UNIVERSITAT DE BARCELONA

El patró de microestriació dental de primats *Catarrhini*: un model ecològic per primats fòssils i homínids

Memòria presentada per

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"Hipòtesis, prediccions, experiments i respostes:
és el mètode científic"

Stephen J. Gould, 1980

4. Publicacions

4.1 La replicació de les dents i l'obtenció d'imatges

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L'anàlisi del microdesgast dental és una de les tècniques més utilitzades per interpretar la dieta dels homínids i els primats fòssils a partir de la caracterització i comparació dels patrons de microestriació dental de primats actuals (Puech, 1986b; Ungar, 1994b; Ungar & Teaford, 1996; Teaford et al., 2002; El-Zaatari et al., 2005; Ungar et al., 2006). Les dents dels primats són una font d'informació molt important, no només pels estudis de microestriació, sinó per múltiples estudis de morfologia comparada o taxonomia (Albrecht, 1982), i encara més si es considera el mal estat de les poblacions de moltes espècies de primats (Chapman & Peres, 2001). Una manipulació excessiva dels espècimens osteològics de les col·leccions per part dels investigadors pot suposar un deteriorament dels mateixos i una inevitable pèrdua d'informació. L'alternativa d'estudi podrien ser les rèpliques d'alta resolució obtingudes a les col·leccions osteològiques (Galbany et al., 2004c).

Aquest treball proposa una metodologia que permet l'obtenció de tota la informació morfològica a nivell microscòpic de les corones dentals sense una excessiva manipulació dels espècimens originals. La tècnica consisteix en l'obtenció de motlles dentals d'alta resolució en polivinilsiloxà *President microSystem™* (*Coltène®*), a diferència dels antics motlles parcials a partir de vernissos o Triafol, que no permeten replicar la corona sencera. Aquests motlles s'obtenen després de netejar les superfícies d'esmalt mitjançant l'aplicació de polivinilsiloxà, un material molt emprat en el camp de l'odontologia i ortodòncia, que no danya ni altera les superfícies replicades i ofereix una bona replicació de les superfícies (Beynon, 1987).

Els resultats d'aquest estudi metodològic indiquen que el polivinilsiloxà *President microSystem™ regular body*, així com el *light body* ofereixen bons resultats de replicació de les dents. El polivinilsiloxà *heavy body* no ofereix rèpliques suficientment bones per realitzar posteriors anàlisis amb el Microscopi Electrònic d'Escombrat (SEM) perquè a les superfícies d'esmalt replicades s'hi formen bombolles que impedeixen les anàlisis microscòpiques (Figures 1 i 2).

A partir dels motlles negatius de polivinilsiloxà es van obtenir rèpliques o motlles positius en resina epoxy Epo-Tek #301, que presenta una bona resolució per a la recerca científica a nivell microscòpic (Rose, 1983). També es van

obtenir rèpliques en poliuretà Feropur PR55. Els resultats obtinguts mostren que ambdós materials no presenten cap diferència de resolució respecte l'original ni entre ells fins a 500X de magnificació (Figura 5) i alhora ofereixen una gran duresa i estabilitat temporal. Les rèpliques resultants són aptes per a nombrosos estudis morfològics i permeten estudis microscòpics de les superfícies dentals donada la seva alta resolució.

En aquest treball també s'han considerat altres aspectes de la utilització del Microscopi Electrònic d'Escombrat (SEM). Les anàlisis de superfícies d'esmalt dental requereixen una preparació prèvia de la mostra, que inclou el muntatge sobre un suport d'alumini o llautó i el recobriment o metal·lització amb or. També és necessari evitar l'acumulació de càrregues electrostàtiques eliminant l'acumulació de les partícules de pols dipositades a les superfícies (Figura 3) i afegint un contacte conductor de plata col·loïdal per facilitar la dispersió dels electrons (Figura 4).

Un bon ús del Microscopi d'Escombrat també permet evitar la pèrdua d'informació dels elements lineals de les superfícies d'estudi. Una anàlisi experimental de les microestriacions generades sobre un disc de llautó indica que les imatges obtingudes amb els electrons retrodispersats no sempre són les més adients, ja que els trets lineals paral·lels al detector d'electrons del microscopi es perden. Per contra, les imatges obtingudes amb els electrons secundaris no presenten cap pèrdua d'informació (Figura 6). Aquestes proves també es van realitzar sobre superfícies d'esmalt amb patró de microestriació causat per l'alimentació i van oferir els mateixos resultats. Algunes microestries en disposició horitzontal, i paral·lela al detector d'electrons, no apareixien a les imatges obtingudes amb electrons retrodispersats i sí que ho feien a les imatges obtingudes amb electrons secundaris (Figura 7).

Finalment, les imatges obtingudes amb el SEM han de ser retocades digitalment per millorar-ne el seu contrast i expandir els nivells de grisos. El protocol estàndard recomanat és la utilització del programari Adobe Photoshop per retallar l'àrea d'estudi seleccionada, aplicar un filtre *high-pass* a 50 pixels per eliminar les ombres, i un contrast automàtic dels nivells de grisos. Les imatges resultants d'aquest procediment poden ser analitzades amb altres programes, com ara SigmaScan Pro 5 o Microware 4.

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TOOTH REPLICATION TECHNIQUES, SEM IMAGING AND MICROWEAR ANALYSIS IN PRIMATES: METHODOLOGICAL OBSTACLES

ABSTRACT: Dental microwear analyses are among the most significant techniques through which a researcher can make dietary and ecological inferences from primate fossil specimens. Hard particles, such as plant phytoliths or silica-base sands, can scratch tooth enamel surfaces during food mastication producing a dietary specific pattern of microwear on the enamel surface. The density, axis length and orientation of microwear features, either striations or pits, are highly informative of dietary habits in both extant and fossil primates. The analysis of tooth enamel surfaces requires the use of scanning electron microscopy (SEM) techniques, because of its high resolution power, including gold-coating of teeth for observation. Problems arise when specimens to be analysed are unique and there is no possibility of a direct observation with an environmental microscope. Negative moulds must then be made and silicone-base components are indicated for high quality replication of enamel surfaces. A positive cast needs to be obtained, and epoxy-base resins are frequently used for their good quality and durability. However, successive silicon and epoxy replications result in the loosing of surface detail and precision. Surface observation errors can also be caused be the SEM technology itself, especially if back-scattered electrons are used instead of secondary electrons for maximizing the topographical information of enamel images. This paper reviews the most commonly used methodological approaches to tooth moulding and casting, comparing SEM micrographs of casts with actual tooth surfaces, and contrasting the reliability of SEM images for dietary interpretation of tooth microwear in both extant and fossil primates.

KEYWORDS: Scanning Electron Microscopy – Epoxy resin – Tooth cast – Polyvinylsiloxane impression – Microwear – Primates

INTRODUCTION

Museums holding osteological and paleontological collections of primate bones and teeth are storing highly valuable, and often unique, original samples. Wild-caught primate collections constitute an irreplaceable evidence of primate ecology and adaptations, with significant scientific value for systematics, functional anatomy, and evolutionary studies of primates (Albrecht 1982). The *Primate Specialist Group* of the *Species Survival Commission* of the *World Conservation Union* estimated that half of the world's 250 species of primates were of serious conservation concern, 96 of them nowadays considered to be critically endangered (Chapman, Peres 2001). A great effort in the conservation of these worthy specimens is needed. Sometimes, collections of skulls and skins of wild-caught primates are enormous (Tappen 1969), being of great interest to researchers since large samples of skeletal material can be gathered (Almquist 1973). Primate specimens brought-up in captivity are also frequent in museum collections, but are of lesser value because of possible captivity drawbacks or simple lack of information about their provenance (Albrecht 1982). Conservation of fossil hominid specimens is also of great concern, and repeated handling of remains by specialists is among the main causes of their deterioration. Curators tend to prevent this by not providing

access to specimens. This is of special concern for teeth collections, since teeth are the most abundant remain in the human osteological record and many researchers focus their investigations on their evolutionary history. Availability of high quality tooth moulds at museums or research units (Galbany *et al.* 2004) would reduce the handling problem without reducing access to such valuable remains.

