Quantitative Assessment of Interproximal Wear Facet Outlines for the Association of Isolated Molars

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ABSTRACT The determination of the minimum number of individuals can be very challenging, especially in an assemblage of fragmentary bones and isolated teeth. Similarities in tooth morphology, degree of wear, and interproximal wear facets (IPWF) are generally used to associate isolated teeth qualitatively. However, no quantitative method has yet been established for an objective identification and matching of isolated tooth crowns. In this study, we analyze the IPWF morphology of adjacent mandibular molars (17 M_1/M_2 pairs), applying both qualitative and quantitative methods to test a reproducible approach for crown association. The surfaces of distal (for M_1) and mesial (for M2) IPWF were surface-scanned and digitally selected. Three-dimensional (3D) and two-dimensional (2D) outlines of IPWF were analyzed using elliptic Fourier analysis (EFA) and geometric morphometrics methods (GMM).

Teeth are an important source of biological information, and this is of particular interest in paleontological and bioarchaeological studies. Tooth crowns are the most abundant remains in the fossil and archaeological record because of the hardness and resistance of enamel. Determining if two or more isolated teeth belong to one or more individuals is of particular importance for the reconstruction of individual dentitions and the evaluation of the minimum number of individuals recovered. One approach to face the problem of isolated tooth specimens is a simple qualitative assessment by trying to fit them into a jaw bone discovered in the same stratigraphic layer (Weidenreich, 1937). However, this method is limited by the fact that jaw bones are often too fragmentary or absent at fossil sites. Even internal micromorphology has been used for matching adjacent teeth of the same individual. For example, the contour lines of Owen in the dentine and the striae of Retzius in the enamel were used for tooth association (Gustafson, 1947; Lunt, 1974). The latter method, however, is usually invasive, requiring tooth sectioning, and consequently these techniques are not applicable in a rare fossil assemblage. Consequently most tooth associations in fossil hominin collections are done using qualitative identifications. The Neanderthal dental sample from Krapina, Croatia was analyzed by using the orientation and morphology of the interproximal wear areas, the continuity of occlusal wear, and some unique features present in some crowns (Wolpoff, 1978, 1979; see also Radovčić et al., 1988). The Additionally, teeth were qualitatively associated by visual evaluation of the IPWF outline and by physical matching. Unsatisfactory results with less than 50% of tooth pairs correctly associated were obtained by using both methods, shape analysis (digital approach) and the visual evaluation (qualitative assessment) of the IPWF outline. The physical matching of the crowns showed highly variable accuracy ranging between 53% and 77%. The quantitative formspace analysis of 2D IPWF outlines provided the best results (82% of correctly associated teeth), but no statistically significant differences were recorded when compared with the manual matching. Since three tooth pairs out of 17 could not be quantitatively associated, we suggest that the quantitative analysis of IPWF should be used only in addition with other approaches. Am J Phys Anthropol 144:309-316, 2011. \circ 2010 Wiley-Liss, Inc.

same method was applied to identify seven human teeth of uncertain origin from the Tabun Cave in Israel (Coppa et al., 2005). Photographs of interproximal wear facets (IPWF) were used to superimpose outline drawings visually on acetate transparencies to reconstruct tooth rows of the hominoids from Paşalar in Turkey (Gencturk et al., 2008). In all these cases, the authors used the IPWF to fit adjacent teeth together in the same tooth row.

Interproximal tooth wear is commonly an age-related process (Kieser et al., 1985) produced by tooth-to-tooth contact and crown movements occurring during chewing (Fig. 1). Two main forces are involved during masticatory

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Fig. 1. Lower right second molar (RM_2) , mesial view. The arrow points out the mesial interproximal wear facet (IPWF). The scale in the figure is equivalent to 1 cm.

movements: a lateral force that directs bucco-lingually, and a mesial force that pushes the teeth making them migrate anteriorly (Picton, 1962). One can expect that these movements generate a strong correlation in size, morphology, orientation, and angulations between contiguous IPWF, providing a suitable way to determine tooth associations.

