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INTRA- AND INTERPOPULATION HUMAN BUCCAL TOOTH SURFACE MICROWEAR ANALYSIS: INFERENCES ABOUT DIET AND FORMATION PROCESSES

ABSTRACT: This study describes intra- and interpopulation buccal dental-microwear variability in ancient prehistoric and historic farmers, and in a modern (in vivo sample) Spanish population. An attempt has been made to relate microwear patterns to sex-age related factors and dietary habits among human groups from Spain. Casts of mandibular molar teeth of two age-grouped adults (~17–25 and 25–35 years of age) of both sexes were examined with a scanning electron microscope (SEM). Micrographs were taken on buccal surfaces at 100× magnification. Buccal dental-microwear density and length (in micrometers) by orientation classified into four categories from 0° to 180° were recorded on well-preserved enamel micrographs using an image analysis software package. Finally, univariate and multivariate statistics were applied to examine intragroup sex-age factors and interpopulation diet-related differences in buccal-microwear patterns. No significant differences between groups defined by sex and age appeared. Nevertheless, males present a higher microwear density than females. On the other hand, adult age-group results indicate that the buccal-microwear pattern is an accumulative process. Significant differences ($p<0.05$) were found in the buccal-microwear density and length between groups, related to physical characteristics of their diets. An implication of these results is that intra- and interpopulation buccal-microwear might be an indicator of the physical diet nature regarding diet abrasiveness rather than dietary habits during the Holocene. Furthermore, long-term buccal-microwear turnover depends on the abrasive nature of the diet linked to dietary habits and food technical processing methods.

KEYWORDS: Teeth – Microwear – Diet – Formation processes – Sex – Age – Spain

INTRODUCTION

During mastication, the postcanine dentition acts to reduce food toughness. However, food texture – i.e. the physical properties of the food – depends both on its nature and on cooking modes. Hard or elastic foods could show mechanical differences during mastication rather than abrasive effects on the enamel (Romero, De Juan 2005). In fact, many foods are not hard enough to scratch tooth surfaces (Gügel *et al.* 2001, Newesely 1993, Romero, De Juan 2005, 2006, Sanson *et al.* 2007). Only those foods which include intrinsic or extraneous abrasive particles harder than enamel on Moh's scale, such as siliceous opal-phytoliths in monocotyledone plants or exogenous grit from food processing methods can scratch tooth enamel surfaces (Gügel *et al.* 2001, Teaford, Lytle 1996, Romero, De Juan 2006, Romero *et al.* 2007). Therefore, microscopic wear patterns on tooth enamel surfaces (dental microwear) are highly correlated with abrasion (tooth-food-tooth contact), but are also determined by the relative hardness and physical properties of tooth surfaces (Newesely 1993, Teaford 2007, Romero, De Juan 2005).

Experimental *in vivo* microwear studies on occlusal and buccal postcanine teeth surfaces have shown that

the enamel of humans who eat soft-industrialised foods have fewer microwear features than those consuming an induced-abrasive diet, where the rate of wear increases (Romero *et al.* 2007, Teaford, Lytle 1996) with a different turnover that is lower in the buccal surface than in the occlusal one (Pérez-Pérez *et al.* 1994, Romero *et al.* 2006, 2007). Therefore, although ancient human diet behaviour approximation through dental microwear analysis must be understood in terms of abrasiveness, it equally reflects cultural and environmental factors (Pérez-Pérez *et al.* 2003, Romero *et al.* 2003, 2004, Teaford 2007).

Human dental microwear analyses of molar teeth from the museum collection specimens have provided direct evidence of dietary behaviours (Jarošová *et al.* 2006, Mahoney 2005, Pérez-Pérez *et al.* 2003, Romero 2005, Teaford 2007, Ungar *et al.* 2006). However, modern human microwear models and selected prehistoric human groups for dental microwear comparison are necessary (Pérez-Pérez 2004, Pérez-Pérez *et al.* 2003, Romero *et al.* 2004, 2006, 2007, Teaford 2007). Thus, dental microwear studies focused on the variability between hunters-gatherers and farmers correlate microwear density and morphology (pits and scratches) on occlusal surfaces with diet abrasiveness (Mahoney 2005, Teaford *et al.* 2001, Ungar *et al.* 2006). Nevertheless, human dental occlusal-microwear data for that dietary transition is a complex signal (Teaford 2007) derived not only from differences in habitat adaptation, food acquisition or processing techniques among populations (Mahoney 2005, Teaford *et al.* 2001, Ungar *et al.* 2006), but also from intertooth and intrafacet occlusal-microwear variations (Mahoney 2005). In contrast to microwear on the chewing surfaces of teeth, no tooth-to-tooth contact occurs on non-occlusal surfaces during mastication, enamel microwear being directly related to abrasion in the course of an individual's lifetime (Pérez-Pérez 2004, Pérez-Pérez *et al.* 1994, Romero, De Juan 2005). Research on human buccal dental-microwear has shown that variability in intragroup buccal-microwear values seems to be less significant than interindividual and intergroup variability with a correlation between microwear density and dietary habits (Jarošová *et al.* 2006, Lalueza *et al.* 1996, Pérez-Pérez *et al.* 2003, Romero 2005, Romero *et al.* 2003, 2004). Although these studies clearly demonstrate the existence of relationships between diet and interspecific microwear variability, little is known about buccal-microwear formation rate-dietary and age relationships. The reconstruction of human past diets through dental microwear must depend on a comparatively large sample of known dietary habits identified in modern hunters-gatherers and industrialised people, in which both microwear, diet and intrapopulation differences by sex and age are represented. Based on some studies which have suggested that microwear density on buccal tooth surface is due to grooving age-related processes (Pérez-Pérez *et al.* 1994, Romero, De Juan 2006, Romero *et al.* 2006), the present study analyses intra- and interpopulation buccal dental-microwear variability within a sample of ancient and modern human groups from different chronologies. The study aims to augment the buccal-microwear dataset in human groups and interpret microwear variation by sex and age in the populations examined.