Tooth casts are the main source for Scanning Electron Microscopy (SEM) research since tooth observation frequently requires sample metallization and chamber vacuum. However, reliability of casts needs to be ascertained in terms of surface and feature measurements precision compared to the original tooth. In addition, SEM technology shows some other limitations in microwear research that need to be considered. In this paper we analyse the accuracy of various tooth moulding techniques in enamel microwear research and consider the difficulties of obtaining good, high quality digitalized SEM images of enamel surfaces for microwear analyses.

MATERIALS AND METHODS

Moulding of teeth

The main reason for using tooth replication techniques in paleontology and anthropology is because the original specimens are too valuable to be studied directly by SEM (Beynon 1987), and the replicas, which are copies of the original teeth obtained with various reproduction procedures, allow in turn to visualize inaccessible areas on the original specimen (Beynon 1987). Silicon-base replication procedures are frequently used to obtain negative casts from the original teeth by applying hydrophobic polyvinylsiloxane silicones. Polyvinylsiloxane *President microSystem™* (*Coltène*®), usually *Regular Body*, impression material is widely used for odontological practice and dental microwear research (Ungar 1996, Ungar, Spencer 1999) because it reproduces features with resolutions to a fraction of a micron (Teaford, Oyen 1989a) and maintains the resolution for many years (Beynon 1987). *Coltène Light Body* Polyvinylsiloxane provides a more faithful cast, but *Regular Body* shows enough resolution to analysis by SEM at high magnification, whereas *Coltène Heavy Body* shows somewhat less resolution. In the present study, SEM images of enamel surfaces were obtained using all three *Light*, *Regular*, and *Heavy Body* impression materials to test their fidelity against the original enamel surface. Prior to the moulding procedure, all tooth enamel surfaces were cleaned with pure acetone, to remove chemical preservatives, and then rinsed with 70% ethanol, by gently rubbing the enamel surface with a cotton ear-cube. For image analysis, teeth with non-preserved enamel or presence of tartar deposits or enamel defects were discarded. The polyvinylsiloxane was applied with a thin tip (provided by the manufacturer) to reduce the chance of bubble forming in contact to the enamel surface of the studied tooth. Right before the negative casts had completely dried, conserving their flexibility, they were pulled-out from the teeth and then kept in labelled plastic bags, away from dust. This first reproduction of the enamel surface was used to obtain a high resolution positive cast of the tooth, dimensionally precise and capable of resolving fine surface details (Beynon 1987).

This two-stage technique is advantageous because the primary putty impression closely adapts to the specimen surface, and the second stage, a low viscosity pseudo-plastic impression material, is subjected to high shear forces or to centrifugation, being forced into inaccessible areas which may not be replicated by a single stage impression technique (Beynon 1987). All the positive moulds were obtained using epoxy resin Epo-Tek #301 (parts A and B are mixed in a 1:4 weight ratio), yielding faithful replicas with excellent detail for scientific research (Rose 1983) or biological specimens (Benevius, Hultenby 1991), despite Teaford and Oyen (1989a) indicate that the resolution of some impressions/epoxy moulds is not as good as that obtained with other techniques. Still, epoxies are generally the easiest materials to use because they offer the best combination of working time, setting time, viscosity, resolution of detail, and dimensional stability, remaining the material of choice for most high-resolution casting purposes, not only for casting primate or hominid teeth (Teaford, Oyen 1989a) but also for other zoological groups. Epoxy resins have been used to mould teeth from different sources: reptiles – Cuban crocodile *Cocodrylus rhombifer* (Maas 1994), carnivores – Viverridae (Taylor, Hannam 1987), marsupials – American opossum *Didelphis marsupialis* (Kay, Covert 1983), koala *Phascolarctos cinereus* (Young, Robson 1987), or Artiodactyls, wild moose *Alces alces* (Young, Marty 1986), or sheep *Ovis aries* (Maas 1994). However, most applications have focused on Primates, such as Strepsirrhini – brown lemur *Lemur fulvus* (Maas 1994), New World monkeys (Teaford, Walker 1984, Teaford 1985, Teaford, Robinson 1989, Teaford, Glanger 1991, Teaford, Runestad 1992), Old World monkeys (Ryan 1979a, Teaford, Oyen 1989b, Hojo 1991, Ungar, Teaford 1996), *Hominoidea* (Gordon 1984, 1992, Flynn Zuccotti *et al.* 1998), fossil primates (Ryan 1979b, Teaford, Walker 1984, Ungar, Grine 1991, 1996, Teaford *et al.* 1996, Ungar, Teaford 1996, Flynn Zuccotti *et al.* 1998, King *et al.* 1999), fossil hominids (Grine 1986, Beymon 1987, Ungar, Grine 1991, Ungar *et al.* 2001) or *Homo sapiens sapiens* (Peters 1982, Benevius, Hultenby 1991, Maas 1994, Ungar, Spencer 1999, Grine *et al.* 2001, Göhring *et al.* 2002, Hojo 2002).

Before pouring the epoxy resin into the casts, the two components (A+B) were thoroughly stirred, in order to mix them, and centrifuged during 1 minute at 3,000 rpm (Orto-Alresa Digicen centrifuge) to eliminate air bubbles from the mixture. Before casting it is usually necessary to build a wall around the mould, particularly if the mould is very flat or irregular, to prevent epoxy from seeping out of