Two different approaches based on the IPWF are routinely used to determine tooth associations: the IPWF outline can be qualitatively evaluated as an independent feature for tooth association (e.g., Gençturk et al., 2008) or IPWF can be directly employed for the physical matching of adjacent teeth (Wolpoff, 1978, 1979; Coppa et al., 2005). In the latter approach, besides the IPWF surface, other dental features, such as tooth morphology and dental wear stage, are considered. These two approaches are based on a qualitative assessment of the IPWF.

Quantitative data based on measures of shape and size of IPWF provide an objective and reproducible method for tooth association, whereas the results of a qualitative analysis are more subjective, mainly depending on the experience of the worker. In the present study, we tested the utility of IPWF outlines for individual association of isolated molars by means of quantitative analysis. Distal (for M_1) and mesial IPWF (for M_2) were selected and analyzed using elliptic Fourier analysis (EFA) and geometric morphometric methods (GMM). These results were compared with those produced by traditional visual approaches.

MATERIALS AND METHODS

The sample consists of 34 first and second mandibular molars $(17 \text{ M}_1/\text{M}_2 \text{ pairs})$ of modern humans belonging to 15 individuals, 11 of which were collected at the Depart-

Fig. 2. Methodological steps included in the analysis: B, buccal; L, lingual; O, occlusal; b, buccal point; GMM, geometric morphometric methods; EFA, elliptic Fourier analysis.

ment of History and Methods for the Conservation of Cultural Heritage, University of Bologna in Ravenna, Italy. Four individuals were collected at the Department of Paleoanthropology and Messel Research in the Senckenberg Research Institute in Frankfurt am Main, Germany. For each specimen, distal IPWF of M_1 and mesial IPWF of M2 were considered. Only molars with a slight or moderate degree of wear were included in the analysis (wear stage 5 and less; Smith, 1984). We considered the left and right sides of the same individual, when available, as two separate tooth pairs.

The essential steps followed for the digital approach of the present research (casting the teeth, three-dimensional (3D) model generation, IPWF segmentation, outline orientation, and statistical analysis) are summarized in Figure 2 and described in detail in the following paragraphs.

Scanning and digitalization of the models

The transparency and reflection of tooth enamel usually make the direct scanning of original specimens INTERPROXIMAL FACET ANALYSIS FOR TOOTH ASSOCIATION 311

Fig. 3. (a) Distal facet of M_1 and mesial facet of M_2 are visualized; (b) facets are virtually detached from the digital dental models, distal facet of M_1 provides the reference orientation. To obtain the same orientation for the mesial facet of M_2 , the facet is rotated 180° along the *z*-axis, and the internal aspect is considered.

impossible. The use of talc or an ammonium chloride coating to cover the tooth surface was avoided because small particles of the powder might remain on the surface even after cleaning and could prevent future microwear analyses. For this reason, we produced high resolution molds of isolated teeth using an impression material based on A-silicones (Provil Novo Light C.D.2, Heraeus Kulzer GmbH) (Fiorenza et al., 2009). Afterwards, casts with nonreflective, superhard gypsum (Everest Rock, KaVo), a replica material specially developed for surface scanning, were produced. We surface-scanned the casts of the original specimens with a white-light scanner (smartSCAN3D, Breuckmann GmbH) with a x/y-resolution of \sim 55 μ m. The acquisition and alignment of the scan-data were processed with optoCAT software (Breuckmann GmbH), whereas PolyWorks[®] 10.1 (Innov-Metric Software Inc.) was used for the final 3D image analysis. We used left molars as a reference while right molars were mirrored digitally.

The shape and size of the IPWF is usually age-related, resulting in concave, convex, and sinuous three-dimensional surfaces in advanced age (Kieser et al., 1985). Thus, 3D and 2D analysis of the IPWF outlines were carried out to test whether the latter might involve a relevant loss of information when compared with the 3D outlines.

