a "Y-shaped" groove on second premolars. But in our experience, the anatomy of these teeth is more variable. A literature search revealed a paucity of descriptive information on prevalence and features of the coronal morphology of these teeth. The purpose of the present study was to assess the actual variability of the occlusal groove patterns in a sample of contemporary Iranian adolescents.

MATERIALS AND METHODS

This investigation was undertaken in the high schools of Isfahan City, Iran. The students were screened and only those with erupted mandibular first and second premolars present bilaterally were selected. Direct intraoral examination was undertaken. Morphological details of the crown: namely the number, position and height of cusps and the sex of the subjects were recorded on prepared forms. Data were excluded from the investigation in cases where the teeth were restored, worn or heavily broken.

A cusp was defined as a pronounced elevation on the occlusal surface of a tooth terminating in a conical, rounded, or flat surface (Jordan and Abrams, 1992). Four hundred individuals (1,600 teeth: 800 first premolars and 800 second premolars) were analyzed for the present descriptive study.

RESULTS

Mandibular first premolar

Eighty-six individuals (21.5%) had a bilateral H-shaped pattern, 280 (70.0%) had a bilateral U-shaped pattern, and 34 (8.5%) were mixed (Table 1). Chi-square test revealed that there was no sex predilection for pattern of the central groove. But in the mixed

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TABLE 1. *Groove patterns of the mandibular first premolar*

Groove form	Males	Females	Total n	%
H-shaped	41	45	86	21.5
U-shaped	134	146	380	70.0
Mixed	25	9	34	8.5
Total	200	200	400	100.0

TABLE 2. *Cusp number of the mandibular second premolar*

Number of cusps	Males	Females	Total n	%
2-cusp forms	143	149	292	73.0
3-cusp forms	25	38	63	15.7
Mixed	32	13	45	11.2
Total	200	200	400	100.0

group, there was significant difference between males and females (P = 0.0163), with mixed patterns occurring in males more often that females (males = 25, females = 9).

Mandibular second premolar

Most cases (292/400; 73.0%) were bilateral 2-cusp forms; 63 (15.8%) were bilateral 3-cusp forms; and 45 (11.3%) were mixed (Table 2). Chi-square test revealed no sex predilection in the first and second categories but in the mixed groups there was significant difference between males and females (P = 0.004), also with mixed cusp forms occurring more often in males (males = 32, females = 13).

Various occlusal patterns occurred in the 2-cusp premolar (Table 3). The predominant pattern (44.0%) was a U-shaped groove form (P < 0.001).

DISCUSSION

In the Iranian sample, 70.0% of cases had a U-shaped central groove pattern on the mandibular P1. But in a study from the Ivory Coast (Adiko *et al.*, 1999) the occlusal morphology of this tooth tended toward the 2-cusp mandibular second premolar. Among bilaterally symmetric cases the 2-cusp forms were far more common in the Iranian that the 3-cusp forms (15.8%). This finding is consistent with other population studies. Pederson (1949) gave a frequency of 63.8% in his series of 188 casts of East Greenland Eskimos. He quoted frequencies of 16.8% and 25.6 % in European (de Terra, 1905) and Finnish (Kajava, 1912) dentitions, respectively. However, in the present study 11.3% of cases were asymmetric (2 cusps on one tooth and 3 cusps on the homologue); data on asymmetry in the other studies were not reported. The LP2 3-cusp form occurred in nearly 16.0% of the Iranian sample. This occurrence is not very high. In Loh's study of Chinese from Singapore (1993), the 3-cusp form constitutes a quarter of the cases studied, and Loh considered this trait to be a North Asians characteristic.

The H-shaped pattern in the 2-cusp form of LP2 might also be an ethnic feature Loh (1993), stated that the 3-cusp forms (Y-shaped) were an important variation in that (1) no sex predilection is found for its occurrence; (2) development of structures with bilateral presence usually shows minor variations in size and shape; (3) different forms on each side is unusual, and (4) when asymmetry occurs in pattern, it is seen significantly more often in males.

CONCLUSION

In this study of Iranian adolescents the predominant occlusal pattern was U-shaped in both the first and second premolar samples. Thus in this population, occlusal morphology of first premolars was like that explained in dental textbooks, but the occlusal anatomy of second premolars is more variable that expected.

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TABLE 3. *Groove patterns of the mandibular second premolar*

Groove pattern	Males	Females	Total n	%
H-shaped	32	52	84	21.0
U-shaped	85	91	176	44.0
Y-shaped	25	38	63	15.7
Mixed	58	19	77	19.2
Total	200	200	400	100.0

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Thanks to the efforts of Sally Graver (Ph.D. Student, Ohio State), the Dental Anthropology Association Web site has a new home. The new Web site address is:

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This is located on the Ohio State University Department of Anthropology's server.

Alma Adler (Ph.D. Candidate, Arizona State University) designed the new Web site, which currently has links to the membership form, Dahlberg Prize announcement, and to Phil Walker's and Ed Haagen's quick-time movies of the dentition. In addition, we plan to make past issues of *Dental Anthropology* available on the Web site.

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