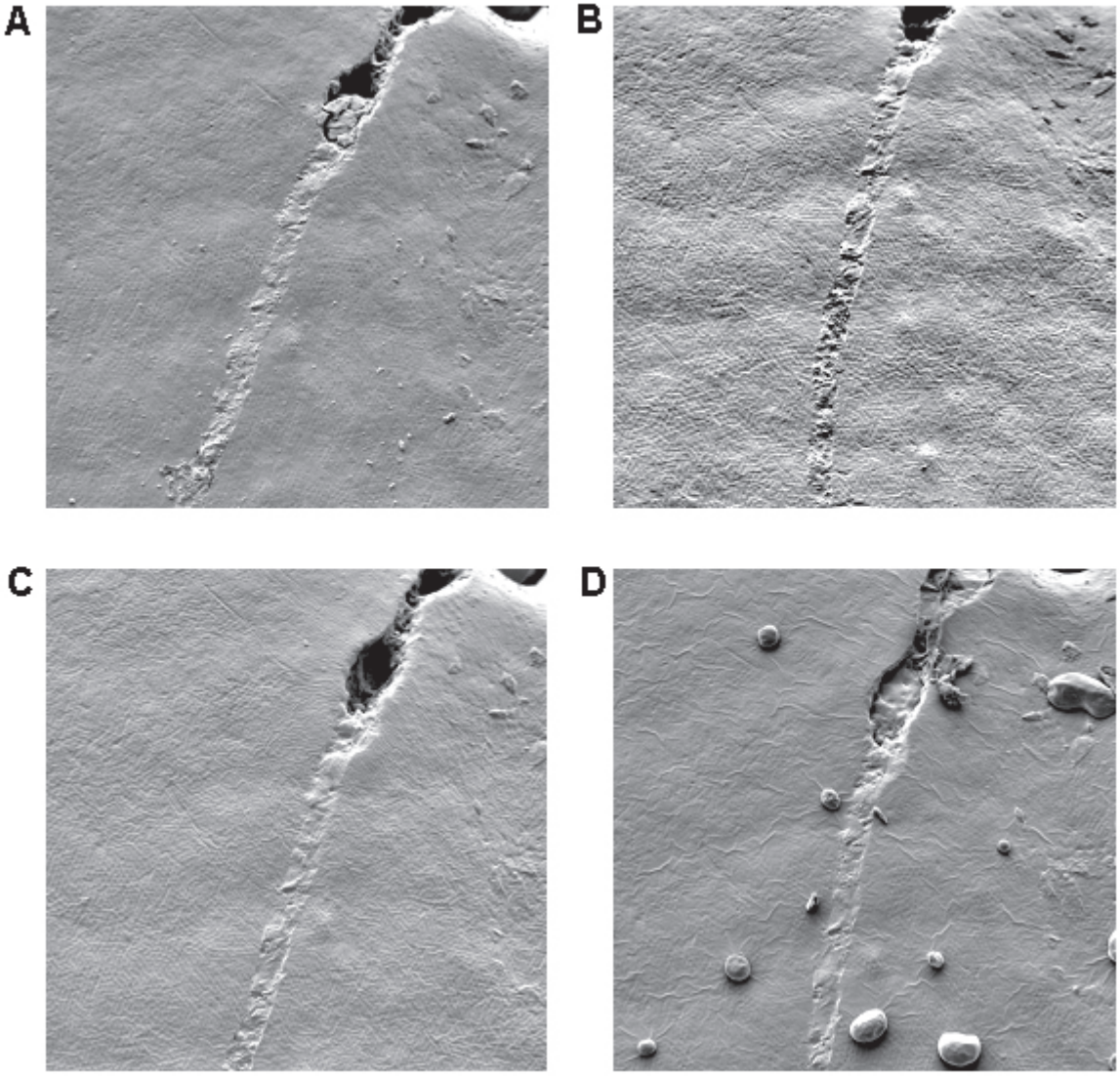

FIGURE 1. Four images of the same enamel surface (lingual surface of the lower left first molar belonging to a modern *Homo sapiens* from La Olmeda, Spain ca XII–XV BC), moulded with different *President microSystem*™ (*Coltène*®) polyvinylsiloxane viscosities. A: original tooth surface, B: *Light Body*, C: *Regular Body* and D: *Heavy Body*. All images are at 100× magnification.

the mould before it sets (Rose 1983). *President microSystem* was used because of its easy and clean application. Immediately after, the resin was poured carefully into the negative casts by using a Pasteur pipette and centrifuged again during 3 minutes at 2,500 rpm (Meditronic Selecta centrifuge), in order to remove any possible air bubbles in contact with the enamel surfaces. The centrifugation reduces the number of bubbles trapped at the mould/cast interface and ensures a good cast (Waters, Savage 1971, Rose 1983). When the epoxy is fully cured, its surface is not tacky to the touch, usually in 6 to 8 hours (Rose 1983), although in our experience epoxy replicas do not completely dry until at least 48 hours. Speeding this step by oven 'cooking' is not advisable. Finally, the tooth replicas were mounted with term fusible gum in either a brass disc or on aluminum stubs. In any case, a colloidal argent belt (Electrodag 1415M-Acheson Colloiden) solution was applied for electron dispersal in the plastic cast, to prevent accumulation of electrostatic charges during SEM observation (Rose 1983). Finally, the sample was sputtered coated with a 400 Å gold layer to allow observation into the SEM and kept clean, dry and dark, preferably inside a storage case, to maintain it dust-free.

In addition to the extended use of epoxies for enamel microwear or abrasion analyses, other materials have also been used for the same purpose. Epoxy resins provide high quality casts but lack some advantages that other materials have. In our initial studies we used Triafol (Balzers Union BU 008 002-T) dissolved in chloroform, that provided very good quality one-step casts (Lalueza, Pérez-Pérez 1993, Lalueza *et al.* 1993, Pérez-Pérez *et al.* 1994, Lalueza *et al.* 1996), which could be directly observed under SEM. Despite it was a fast and easy method, it could only be applied to small enamel surfaces. In the present study we

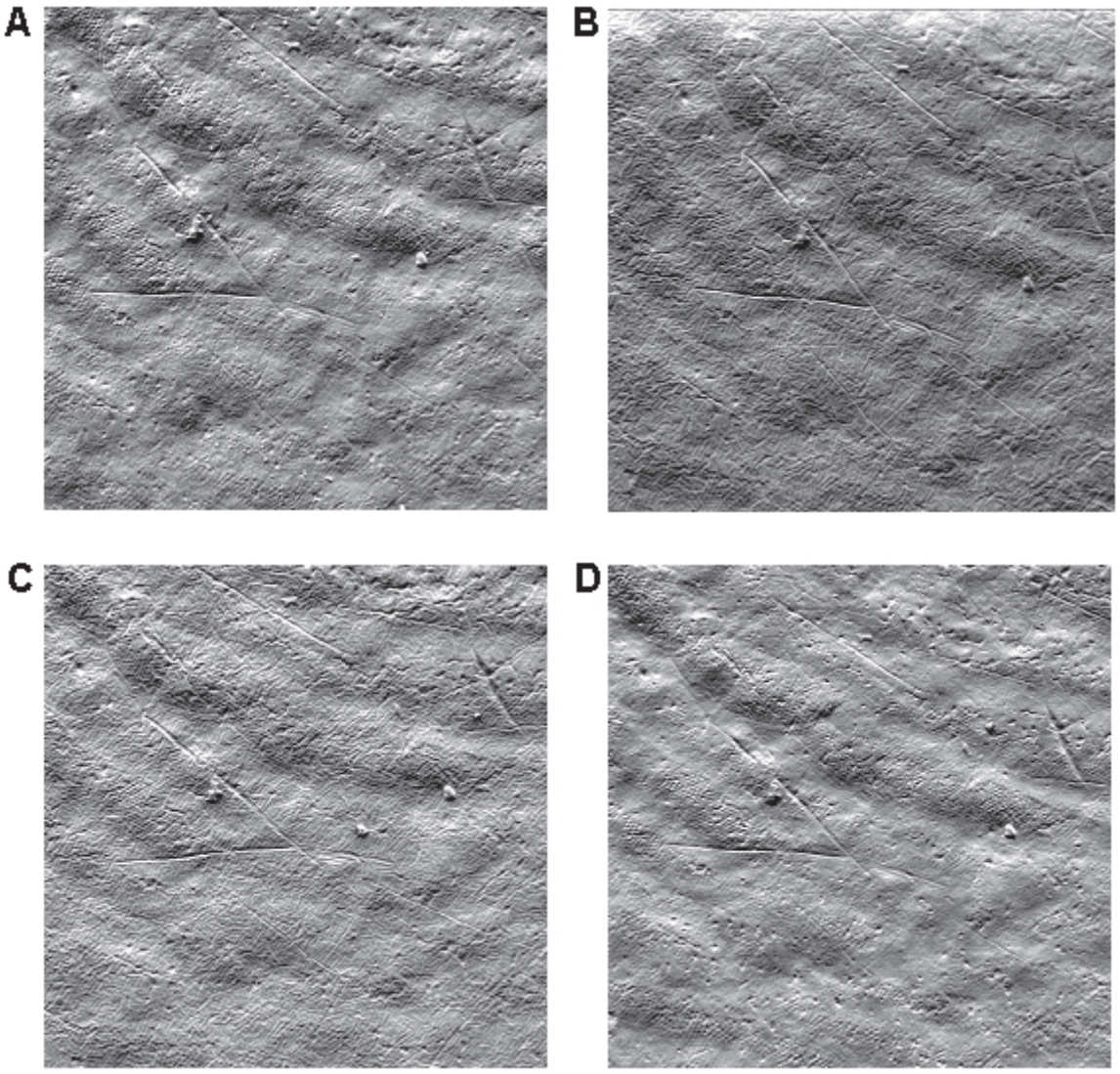

FIGURE 2. Four images of the same enamel surface (buccal surface of lower right second molar belonging to a modern *Homo sapiens* from La Olmeda, Spain ca XII–XV BC), moulded with different *President microSystem*™ (*Coltène*®) polyvinylsiloxane viscosities. A: original tooth surface, B: *Light Body*, C: *Regular Body* and D: *Heavy Body*. All images are at 100x magnification.

have also used Feropur PR-55 (Feroca SL), a fast, bicomponent polyurethane that hardens at room temperature in only five minutes. It has excellent fluidity, it is clean and easy to work with, and only takes some minutes to harden completely. The two components have to be mixed at equal proportions (1:1 in volume) before pouring it inside the negative cast. Centrifugation is recommended for removing air bubbles from the negative mould surfaces. Feropur replicas do not differ in size and form from the original teeth and the cost per cast is significantly lower compared to the epoxy ones.