Orientation of the IPWF

The digital models were approximately oriented manually on the screen by rotating the occlusal surface parallel to the xy-plane and the mesio-distal and bucco-lingual crown diameters parallel to the x -axis and y -axis of the Cartesian coordinate system (Fig. 2). We considered the distal IPWF for M_1 and the mesial IPWF for M_2 (Fig. 3a). In the IMEdit module of $PolyWorks^{\circledR}$ the outline of the facets was marked and segmented with the polyline tool (Fig. 3a). Subsequently, independent digital models of the corresponding facets were created (Fig. 3b). To investigate the outline shape and size correspondence of adjacent teeth, the M_2 IPWF was rotated 180° along the z-axis (Fig. 3b). The facets were imported into the IMInspect module of PolyWorks[®] while maintaining the previous orientation defined in IMEdit, with the buccal side on the left, lingual side on the right, and the occlusal side on the top of screen. Depending on the masticatory forces and on the degree of wear, the molar's IPWF show differences in shape. A circular shape, in

which a major axis is not distinguishable, is often dominant in unworn or moderately worn teeth, whereas in teeth characterized by advanced wear stages the IPWF tend to be bucco-lingually elongated and occluso-cervically narrowed (subrectangular shape). For this reason the first and second eigenvector of the facet were computed by fitting all the data points of the digital model using the singular value decomposition of the data points distribution (Fig. 2). Each facet was subsequently rotated until the plane identified by the first two eigenvectors was parallel to the xy-plane of the Cartesian coordinate system. The 2D outlines were obtained by projecting the 3D outlines orthogonally onto the xyplane, and the buccal endpoint of the first eigenvector allows identification of the buccal point, b (Fig. 2). For the identification of the similar points in the 3D outline, the buccal endpoint of the first eigenvector was orthogonally projected onto the 3D facet outline.

Afterwards, the outlines (2D and 3D) were imported into Rhinoceros 3.0 (Robert McNeel & Associates, Seattle, WA) and standardized for rotation and translation. Each outline was oriented with its first two eigenvectors onto the Cartesian coordinate system, rotating the first eigenvector parallel to the x-axis and the second eigenvector along the y-axis. Accordingly, the outlines were translated to overlap the buccal point from which 30 equidistant points were created (Fig. 2).

Statistical method

Statistical analyses were performed in R software (R Development Core Team, 2008). Shape and form analyses of the outlines were performed using EFA and GMM. EFA provides an orthogonal decomposition of each curve into a sum of harmonically related ellipses that progressively describe the outlines in detail (Kuhl and Giardina, 1982; Ferson et al., 1985; Rohlf, 1990). As mentioned above, the outlines were already invariant for translation, rotation, and starting point. Invariance of size was obtained by dividing each point's coordinate (the 30 equidistant points) with the outline's centroid size (CS). The first seven harmonics of the elliptical Fourier series were sufficient to provide a comprehensive description of IPWF outlines, explaining almost 98% of the IPWF outlines. A set of 30 EFA coefficients in 2D (two coefficients for the zeroth harmonic and four coefficients for each other harmonic) and 45 EFA coefficients in 3D (three

coefficients for the zeroth harmonic and six coefficients for each other harmonic) was obtained.

Moreover, the equidistant points (semilandmarks) on the target outlines were iteratively slid along the outline (both in 2D and 3D), based on bending energy, to create geometrically homologous points (Bookstein, 1991, 1997; Gunz et al., 2005). The sliding was done considering points on a reference outline. Using this algorithm, the bending energy between the reference and target outline was minimized and artificial deformation removed. In the first step, the first outline was chosen as a reference outline and the rest of the outlines (target outlines) were slid according to this one. In the second step, GPA (Generalized Procrustes Analysis) was performed to obtain a Procrustes mean outline as a second-step-reference outline, and the sliding procedure was repeated considering this reference. Furthermore, GPA and sliding were repeated iteratively until convergence. Accordingly, final alignment among outlines was performed by GPA, which standardizes the size of the objects and optimizes their rotation and translation so that the distances between corresponding (semi)landmarks are minimized (Bookstein, 1991; Dryden and Mardia, 1999; Zelditch et al., 2004). The centered Procrustes shape coordinates (Procrustes fit coordinates) were augmented by natural logarithm of centroid size, log CS.