MATERIAL AND METHODS

Buccal tooth surface microwear was analysed in a sample of 80 individuals from different chronological periods (*Table 1*). Skeletal remains from three ancient populations recovered in archaeological sites from Villena (Alicante, Spain) – two prehistoric (3rd and 2nd millennia BC) and one historic (10th–13th centuries AD) – were selected from the anthropological collection housed at the Archaeological Museum of Villena (Alicante, Spain). The collection boasted a large well-preserved sample of skeletal remains (Romero 2005). The populations were selected to represent different archaeological periods from the same territory. In addition, an *in vivo* human sample was chosen for buccal-microwear analysis purposes (Romero *et al.* 2006) seeking to obtain a modern human model to microwear-related comparison.

The skeletal sample

The skeletal remains used for this study come from Villena, a town situated in the Vinalopó river Valley (eastern Spain). The Vinalopó river Valley has a great number of anthropological remains and artefacts, from hunter-gatherer societies of the Upper Paleolithic to historic agro-pastoral populations, which started to be recovered in 1950 (Soler García 1993). The archaeological sites have higher percentages of skeletal remains (Romero 2005) than are used in this study, which has grouped them in order

TABLE 1. Population sample size studied across chronology, sex and age groups.

Population	Symbol	Period	Chronology	Male		Female		?		All
				~17–25	25–35	~17–25	25–35	~17–25	25–35	~17–35
Molinico	M	Chalcolithic	3000–1800 BC	2	3	1	8	0	2	16
Cabezo Redondo	C	Bronze Age	1800–1500 BC	1	6	3	4	0	2	16
Losilla	L	Islamic	900–1200 AD	2	4	2	3	0	1	12
In vivo[*]	V	Modern	2004–2005 AD	11	6	9	10	0	0	36
Total				16	19	15	25	0	5	80

[*] The *in vivo* sample was obtained from 19 female and 17 male students from the Universidad de Alicante and Universidad de Barcelona in Spain. Age groups are defined by 18–25 and 26–35 years based on their known age.

to obtain a standardised sample by age. The Chalcolithic human remains come from El Molinico (3000–1800 BC) (M) – a multiple inhumation cave (Soler García 1986). As for the Bronze Age sample, it was taken from Cabezo Redondo (1800–1500 BC) (C) (Soler García 1987) – an important economic and commercial site during the Bronze Age in eastern Spain (Martínez, Iborra 2001, Soler García 1987). Prehistoric sites are located in hills near (2–3 km) the current town of Villena. Finally, an Islamic necropolis in La Losilla (900–1200 AD) (L) recovered from the ancient urban structure of Villena (Soler García 1977) was selected too.

The human population sample based their subsistence economies on animal husbandry and plant cultivation, which reflects their specific environmental conditions and adaptations (Del Rincón 1998, Martínez, Iborra 2001, Romero 2005, Soler García 1987). Nevertheless, changes in the percentages of domestic or cultivated products and food technical processing methods are evident across periods (Del Rincón 1998, Romero 2005, Soler García 1993). From the Chalcolithic onwards, documents reveal a reduction in the frequency of animal hunting and an increase in the husbandry of domestic animals, with a predominance of ovicaprids (sheep and goats) (Del Rincón 1998, Romero 2005). On the other hand, the percentages of cultivated products such as wheat and occasionally barley ground in stone mortars during the Chalcolithic change during the Bronze Age. Indeed, since the Bronze Age there is evidence of both greater society stratification and growth in the availability of agricultural products (barley, legumes, emmer wheat, spelt wheat, bread wheat or millet), which helped to minimise seasonal fluctuations in the food supply and the use of more refined sowing, weeding and grinding techniques.

Sex and age in the skeletal remains was determined using physical-anthropological methods already described (Buikstra, Ubelaker 1994, Cox, Mays 2000). Sex was mostly determined using the cranial, postcranial skeleton, along with the pattern of sexual dimorphism present in cranial features and the mandibular bone. The age at death was estimated using cranial sutures, tooth development and eruption (Buikstra, Ubelaker 1994), as well as dental wear (Brothwell 1981). Each individual belonging to ancient human groups was allocated to one of the two dental age stages defined by molar wear from ~17–25 to 25–35 years of age (Brothwell 1981) (*Table 1*). Relatively small differences became visible as noted by Oliveira *et al.* (2006) when comparing the dental wear age range assigned to each individual with the limits indicated by age classification skeletal methods.