SEM imaging of enamel surfaces

Before SEM imaging, all teeth were observed at 40× magnification with a VMT Zeys binocular magnifying glass in order to detect well-preserved enamel surfaces on each tooth. Although initially all of the teeth seemed to be in good condition, some of them proved not to be useful because their enamel had extensive microscopic damage. SEM pictures were obtained with two different Scanning Electron Microscopes, a Hitachi 2300 and a Cambridge Stereoscan 120, at the *Serveis Cientifico-Tècnics* (SCT) of the University of Barcelona. In all cases, casts were placed in a horizontal position, with zero degrees of tilt. SEM pictures were taken at 100× magnification on the medial surface of the buccal surface of the tooth, avoiding both the occlusal and cervical thirds of the tooth. This is the standard procedure that we are widely using for microwear analysis on the buccal surface of teeth to infer dietary habits because it covers a significantly broad patch of enamel, where striations of various lengths are the main feature that can be observed (Pérez-Pérez *et al.* 1994). Gordon

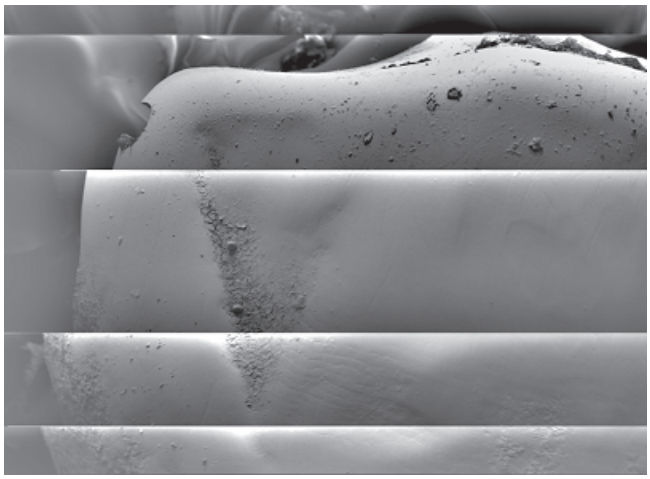

FIGURE 3. SEM image affected by high electrostatic charge caused by an incorrect colloidal argent belt between the stub and the mould, necessary for electron dispersal.

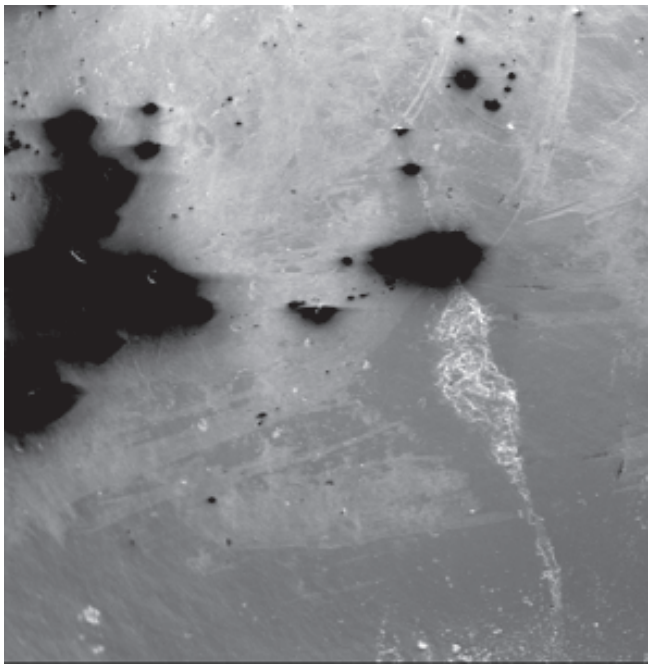

FIGURE 4. The dark areas on the enamel surface are formed by the accumulation of dust on the mould, not allowing for electron dispersal inside the SEM.

(1988) indicated that 120–130× magnification is a good compromise between the covered area and clarity of image. At higher magnifications (i.e. 500×) on the buccal surface of teeth, only a few fragmented fine striations can be distinguished underneath more recent scratches, and the covered area is too small to yield meaningful results (Pérez-Pérez *et al.* 1994). Electron acceleration used was relatively low, 10–15 KV, and working distance (WD) ranged between 15–25 mm, depending on the size of the tooth. SEM pictures were digitalized with SEM Image Slave software, obtaining a 1024×832 pixels image in both the H-2300 and S-120 SEM microscopes used.

Both secondary and back-scattered electrons were used. Back-scattered SEM electrons provide a topographic image in which surface relief is maximized and, therefore, this may be the choice for some morphological studies. Recently, the use of back-scattered electrons has also been advised for SEM microwear analyses on enamel surfaces, since teeth generally show flat surfaces with low relief of microwear features, such as striations, pits, furrows, perikymata, or enamel defects. However, several authors have shown that the use of other than secondary electrons introduces a great methodological error because extinction of linear features occurs when feature orientation parallels that of the SEM electron detectors (Pérez-Pérez *et al.* 2001). An experimental analysis was designed to provide evidence of such feature extinction in two different SEM microscopes (JEOL-840 and Cambridge Stereoscan-120). A metallic disc was gently scratched in a single direction with abrasive glass paper, producing strictly parallel

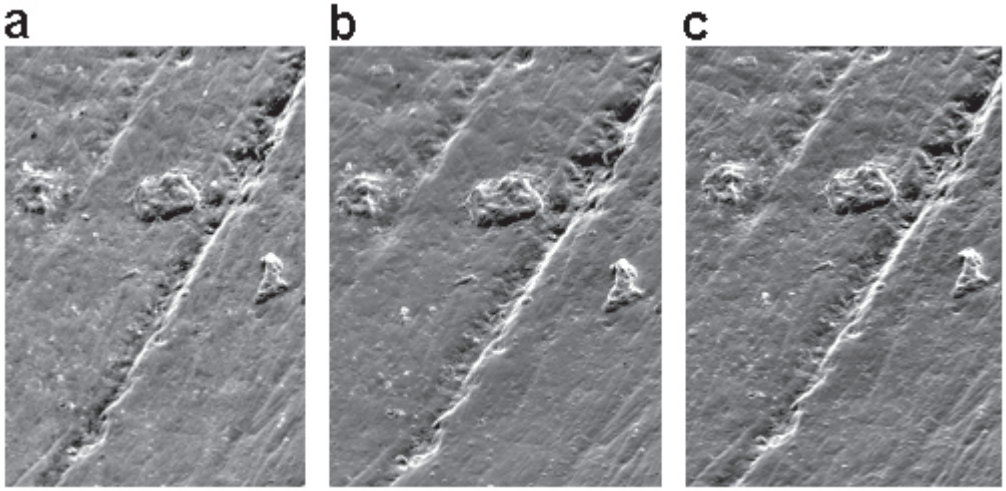

FIGURE 5. Three images of the same enamel surfaces obtained with the original teeth (a), the Epo Tek #301 cast (b), and the Feropur PR-55 cast (c). All images were obtained at 500× magnification.