EFA coefficients, squared Procrustes shape, and form distances were submitted to hierarchical cluster analysis (complete, single, average, and median linkage) (Everitt, 1974) to quantify the percentage of correctly associated teeth. Since this analysis is not a scale free method, the result depends on the units of the variables. Therefore, EFA coefficients cannot be augmented by log CS. The combination between EFA coefficients (artificial distances) and log CS do not create any reasonable space that could be used for hierarchical cluster analysis.

While intraobserver errors can arise during facet marking, the quality of the dental 3D models is assumed from the feature accuracy information of the smartS-CAN3D $(\pm 12 \mu m)$ provided by the Breuckmann GmbH developer (www.breuckmann.com). Once the IPWF outline has been marked and segmented, the computation of the first two eigenvectors and the standardization process (for translation, rotation, and scaling) of the outlines is a highly reproducible method. Accordingly, for the intraobserver error evaluation during facet outline digitization, the IPWF of eight teeth were marked and segmented twice. The obtained outlines were compared with the sample variance (where the variance is calculated for all teeth together, $n = 34$). We found that the mean intraobserver error rate (mean intraobserver squared error divided by the sample variance, in percent) is equal to 2.44%, an error that could be considered acceptable. For this reason, repeating the entire procedure to test the results was not required.

Visual approach

The 34 isolated lower molars were analyzed by one observer using traditional visual methods. The first method is based only on the visual assessment of IPWF outlines. The isolated teeth were arranged randomly on the table with the correct IPWF oriented upwards. In addition, the molar roots were covered with adhesive paper tape, used to hide the specimens' label. The observer was only allowed to look at the tooth wall of the IPWF under study. Instead, the second visual method is based on the physi-

 $A_n = \frac{1}{2}$

cal contact of the isolated molars, where, beyond the IPWF profiles, continuity in dental morphology, tooth size, and occlusal wear is also important. In both methods, the analyses were carried out at different time intervals of one week and repeated three times to avoid a familiarization with the teeth during consecutive days.

Comparison of the methods

Finally, to quantify the difference between all the methods, we used the McNemar chi-square test of symmetry. It is a nonparametric method for dichotomous (binary) data (good classification/misclassification) applied to 2×2 contingency tables for matched-pair problems. In our setting, rows and columns of 2×2 contingency tables correspond to compared methods (for each visual approach, the best result was used in the analysis). The tested hypothesis regards "marginal homogeneity" (compared methods are given the same results), which is equivalent to the test of equality of row and column marginal frequencies (McNemar, 1947).

RESULTS

Based on the visual assessment of the IPWF outline, not more than eight individuals (47%) were correctly associated in all three independent tests (Fig. 4). The percentage of correctly associated teeth increases through the physical-contact matching of the molars, even if the results are highly variable: in the first test, 13 individuals (77%) were correctly recognized, but in the second and the third experiments, the number of correctly matched molars decreases to nine individuals (53%).

For the digital approach, the cluster analysis method with the largest number of true tooth pairs at the single cluster (the so called 'most similar') was chosen. Using the four hierarchical cluster analyses, we found that complete linkage presented the highest number of correctly matched individuals.

Shape-space sliding (semi)landmarks and EFA (for 2D and 3D outlines) provide almost the same results, with not more than eight individuals correctly coupled (47%). The number of correctly associated teeth increases to 14 individuals (82%) in 2D form-space analysis when log CS is included (Fig. 4). In general, the association of crowns showed no improvement when using 3D outlines compared with 2D projected outlines.

Regarding these considerations, we only present the cluster dendrogram obtained when Procrustes form distances were submitted to hierarchical cluster analysis. Among the three misclassified pairs, it is worthwhile pointing out that only one pair is totally mismatched (pair 16), the other two pairs being partially clustered (pairs 9 and 13).