In vivo sample

A sample of 36 adult individuals of both sexes, aged 18–35 years (*Table 1*), with the permanent teeth fully erupted, non bruxism and no history of orthodontic implants, was selected (Romero *et al.* 2006). Each subject made a test devised to collect information about dietary habits. The results show a Mediterranean diet similar to the weekly results previously obtained from adult human people (20–50 age range) of both sexes (Medrano *et al.* 1994) with a higher consumption of cereal, vegetables, fruits and meat than of fish, pulses or green vegetables.

Buccal-microwear analysis

Only one left-lower molar tooth per individual – preferably the lower-left M1 – was selected for a microwear analysis that showed well-preserved enamel surfaces and non *post-mortem* microscopic abrasion and erosion (see below). The teeth sample (*Table 1*) is represented by left-lower Pm4 (6.2%), M1 (82.5%) and M2 (11.2%).

High-resolution dental impressions of mandibular molar teeth from the human skeletal remains and the *in vivo* sample were taken following buccal-microwear analysis procedures (Galbany *et al.* 2004, 2005, 2006, Romero 2005). The mandibular molar teeth were selected for standardisation reasons. Previous studies had shown that buccal microwear should preferably be studied from lower molar teeth (Lalueza *et al.* 1996, Pérez-Pérez *et al.* 1994).

The enamel surfaces from the ancient teeth sample were cleaned with acetone and ethanol, and later air-dried. On the other hand, prior to obtaining silicone-based moulds from the *in vivo* human sample, the volunteers' mouth was wiped with a buccal cleaning product (Lacer®, Barcelona, Spain) and dried with an air compressor for approximately 30 seconds. Dental impression moulds were made using a hydrophobic polyvinylsiloxane silicone-based impression material (President Jet-Regular body, Coltène®) into which two-base component epoxy resin (Araldite® 2020, Vantico AG, Basel-Switzerland) or polyurethane (Feropur PR-55, Feroca®, Spain) was poured in accordance with the methods described by Galbany *et al.* (2004, 2006). The resulting high-resolution tooth replicas were mounted on brass stubs with fusible glue. A colloidal silver solution (Silver Conductive Adhesive 416, Electron Microscopy Sciences-Washington, PA) was applied to improve conductivity during the scanning electron microscope (SEM) observation (Galbany *et al.* 2004).

Finally, the replicas were coated with gold-palladium alloy to prepare them for viewing under a SEM Hitachi S3000N recorded well-preserved buccal enamel micrographs in secondary mode (SE) and 10–15 kV at 100× magnification (1,280×960 pixels – BMP file format), under protoconid or hypoconid cusp tips depending on the level of enamel preservation on the medial third of the buccal surface. A semi-automated image analysis system (Microware 3.0; Ungar 1995) was used to count and measure the buccal microwear pattern on micrographs of a 0.56mm^2 area (Galbany *et al.* 2005, Pérez-Pérez *et al.* 1999) processed with Adobe Photoshop™ 6.0 (*Figure 1*). Only buccal tooth surface lineal striations $\geq$10μm with a length/width ratio of at least 4:1 were counted and measured; fragments of striations crossed or broken by others were considered separately. No visible trampling features such as parallel distribution, fractures or curved scratches were analysed