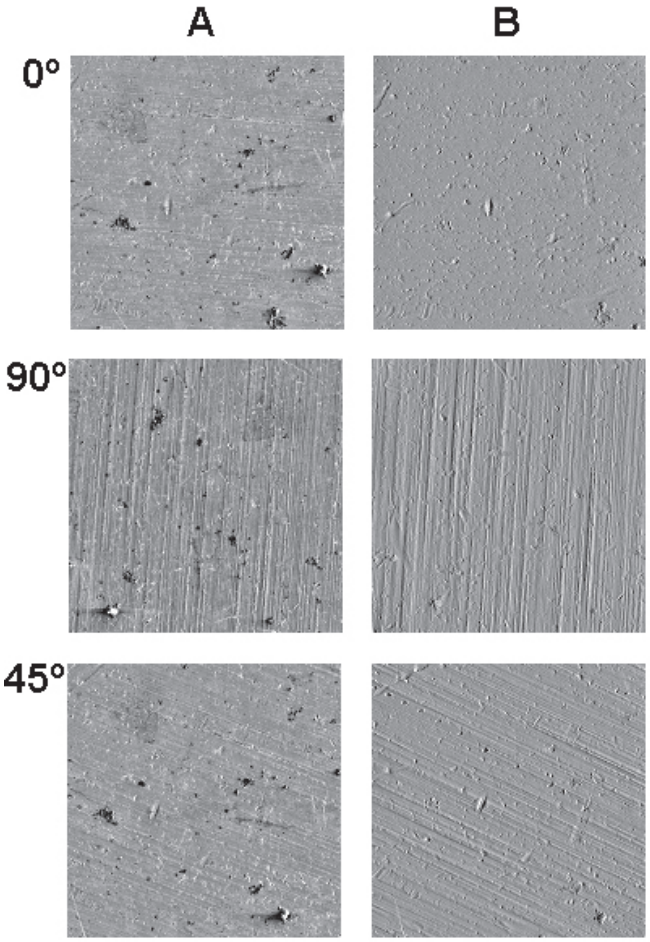

FIGURE 6. Feature extinction experiments made with an artificially parallel-scratched disc. Images were obtained with SEM (JEOL-840) using secondary (A) and back-scattered (B) electrons at different orientation angles (0º, 45º, and 90º) with regard to the electron detector alignment.

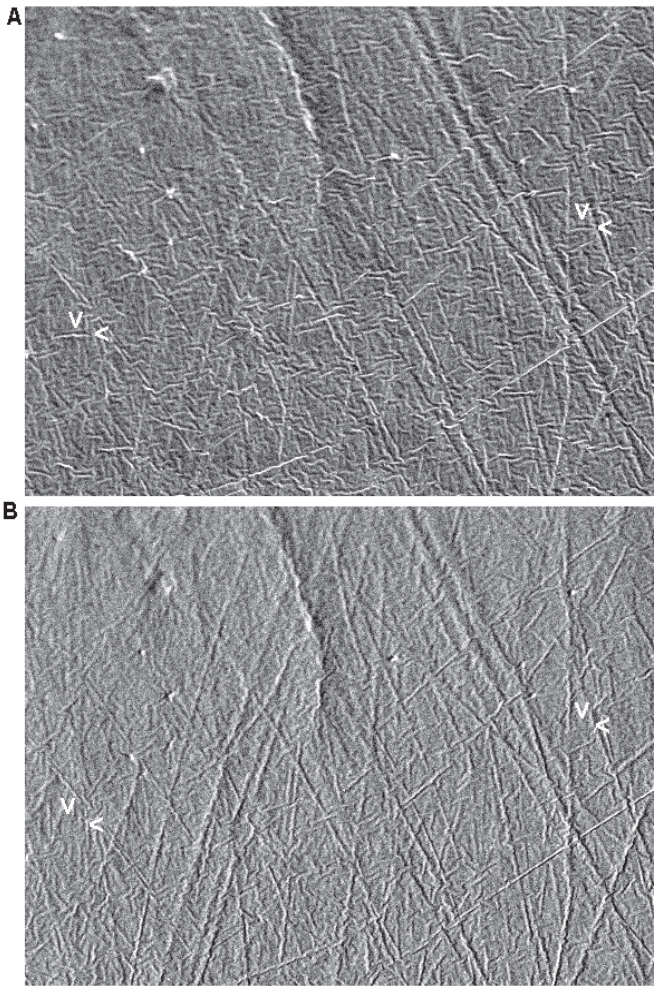

FIGURE 7. SEM images of the same enamel surface obtained with secondary (A) and back-scattered (B) electrons. Some horizontal features are not present in the back-scattered electron image.

striations. The disc was then photographed at 100× magnification at three different angles of orientation (0º, 45º and 90º) with respect to the electron detector plane in the two SEM microscopes, both using secondary and back-scattered electrons. The digitalized images were enhanced with Adobe Photoshop v.5. First of all, the selected area was cropped to include an enamel surface area of 0.56 mm^2. Next, a high-pass filter at 50 pixels was used to remove shade effects in the image and an automatic adjustment of grey levels was applied to increase image contrast. This procedure provides a great contrast for microwear feature measuring. This is usually done with a semi-automatic *software*, usually Microwear 4.0 by P. Ungar or a standard package such as SigmaScan Pro 5.0 by SPSS. The Microwear program automatically discriminates between pits and scratches using a 4:1 length/width ratio. Various length-to-width ratios have been proposed. Teaford and Walker (1984) considered as scratches features above the 10:1 ratio, considering objects with smaller ratio as pits or relatively broad scratches. It is clear that pits and scratches are found at opposite ends of a continuum of surface wear phenomena, and the decision about where to divide the continuum is always arbitrary (Gordon 1988), although pits are generally considered as features having approximately equal length and breath, from 1:1 to 2:1, with no discernible axis of orientation (Gordon 1988).

RESULTS AND DISCUSSION

The comparison of the different polyvinylsiloxane moulds has shown differences in fidelity and replicability of enamel features. SEM images of the original tooth surfaces were always of highest quality (*Figures 1A* and *2A*). Some moulds made with *Light Body* polyvinylsiloxane seemed to more accurately replicate small features, such as enamel prisms (*Figure 1B*). *Regular Body* polyvinylsiloxane proved to be highly reliable, accurately replicating all microwear striations on the enamel surfaces (*Figures 1C* and *2C*). The *Heavy Body* polyvinylsiloxane cast showed

some degree of feature obliteration (*Figure 1D*). The samples were cleaned before starting a SEM session in order to prevent dust accumulation or a lack of metallic contact between the mould and the stub (*Figures 3* and *4*). Additional colloidal argent and gold coating was applied whenever necessary and an air duster was always used prior to SEM observation. No differences in surface fidelity were observed between the original tooth surface observed at 500× and the surfaces replicated with epoxy Epo-Tek #301 (*Figure 5B*) or Feropur PR55 (*Figure 5C*), both made from the *Regular Body* negative. The hardening speed and the reduced cost of Feropur make this material a good alternative to Epoxy resins. For this reason, in order to obtain a quality result, negative moulds should always be taken at least with a polyvinylsiloxane material similar to *President microSystem™ regular body* (*Coltène®*) and then positive moulds be made with a high quality resin, such as Epoxy resins or Feropur PR-55.

The sample was properly oriented inside the SEM, standardizing SEM parameters throughout the whole analysis. 100× magnification is suitable for buccal surface analyses, whereas 200× and 500× are frequently applied to occlusal facets. Scanning Electron Microscopes provide a wide set of image composition possibilities. The most common is the use of secondary electrons, although back-scattered electrons can also be used. The experimental analyses showed that total feature extinction is of major concern for the back-scattered electrons images whenever the major orientation axis of the striations and that of the SEM detector were coincident (*Figure 6*). Less extinction appeared in the secondary electron images, though a somewhat reduced image relief was observed. Some degree of feature extinction was also observed in back-scattered mode for some non-experimental enamel tooth surfaces (*Figure 7*). This effect was, however, less significant since tooth enamel shows more randomly oriented striations, and only those scratch segments with parallel orientations to the detector were affected. Therefore, the use of back-scattered electrons should be avoided in SEM enamel tooth microwear analyses where feature density and length by categories of orientations are to be measured for intra- or inter-population comparisons, despite secondary electron images show somewhat less surface relief.

In future research the use of tooth crown moulds collections (Galbany *et al.* 2004) will increasingly be the first choice since original fossil specimens need to be preserved from handling damage. Accuracy of replication and conservation of moulds will also be required. In addition, methodological standardization of SEM research should also be considered.