The McNemar chi-square test emphasizes that the result of hierarchical cluster analysis for 2D form-space data is significantly different from all the other methods except the physical manual matching of the molars (Table 1). The matching success rates obtained for the latter are significantly different from those obtained by EFA (both 2D and 3D), 3D shape-space, and visual matching based on IPWF outlines, and are near the significant level for 2D shape-space.

Finally, it is important to emphasize that the results are less consistent when only the size (area of the IPWF, Table 2) is considered in the hierarchical clustering. In

Fig. 4. The bar plots display the percentage of correctly matched teeth in the traditional (top) and digital (bottom) approach, respectively. Number of associated tooth pairs are inside the bars.

TABLE 1. McNemar chi-square test of symmetry among the methods used for the association of isolated molars

List of methods								
	2D EFA	3D EFA	2D shape-space	2D form-space	3D shape-space	3D form-space	Visual matching ^a	Physical contact ^b
2D EFA		0.655	0.655	0.020	1.000	0.317	0.739	0.034
3D EFA				0.034	0.705	0.527	1.000	0.025
2D shape-space				0.014	0.564	0.480	1.000	0.059
2D form-space					0.008	0.046	0.014	0.564
3D shape-space						0.18	0.763	0.034
3D form-space						0	0.414	0.180
Visual matching ¹								0.025
Physical contact ²								

 $\mathrm{^{a}}$ Visual matching of IPWF outlines.
 $\mathrm{^{b}}$ Physical contact of the molars.

Significant P -values <0.05 are given in bold.

fact, in this case, only four individuals were correctly associated (11.8%).

DISCUSSION AND CONCLUSIONS

Interproximal wear facets are considered a valuable feature for the association of isolated teeth (Wolpoff, 1978, 1979; Radovčić et al., 1988; Coppa et al., 2005; Gencturk et al., 2008). Currently, two different methods

Fig. 5. Complete linkage cluster analysis of the form differences between 2D IPWF outlines. Shadowed specimens are mismatched, and their outlines are shown to describe the degree of form discrepancy (outlines are at scale). The 17 specimens were labeled using progressive numbers: specimen $1 = 1.1$ (M₁) and 1.2 (M₂); specimen $2 = 2.1$ (M₁) and 2.2 (M₂); specimen 3 = 3.1 (M₁) and $3.2 \, (M₂)$; etc.

for tooth association are in use: 1) the visual evaluation of the IPWF outlines, 2) the manual matching of the teeth by physical contact of two dental walls, plus the evaluation of dental features, such as general dental morphology, tooth size, and the occlusal wear. Nevertheless, almost nothing is known about the correctness of IPWF outline matching by the above mentioned methods. In all the previous works, the IPWF outline was only qualitatively evaluated (e.g., Wolpoff, 1978, 1979; Coppa et al., 2005; Gençturk et al., 2008). In physical anthropology, the necessity to support qualitative descriptions with quantitative data is well recognized (e.g., Pietrusewsky, 2008). In this respect, a new digital approach does not only provide the opportunity to quantify IPWF outlines and to compare them objectively for tooth associations, it also allows a statistical description and interpretation for the use of IPWF in the future determination of the minimum number of individuals in an assemblage of isolated teeth.

In our pilot study, we observed that the IPWF of adjacent teeth belonging to the same individual can show clear differences in outline shape, and an almost similar shape can be shared between teeth belonging to different individuals. The results of the shape analysis using a digital approach (EFA and GMM) are relatively consistent with the results obtained by visual assessment of the IPWF outline. In both cases less than 50% of the individuals were correctly associated. Tooth pairs not correctly associated (the residual 50%) have different IPWF outlines. A higher rate of correct matches was obtained by manually testing the molar fitting, although this approach showed a high variation in success (between 53% and 77% in the correctness of the results). This variation could depend on subjective evaluations, where the success of tooth association is influenced by individual experience and knowledge of interproximal wear. In spite of this, the physical manual matching is a simply applied method which, in contrast to the computer-based