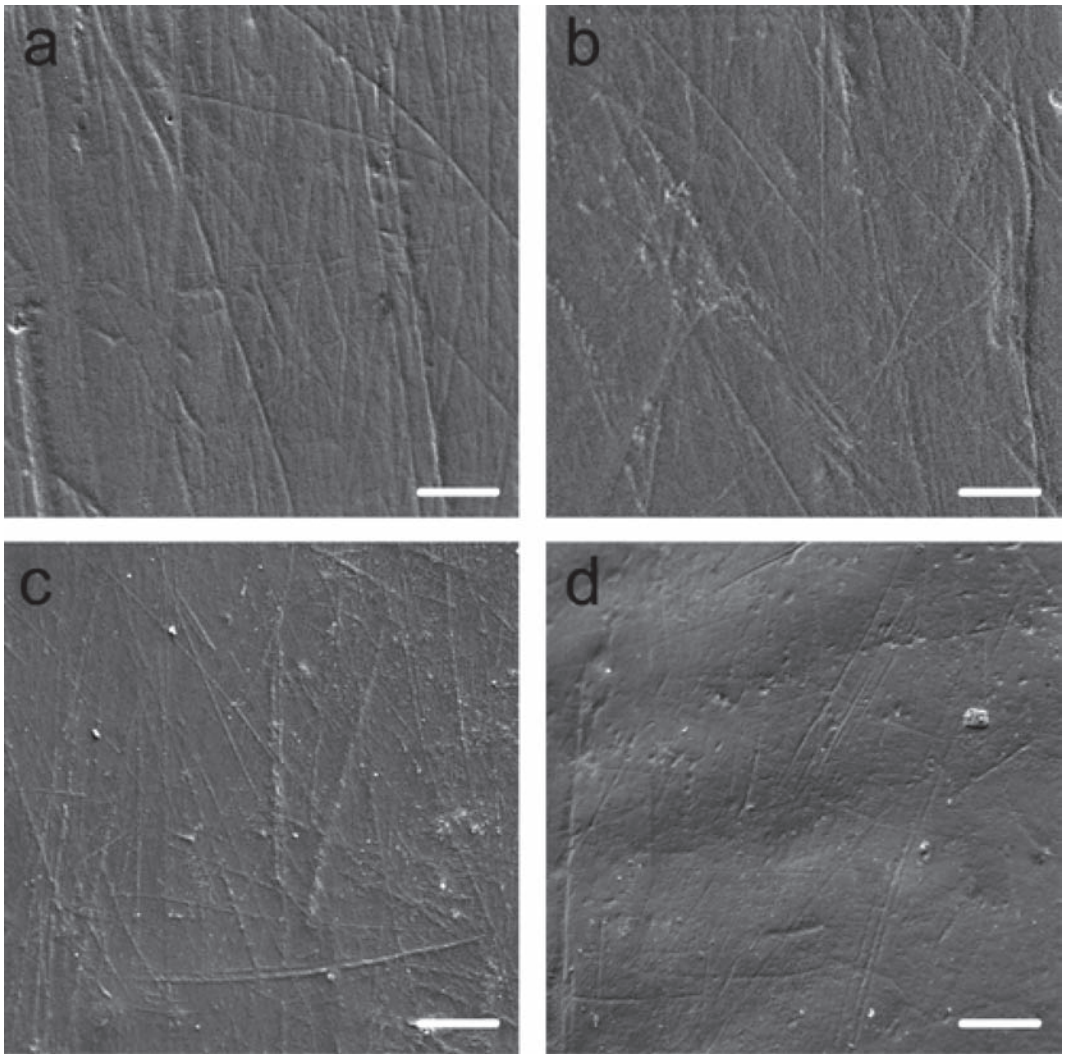

FIGURE 1. Selected buccal surface SEM micrographs at 100× of adult individuals from the human populations examined: Chalcolithic (a); Bronze Age (b); Islamic period (c); and a modern human (*in vivo* sample) (d). Preferential striation orientation and lengths can be observed. Bar: 100 µm

(Galbany *et al.* 2004, Pérez-Pérez *et al.* 2003, Romero *et al.* 2003, 2004).

The number of striations (N), their length (X) in micrometers (µm), the standard deviation of length (S), and the preferred orientation distribution (PO) from 0° to 180° were all recorded for each one of the tooth-micrographs examined. Summary statistics of total N, X and S were ultimately obtained and classified into four orientation categories (in 45° intervals) (Pérez-Pérez *et al.* 1994) for left lower molars as follows: mesio-distal (M): 112.5°–157.5°, V (vertical): 67.5°–112.5°, H (horizontal): 0–22.5° and 157.5°–180.0°, and disto-mesial (D): 22.5°–67.5°. Every variable considered passed the Kolmogorov-Smirnov normality test ($p>0.05$). One-way analysis of variance (ANOVA) tests served to explore differences ($p<0.05$) in NT and XT variables with a Bonferroni *post-hoc* test.

In order to study the effects of period, sex and age on buccal-microwear among populations, multivariate analysis of variance (MANOVA) tests using Wilks' lambda to determine significant effects were performed. Three dependent variables – namely microwear density (NT), average microwear length (XT) in µm, and preferred microwear orientation distribution (PO) from 0° to 180° – were used in the three-factor MANOVA. A number of discriminant analyses helped to determine the interpopulation dietary-related variability using 15 buccal microwear variables obtained (NM, XM, SM, NV, XV, SV, NH, XH, SH, ND, XD, SD, NT, XT, ST) and discriminant functions derived (F). All statistical tests were conducted using SPSS (version 12.0), and graphs were created with SYSTAT (version 11.0).

RESULTS AND DISCUSSION

Descriptive statistics for buccal dental-microwear pattern, microwear density (NT) and average microwear length (XT) in µm, along with differences (one-way ANOVA and Bonferroni multiple comparison test) between populations are shown in *Table 2*. *Table 3* in turn provides three-factor MANOVA test results for interaction between period, sex, and age on buccal-microwear pattern (NT, XT and

TABLE 2. Descriptive statistics for buccal microwear density (a) and length (b) and one-way ANOVA with Bonferroni *post-hoc* test (*) results between populations analysed.

a) Buccal microwear density (NT)

								ANOVA		
Population	**Period**	**n**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**	**Std. error**	**df**	**F**	**p**
M[a]	Chalcolithic	16	177	435	299.68	79.045	19.761			
C	Bronze Age	16	54	151	110.062	27.433	6.858	3, 76	84.946	0.000
L	Islamic	12	76	191	120.416	31.953	9.224			
V	Modern	36	46	166	91.611	32.155	5.359			

Bonferroni *post-hoc* test:
[a] M significantly greater than C (p<0.000), L (p<0.000), and V (p<0.000).

b) Buccal microwear length (XT)