AKNOWLEGMENTS

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4.2 La qualitat de les rèpliques

Galbany J, Estebaranz F, Martínez LM, Romero A, De Juan J, Turbón D & Pérez-Pérez A (2006) Comparative analysis of dental enamel polyvinylsiloxane impression and polyurethane casting methods for SEM research.

Microscopy Research and Technique 69(4): 246-252.

L'ús de rèpliques d'alta ressolució, tant per a l'estudi morfològic com per a l'anàlisi del patró de microdesgast dental, està molt estès entre els científics i ha substituït l'estudi de les dents originals (Goodwin & Chaney, 1994), així com altres fòssils (Purnell, 2003). El polivinilsiloxà *President microSystem™* (*Coltène®)* presenta bons resultats de replicació a nivell microscòpic i és molt utilitzat en estudis de microdesgast dental (Ungar, 1996; Galbany et al., 2004a) i alhora presenta un gran estabilitat temporal (Andritsakis & Vlamis, 1986; Beynon, 1987).

Tot i la gran importància dels motlles i les rèpliques en aquests estudis, hi ha poques anàlisis sobre la qualitat dels productes emprats, i tampoc no hi ha estudis on es comparin els diversos materials de replicació existents. Per altra banda, també manca informació de quina és la capacitat d'obtenció de rèpliques a partir d'un mateix motlle negatiu, sense que aquestes no perdin resolució.

Aquest treball s'ha centrat en aquests dos punts. En primer lloc, i després de revisar els estudis existents, s'ha comparat la qualitat de replicació de dos polivinilsiloxans de cases comercials diferents, a partir dels motlles fets amb poliuretà Feropur PR55 (Galbany et al., 2004a). Alhora s'han realitzat sèries de rèpliques successives de poliuretà d'un mateix motlle negatiu per determinar a partir de quina rèplica es comença a perdre la resolució a nivell microscòpic.

Les imatges obtingudes al Microscopi Electrònic d'Escombrat (SEM) indiquen que ambdós polivinilsiloxans utilitzats, *President microSystem™* (*Coltène®*) i *3M Imprint II*, ofereixen una excel·lent replicació de les superfícies d'esmalt a nivell microscòpic (Figura 1) quan s'utilitza la de gra fi, *light body*, tal i com succeïa amb les de gra mig *regular body* (Galbany et al., 2004a). Les rèpliques obtingudes amb polivinilsiloxans de gra gruixut, *heavy body*, presenten diferències en la qualitat de la replicació i mostren menys detall de resolució a ambdues marques comercials.

La observació directa dels negatius de baixa viscositat, *light body*, directament al SEM mostrava imatges amb una bona resolució i estabilitat (Figura 2) i l'absència total de danys produïts per la manipulació dels motlles, tot i que en ocasions s'ha descrit la presència d'esquerdes longitudinals causades per les forces de compressió i tensió (Hillson, 1992). Així mateix, no sembla que

el buit generat a la cambra afecti a l'estabilitat ni a la deformació del motlle de polivinilsiloxà.

En segon lloc, s'han realitzat sèries de 6 rèpliques successives en poliuretà a partir dels mateixos motlles de polivinilsiloxà, obtinguts de dents originals de sèries arqueològiques, per determinar a partir de quina rèplica es comença a perdre resolució de les microestructures observades a la superfície dental original. Totes les rèpliques successives obtingudes presentaven una elevada qualitat fins els 40X augments. Les anàlisis de les imatges obtingudes a 100x augments al SEM indiquen que les quatre primeres rèpliques successives d'un mateix motlle negatiu presenten la mateixa resolució entre elles i igual a la dent original, sense que hi hagi pèrdua d'informació a nivell microscòpic (Figura 3). La pèrdua de resolució a 500X augments també es detecta a partir de la cinquena rèplica successiva a les vores de les microestriacions, que estan menys definides, i la superfície de l'esmalt presenta més rugositat (Figura 4). A 1000X augments la pèrdua de resolució a partir de la cinquena rèplica successiva és evident. Les microestriacions dentals esdevenen més gruixudes i amb vores menys definides, i l'esmalt és rugós i irregular (Figura 5).

Els motlles obtinguts a partir d'originals, doncs, podrien ser utilitzats en diverses ocasions, entre un i quatre cops, fins i tot per part de diferents investigadors. D'aquesta manera s'evitaria una manipulació excessiva de les dents de primats i homínids originals dipositats a les col·leccions.

Comparative Analysis of Dental Enamel Polyvinylsiloxane Impression and Polyurethane Casting Methods for SEM Research

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KEY WORDS dental microwear; polyvinylsiloxane; epoxy; SEM

ABSTRACT Dental casting is a very common procedure for making high-quality replicas of paleo-anthropological remains. Replicas are frequently used, instead of original remains, to study both fossil and extant Primate teeth in morphological and metrical analyses. Several commercial products can be used in molds. This study analyzed SEM image resolution and enamel surface feature definition of tooth molds at various magnification levels and obtained, with both Coltène® and 3M™ low-viscosity body polyvinylsiloxane impression, materials and polyurethane casts. Results, through comparison with the original teeth, show that both the negative molds and the positive casts are highly reliable in replicating enamel surfaces. However, positive cast quality is optimal for SEM observation only till the fourth consecutive replica from the original mold, especially at high SEM magnification levels. *Microsc. Res. Tech 69:246–252, 2006.* © 2006 Wiley-Liss, Inc.

INTRODUCTION

Although teeth constitute the most abundant remains of the paleontological record of *Homininae*, they are not easily accessible to all scientists. As repeated handling and measurement of fossils are among the main causes of deterioration, museum curators tend to limit access to specimens. Wild-caught primate collections, irreplaceable evidence of primate ecology and adaptation (Albrecht, 1982), are more easily accessible to researchers, but tend to suffer from increasing relapses due to the great interest of large-skeleton collection "hunters" (Almquist, 1973). Tooth cast availability offers a solution to this problem.

Although plaster molds of fossil remains are suitable for museum exhibition, high-quality tooth casts are required if replicas need to be studied instead of original specimens. This is especially so if high-resolution electron microscopy procedures are to be applied, as in dental microwear research. Original specimens are too valuable to be studied directly by SEM (Beynon, 1987), particularly when such procedure requires irreversible gold coating. Environmental microscopy (ESEM) can be used for isolated teeth or small specimens whenever this technology is available (Romero et al., 2004). Therefore, high-quality replication of fossil teeth is an effective way of providing the scientific community with access to rare and important specimens (Goodwin and Chaney, 1994), as well as an alternative method of studying microfossils or microscopic details (Purnell, 2003). Museum curators can then loan tooth casts to researchers for their analyses without any risk of further damage to the original specimens.