approach, is less time consuming and does not require expensive equipment. Consequently, one has to consider why a more careful and objective evaluation of the IPWF outline, using both shape and size information, will be advantageous for tooth associations. In our study, the percentage of correctly associated teeth increased to 82% when applying 2D form-space analysis, including log CS. Accordingly, about 35% of the individuals (the percentage of individuals obtained by subtracting the result of 2D shape-space (47%) from 2D form-space (82%)) were matched mainly with regard to their IPWF outline size, though they present a different shape. In addition, it is important to mention that 18% of the tooth pairs possess a completely different IPWF outline (Fig. 5). Accordingly, based on our preliminary results, we suggest that in a general molar sample, at least 20% of IPWF pairs could be completely different, limiting the performance of any digital approach for tooth association. This limit does not regard the digital method itself but depends on the intrinsic biological difference of some IPWF belonging to adjacent teeth. Why complementary IPWF display differences in their shape and size were not the focus of this analysis, but our digital results perhaps will spark some interest for future investigation to answer this question. It is worthwhile emphasizing that a quantitative morphometric analysis can be useful for investigations of IPWF development and variation (Wolpoff, 1971). Shape and size variation in IPWF outlines of adjacent teeth could provide interesting information regarding the relationship between IPWF development and masticatory forces that are dependent on dietary and nondietary habits (Hinton, 1982).

Additionally, we have observed that a 2D digital approach provides fairly good results because the facets possess only a shallow concavity (at least in our modern human sample), with IPWF outlines usually located on a plane. The 3D outline variability can be decomposed into two parts: first, the variability in the plane defined by the first two eigenvectors, which basically accounts for variability of the 2D outline (as described above, for each facet the 2D outline is obtained projecting the 3D outline orthogonally in the plane identified by the first two eigenvectors); second, the information perpendicular to this plane. We suggest that adding information from the third dimension to the 2D outlines creates noise that could also affect the sliding algorithm, which in the end might interfere with the number of correctly associated teeth.

As discussed above, interproximal wear is an agerelated process (Kieser et al., 1985), depending also on the magnitude of the masticatory forces because of diet and food preparation (Hinton, 1982). Unworn or slightly worn teeth are characterized by small and subcircular IPWF. In this circumstance, the positive coupling mainly depends on the facet's size, whereas the IPWF shape provides small contributions. The digital approach cannot overcome this problem. When facets are small with a subcircular shape, the first eigenvector can have unpredictable directions, affecting the orientation of the IPWF outline. Consequently, for both manual matching and a digital approach, a low success rate in correctly associated teeth is to be expected in young individuals and in populations with soft diets.

On the contrary, both methods work better with moderately or heavily worn teeth, where IPWF usually have subrectangular shapes, which are bucco-lingually extended. This is the case with adult individuals, and in populations, such as modern and ancient hunter-gatherers, where diets required powerful mastication. Nevertheless, both the approaches show advantages and disadvantages. It is an advantage of form-space analysis that results are reproducible and variation in shape and size can be quantitatively evaluated. On the other hand, the manual approach accounts for important details in crown morphology, dental wear, etc., which are not considered in the digital approach. Accordingly, we suggest combining qualitative and quantitative approaches for tooth association derived from manual matching and form-space analysis of 2D outlines.

As argued by Gencturk et al. (2008) and supported by our results, the correspondence and similarity of two IPWF outlines is possibly not sufficient to justify an association. This becomes even more apparent if we look at the distribution of correctly identified adjacent tooth pairs in our sample. Only one tooth pair was consistently recognized correctly in all the methods applied. The results of our study suggest caution when using IPWF analysis as a stand-alone feature for determining tooth association. Instead, IPWF analysis should be combined with other approaches such as occlusal fingerprint analysis (OFA) (Kullmer et al., 2009; Fiorenza et al., 2010), morphological features, and enamel hypoplasia occurrence.

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