								ANOVA		
Population	**Period**	**n**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**	**Std. error**	**df**	**F**	**p**
M[b]	Chalcolithic	16	38.45	70.09	55.629	9.071	2.268			
C[c]	Bronze Age	16	78.3	108.83	93.325	9.260	2.315	3, 76	34.829	0.000
L[d]	Islamic	12	83.05	105.6	90.843	7.549	2.179			
V[e]	Modern	36	38.43	102.22	68.765	15.411	2.568			

Bonferroni *post-hoc* test:
[b] M significantly lower than C (p<0.000), L (p<0.000), and V (p<0.004).
[c] C significantly greater than M (p<0.000), and V (p<0.000).
[d] L significantly greater than M (p<0.000), and V (p<0.000).
[e] V significantly lower than C (p<0.000), and L (p<0.000).
* The mean difference is significant at a 0.05 level.

TABLE 3. Three-factor multivariate analysis of variance (MANOVA) results*.

Main effects	**Statistic**	**F**	**p**
Period by sex by age	0.9352	0.4301	0.917
Period by sex	0.9176	0.5545	0.832
Period by age	0.8494	1.0698	0.388
Sex by age	0.9725	0.5353	0.659
Period	0.1357	19.6248	**0.000**
Sex	0.9873	0.2443	0.865
Age	0.9358	1.3033	0.282

* Results showing interactions of the main effects related to period, sex, and age using Wilks' lambda to determine significance (p<0.05). Dependent variables included microwear density (NT), average microwear length (XT) in µm, and preferred microwear orientation distribution (PO) from 0° to 180°.

PO variables). Descriptive statistics for buccal-microwear pattern (NT and XT) and differences (one-way ANOVA) related to sex and age-groups factors in the populations analysed are reflected in *Tables 4–5*, and are equally illustrated in *Figure 2*. Mean NT and XT values between populations can be found in *Figure 3*. Finally, a plot of the discriminant function analysis is represented in *Figure 4*.

Intrapopulation buccal-microwear variability

Intragroup differences based on sex and age between populations on buccal-microwear showed no statistically significant differences using a three-factor MANOVA to test interaction between these factors (*Table 3*). Significant differences were only detected between the combinations of the main effects related to period, a finding supported by the one-way ANOVA applied to intergroup microwear differences (*Table 2*), as will be discussed later. No sex-related differences in terms of NT and XT arose in the adult samples of each group. Overall microwear density (NT) was generally higher on male teeth than on female teeth (*Table 4*, *Figure 2*). These results are similar to those obtained in other intrapopulation human buccal-microwear studies (Jarošová *et al.* 2006, Lalueza *et al.* 1996, Pérez-Pérez *et al.* 1994).

Instead, microwear density tends to increase with age while striation length decreases. Significant differences additionally appear in the microwear age-related patterns of the Islamic (NT: F=14.252; df=1, 10; p<0.003) and modern human (XT: F=4.320; df=1, 34; p<0.045) samples (*Table 5*, *Figure 2*). The buccal-microwear patterns from the adult age group category (from 18 to 35 years) show a rate of microwear density that remained constant depending

TABLE 4. Descriptive statistics for buccal microwear pattern (NT and XT) and one-way ANOVA results from sex comparisons.

							ANOVA		
Population (Period)	**Sex**	**Variable**	**n**	**Mean**	**Std. deviation**	**Std. error**	**df**	**F**	**p**
M (Chalcolithic)	Male	NT	5	331.6	102.101	45.661	1, 12	1.271	0.281
	Female	NT	9	282.111	63.832	21.277			
	Male	XT	5	54.278	6.822	3.050	1, 12	0.313	0.585
	Female	XT	9	56.682	8.100	2.700			
C (Bronze Age)	Male	NT	7	120.857	24.956	9.432	1, 12	2.551	0.136
	Female	NT	7	98.857	26.548	10.034			
	Male	XT	7	94.697	9.302	3.515	1, 12	1.134	0.307
	Female	XT	7	89.544	8.789	3.322			
L (Islamic)	Male	NT	6	116	21.419	8.744	1, 9	0.129	0.727
	Female	NT	5	123.6	46.522	20.805			
	Male	XT	6	93.558	9.738	3.975	1, 9	1.363	0.273
	Female	XT	5	88.09	4.006	1.791			
V (Modern)	Male	NT	17	95.705	33.092	8.026	1, 34	0.515	0.477
	Female	NT	19	87.947	31.734	7.280			
	Male	XT	17	66.547	11.363	2.756	1, 34	0.660	0.422
	Female	XT	19	70.749	18.390	4.219			

TABLE 5. Descriptive statistics for buccal microwear patterns (NT and XT) and one-way ANOVA results from age-group comparisons.