Science would certainly benefit from the existence of high-quality cast collections, and contesters and colleagues could independently test scientific hypotheses. However, accurate tooth crown molding is difficult (Teaford and Oyen, 1989) and may cause unwanted damage to the original teeth if it is not done properly (Hillson, 2002). High-quality, easy-to-use tooth molding materials are required for optimum and durable results. Silicon-base replication procedures are widely used to obtain negative impressions of original teeth, especially in odontological practice and dental microwear research. Hydrophobic polyvinylsiloxanes, either epoxy-resin or polyurethane replicas, produce high-quality tooth casts (Goodwin and Chaney, 1994) that are very precise replicas of the original specimens (Galbany et al., 2004b) and which provide the detailed resolution necessary in quantitative studies of tooth morphology and wear (Teaford and Oyen, 1989). Dental casts are widely used both for classical morphological/topographic analyses (Dennis et al., 2004; Egocheaga et al., 2004; Jernvall and Selänne, 1999; M'Kirera and Ungar, 2003; Pérez-Pérez et al., 2003a; Ungar, 2004; Ungar et al., 2001; Ungar and Williamson, 2000; Zuccotti et al., 1998) and in dental microwear research (Galbany et al., 2002; Galbany and Pérez-Pérez, 2004; Gordon, 1982, 1984a; Grine, 1986; Hojo, 1991, 2002; Martínez et al., 2004; Pérez-Pérez et al., 1994, 1999, 2003b; Puech et al., 1983; Ryan, 1979a,b; Teaford,

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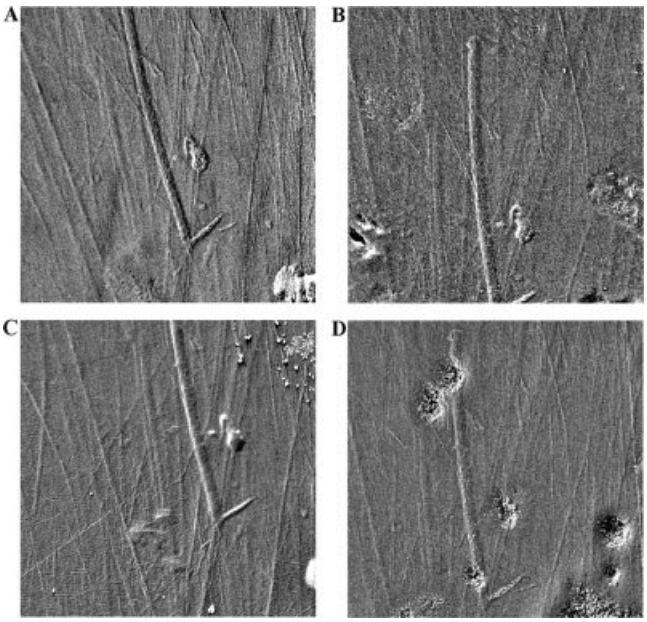

Fig. 1. SEM images at 100× of the buccal surface of the left M_3 tooth from Cueva del Molinico, obtained with various polyvinylsiloxane impression materials: (**A**) 3M Imprint II Light Body; (**B**) 3M Imprint II Heavy Body; (**C**) Coltène President Plus Jet Light Body; and (**D**) Coltène President Plus Jet Heavy Body. The SEM working distance ranged between 13.3 and 22.7 mm. The heavy-body molds show reduced detail resolution, which affects molding reliability.

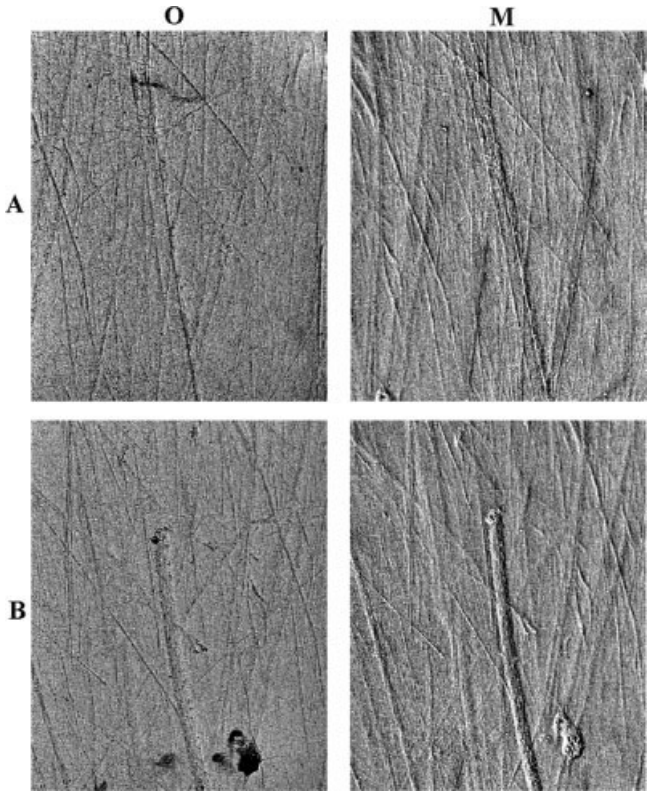

Fig. 2. 100× SEM images of the buccal enamel surface of a left M_3 tooth from Cueva del Molinico, with comparison of original surfaces and silicon-base molds. O, SEM images obtained from original tooth surfaces with BSE in an ESEM; M, images obtained from silicon-base molds with SE. Molds were obtained with 3M Imprint II Regular Body (**A**) and Coltène President Plus Jet (**B**). Regular body molds compared enamel replicas with molds of the original tooth surface and found them highly reliable.

1985, 1988a,b; Teaford and Glander, 1991; Teaford and Oyen, 1989; Teaford and Ungar, 2000; Ungar, 1992, 1998; Ungar et al., 1995, 2004; Ungar and Grine, 1991). Independent molding of dental specimens by each research group is the norm in such studies, but there are serious difficulties in the sharing of casts. Most of these difficulties, such as the useful life of the impression materials, the reliability of successive impressions from the same mold, the accuracy of different replicating materials for SEM research, the standardization of molding procedures for a variety of purposes, or researchers' diverse interests, have never been thoroughly analyzed.

President microSystem™ (Coltène®) polyvinylsiloxane impression material is widely used among dental researchers (Ungar, 1996) because it has excellent dimensional stability and reproduction detail (Andritsakis and Vlamis, 1986) and replicates surface features with resolutions to a fraction of a micron (Teaford and Oyen, 1989). Negative molds made of polyvinylsiloxane maintain SEM resolution for many years (Beynon, 1987), with over 99.5% recovery after deformation (Coltène, 2002). Nevertheless, the ability to make several accurate casts from a single mold (Gordon, 1982) is what determines the usefulness of curating polyvinylsiloxane collections of tooth casts that are easily accessible to researchers, along with the original specimens. Some research has been done in this regard by comparing the reliability of different impression materials (either 3M™ or Coltène, among the most common ones). Beynon (1987) used Coltène Putty to build a primary base holder where Coltène President Light Body was poured to obtain the final negative impression. Hillson (1992) indicated that application of Light Body material directly on the crown sufficed for accurate surface replication, simplifying the molding technique and preventing possible changes in surface details. However, Hillson (1992) pointed out the difficulties in examining negative molds directly under SEM, i.e., negative molds tend to crack when pouring out the impressions, they are difficult to attach to the stub and to coat with gold-palladium, and linear enamel features are difficult to observe on them (Hillson, 1992). Although some observations indicate that the flexibility and porosity of negative silicon-base molds tend to cause mold shrinking under SEM vacuum (Galbany et al., 2004a,b), silicon negatives are adequate for SEM characterization of linear enamel hypoplasia on tooth surfaces (Guatelli-Steinberg, 2004; Guatelli-Steinberg and Mitchell, 2002). Positive casting from negative molds might be a more stable and lasting solution, though little has been published yet on the accuracy and reliability of casting materials. This study focuses on some of the methodological drawbacks of tooth cast sharing among researchers and institutions, such as the accuracy of SEM research on tooth replicas as against origi-