							ANOVA		
Population (Period)	**Age**	**Variable**	**n**	**Mean**	**Std. deviation**	**Std. error**	**df**	**F**	**p**
M (Chalcolithic)	17–25	NT	3	304	119.025	68.719	1, 14	0.010	0.920
	25–35	NT	13	298.692	73.778	20.462			
	17–25	XT	3	50.506	2.804	1.618	1, 14	1.192	0.293
	25–35	XT	13	56.811	9.668	2.681			
C (Bronze Age)	17–25	NT	4	90.75	3.862	1.931	1, 14	2.994	0.105
	25–35	NT	12	116.5	29.006	8.373			
	17–25	XT	4	89.53	1.244	0.622	1, 14	0.889	0.361
	25–35	XT	12	94.590	10.465	3.021			
L (Islamic)	17–25	NT	4	87.25	14.221	7.110	1, 10	14.252	**0.003**
	25–35	NT	8	137	23.976	8.476			
	17–25	XT	4	89.582	5.021	2.510	1, 10	0.154	0.702
	25–35	XT	8	91.473	8.797	3.110			
V (Modern)	18–25	NT	20	84.5	32.451	7.256	1, 34	2.281	0.140
	26–33	NT	16	100.5	30.445	7.611			
	18–25	XT	20	73.328	11.024	2.465	1, 34	4.320	**0.045**
	26–33	XT	16	63.060	18.377	4.594			

on the diet and the age of the individual. Furthermore, intrapopulation studies have identified lower microwear density values in adults than in subadults (Pérez-Pérez *et al.* 1994, Romero 2005) probably related to the amount of enamel lost in older individuals (Romero *et al.* 2003, 2006). In this sense, interindividual and intergroup human buccal-microwear variability may differ according to climatic or ecological food resources exploitation, but intragrup uniformity is remarkable across sex and age factors, which shows a characteristic microwear pattern of the sample under analysis (Pérez-Pérez *et al.* 1994, 1999, 2003, Romero 2005, Romero *et al.* 2006).

Interpopulation buccal-microwear variability

The one-way ANOVA between populations showed significant differences ($p<0.05$) in microwear density (NT) and average length (XT) (*Table 2*, *Figures 2* and *3*). However, no differences were observed for the striation orientation distribution (n=80; PO: F=1.266; df=3,76; $p<0.292$), characterised mainly by vertical values (PO=83.128°±12.535°) with regard to occlusal-to-cervical distribution. The preferred occlusal-to-cervical striation orientation shown on non-occlusal crown surfaces draws in this sense abrasive particle attacks related to jaw biomechanics rather than rates of dentine exposure on

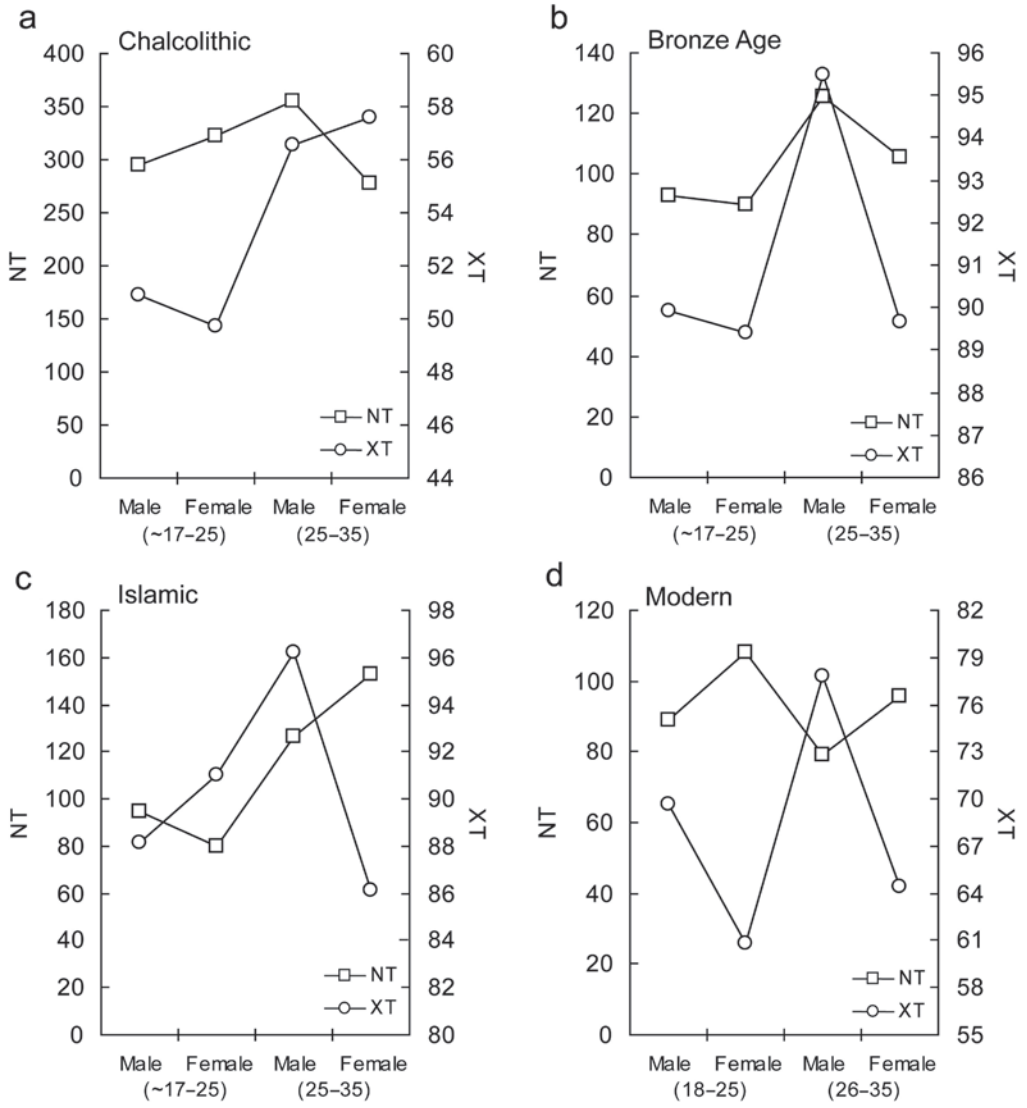

FIGURE 2. Scatterplots showing sex by age-group-related variability of microwear density (NT) and average microwear length (XT) in µm for the chronological (a–d) groups analysed.

occlusal surfaces (Romero *et al.* 2003, 2006, 2007, Pérez-Pérez *et al.* 1994, 2003). The buccal-microwear pattern of the Chalcolithic population (M) is characterised by a high density of short striation and seems to have a more abrasive diet than that of their Bronze Age (C), Islamic (L), and modern (V) counterparts (*Table 2*, *Figures 2* and *3*). Instead, the prehistoric Bronze Age population presents a microwear rate similar in density and length to that of its historic counterparts.

These groups (C and L) differ in microwear density (NT) and their average microwear length (XT) with regard to the *in vivo* sample. Three discriminant factors (F) resulted from the discriminant analyses between populations; the first two F explain 97.1% of total variability (*Figure 4*). The joint discrimination power of the three functions (λ=0.051, χ^2=208.142) was highly significant (p<0.000). The structure matrix showed that the first function (F1) accounted for 60.4% of the total variance and was significantly correlated with NV (r=0.703), NH (r=0.640), ND (r=0.477), NM (r=0.407) and ST (r=–0.202); NT (r=0.926) did not pass the stepwise tolerance test because the variable was strongly correlated with other density variables. As for the second function (F2), the highest correlations were obtained with the variables XM (r=0.660), XH (r=0.608), XT (r=0.595), XD (r=0.588), XV (r=0.355) and SD (r=0.204) and accounted for 36.7% of the total variance.

The results across the different chronological groups clearly suggest changes in the food abrasiveness related to foodstuff technical processes, since a high buccal striation density can be caused by factors other than dietary-related ones (Romero *et al*. 2006, 2007). Different experimental *in vivo* and *vitro* microwear studies on tooth surfaces show that exogenous abrasives such as environmental or milling derived grit are important agents which scratch tooth

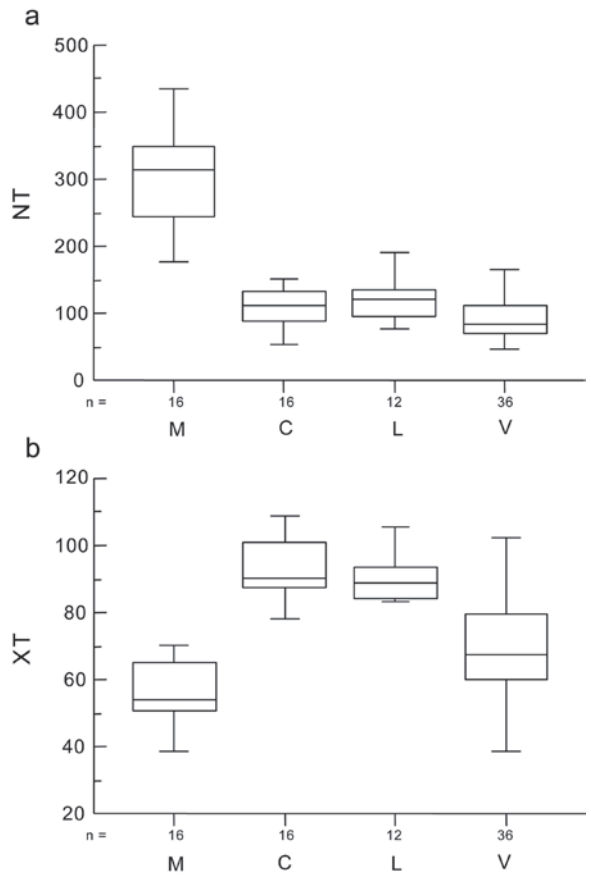

FIGURE 3. Box plot showing buccal microwear density (NT) (a) and length (XT) in μm (b) among the human populations considered: Chalcolithic (M), Bronze Age (C), Islamic period (L), and modern human (*in vivo*) sample (V). The central line in the boxes indicates the sample median; the boxes include 25–75 percentiles; and the whiskers represent the minimum and maximum values identified. Population sample sizes are shown on the x-axis.

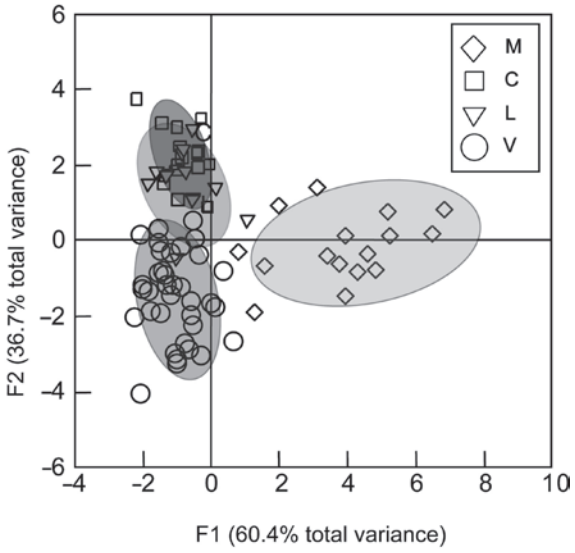

FIGURE 4. Plot of the first two functions based on the discriminant analysis of the human populations under study. The ellipses include 85% confidence regions of the samples compared. Chronological human groups: Chalcolithic (M); Bronze Age (C); Islamic (L); and *in vivo* sample (V).

enamel surfaces (Gügel *et al.* 2001, Teaford, Lytle 1996, Romero *et al.* 2007).

The inferences about buccal-microwear patterns of the populations examined in this study lead the researchers to consider significant differences in food technical processing and environmental exploitation methods rather than general dietary regimes. Buccal-microwear patterns from Pleistocene hominids compared with the modern hunter-gatherer groups from different environments with relatively well-known diets have proved that a higher microwear density upon buccal tooth surfaces appears to be associated with diets that were much more abrasive than those of modern populations (Pérez-Pérez *et al.* 1999, 2003). However, certain groups have been identified with high microwear density values that did not live under similar habitat and climatic conditions as Pleistocene human groups. The high microwear density seen in the Chalcolithic population might actually suggest that exogenous abrasive agents are involved in microwear formation rates, because the average length of striations on abraded surfaces decreases when their number increases (Galbany *et al.* 2005, Pérez-Pérez *et al.* 2003, Romero *et al.* 2003). On the other hand, the increased pitting seen in a microscopic examination of the molar occlusal surface among different populations supports the hypothesis according to which pitting is associated with the consumption of hard foods (Mahoney 2005, Teaford *et al.* 2001, Ungar *et al.* 2006). Nevertheless, the microscopic examinations of occlusal and non-occlusal surfaces clearly show different microwear patterns (Pérez-Pérez 2004, Pérez-Pérez *et al.* 2003, Romero *et al.* 2003).

The differences in microwear variability level during the Bronze Age and Islamic periods with respect to the Chalcolithic population are probably due to the degree of meat consumption or the development of more refined milling techniques. The results obtained in this paper seem to confirm this hypothesis as a piece documented archaeological evidence that includes paleoethnobotanic and zooarchaeologic analyses (Martínez, Iborra 2001, Soler García 1987). Moreover, chewing hard food items may require a large bite force during food breakdown (Romero, De Juan 2005), and this could explain the effect of longer striations on the not-too-highly abraded surfaces found in the ancient populations analysed as well as in modern hunter-gatherer populations (Lalueza *et al.* 1996, Pérez-Pérez *et al.* 2003). Phytoliths plant species of the same taxon may have an impact on the enamel during chewing with different microwear features. Nevertheless, the size and shape of abrasives may not be reflected in the microwear morphology of buccal tooth surfaces. The effects of the number and types of abrasive particles upon buccal enamel surfaces during mastication are seemingly related to microwear density formation rates during an

individual's lifetime (Pérez-Pérez *et al.* 1994, Romero, De Juan 2006).

The *in vivo* human sample showed a lower buccal-microwear density than in the ancient populations, which clearly has to do with differences in the abrasive content of their foods. Few foods in our diet scratch the enamel related to industrialised food technical methods but oxalate contents of various types of actual cereal grains are evident (Siener *et al.* 2006) and contribute to buccal-microwear formation processes (Romero, De Juan 2006). What is more, rates of microwear formation will not only depend on dietary habits, as shown by this study, because food-processing technology must be closely linked to microwear patterns. In fact, different experimental works (Newesely 1993, Romero *et al.* 2007, Sanson *et al.* 2007, Teaford, Lytle 1996) have concluded that exogenous particles, such as dust or mineral grit, have a greater abrasive potential for dental enamel microwear formation patterns than opal phytoliths. In this respect, experimental *in vitro* microwear studies have revealed differences in abrasivity on enamel molar crown which have to do with historic or modern milling techniques (Gügel *et al.* 2001). In short, these interspecific microwear variability patterns must be based not only on dietary, ecological and climatic adaptations but also, probably, on the economic strategy related to food availability and food processing techniques.

CONCLUSIONS

In the light of the results obtained in this study, the role of food technical processing methods undoubtedly has a potential effect in the abrasiveness of the diet and buccal dental-microwear formation rates. Although intragroup buccal-microwear analyses with a large sample are still necessary, the present study confirms previous hypotheses (Pérez-Pérez *et al.* 1994, Romero, De Juan 2006, Romero *et al.* 2006, 2007) regarding the dynamic and long-term accumulative microwear formation processes on buccal tooth surfaces which have an intergrup dependence on dietary habits and food technical processing methods.

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