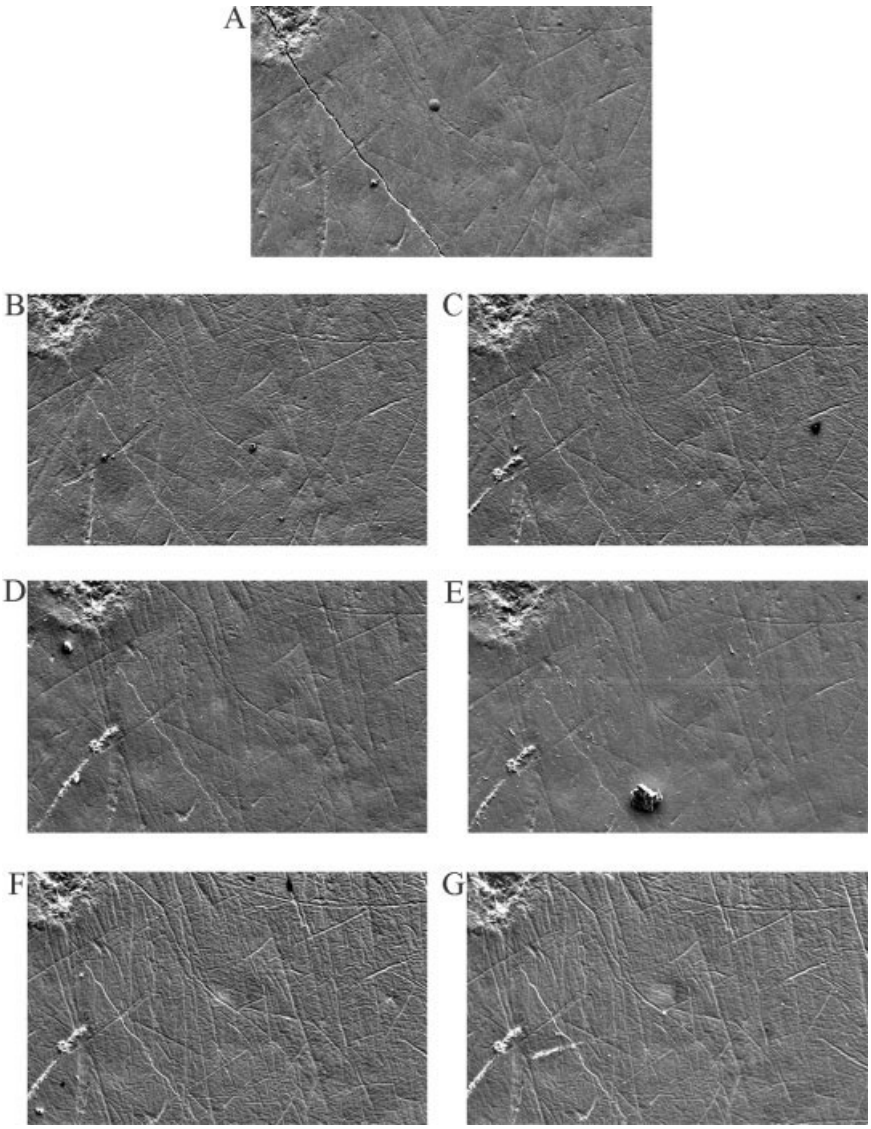

Fig. 3. SEM images at 100× magnification of the same buccal enamel surface of a left M^1 tooth replica from La Olmeda. The mold was obtained with President microSystem (Coltène) polyvinylsiloxane Regular Body. (**A**) Original teeth. (**B**) First replica. (**C**) Second replica. (**D**) Third replica. (**E**) Fourth replica. (**F**) Fifth replica. (**G**) Sixth replica. No differences in surface replication were found between the original tooth surface and the first four replicas obtained.

nal teeth, the reliability of consecutive casting obtaining several replicas from a single mold, and the useful life of tooth molds and casts.

MATERIALS AND METHODS

Four isolated human teeth (an upper left first molar LM^1, a lower right second incisor RI_2, a lower left third molar LM_3, and a lower left canine LC_1) from the medieval site of La Olmeda (Palencia, Spain) c. XII-XVII AD (Pérez-Pérez et al., 1991), and one isolated lower left first molar (LM_1) from Cueva del Molinico (Villena, Alicante, Spain), dating back to the IIIrd millennium BC (Soler García, 1993), were selected for analysis. Original teeth, as well as tooth molds and casts, were studied. Most negative tooth molds were obtained by using Regular Body President microSystem polyvinylsiloxane, since this impression material is widely used in dental studies. For comparative purposes, some molds were also obtained with President Light and Heavy Body bases, as well as with 3M ESPE Imprint II™ polyvinylsiloxane. Tooth-crown molding procedures followed brand indications in all cases and are also described in detail elsewhere (Galbany et al., 2004a, 2005; Pérez-Pérez et al., 1999).

Preparation and Analysis of Negative Molds

Negative impressions of the buccal enamel surface of the lower first molar from Cueva del Molinico were obtained by using both polyvinylsiloxane impression brands (3M® and Coltène). The buccal tooth surface was cleaned with cotton swabs soaked in pure acetone, was then rinsed in 95% ethanol, and was finally air-dried. Two negative impressions were obtained with each silicon brand, and the enamel surface was cleaned again prior to obtaining the second impression. Silicone molds were stuck to brass discs with plastic carbon cement (Leit-C-Plast, Plastic Conductive Carbon Cement, Electron Microscopy Sciences, Washington, PA) and sputter-coated with ~15 nm of gold-palladium. A

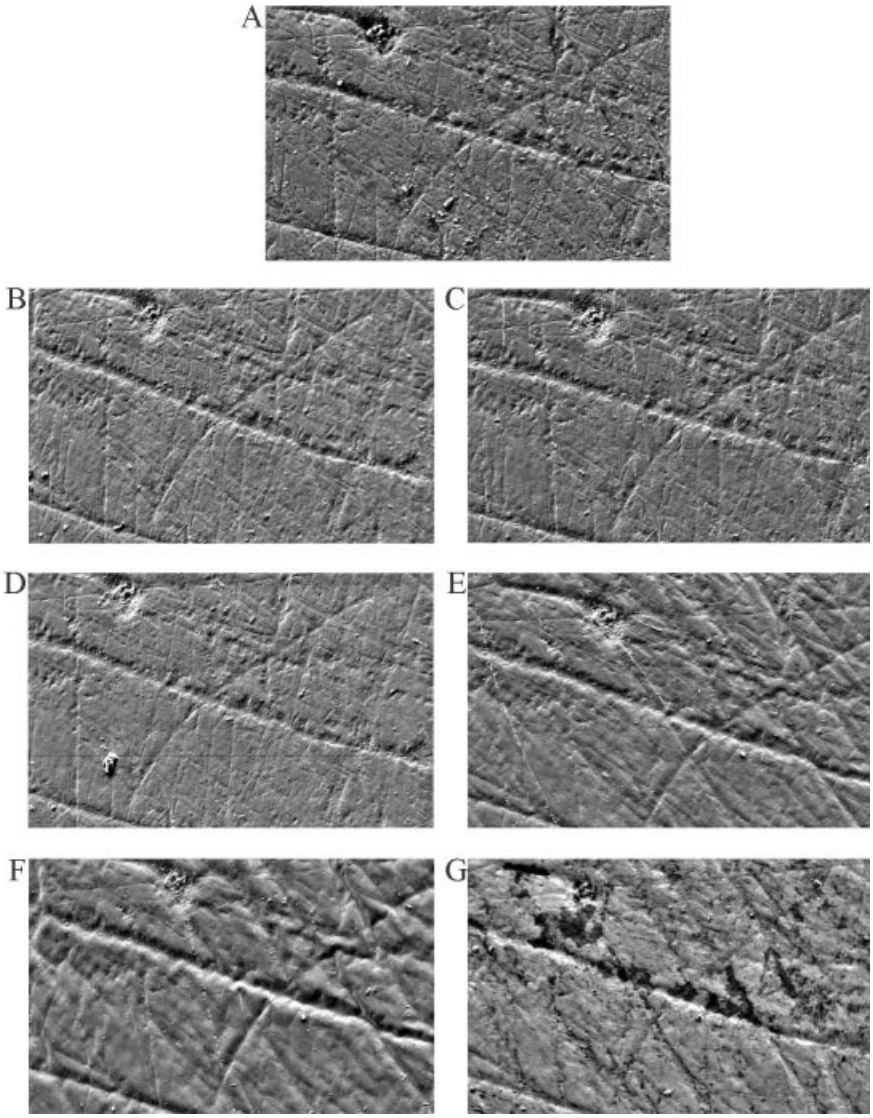

Fig. 4. SEM images at 500× magnification of the same buccal enamel surface of a right I_2 tooth replica from La Olmeda. The mold was obtained by President microSystem (Coltène) polyvinylsiloxane Regular Body. (**A**) Original teeth. (**B**) First replica. (**C**) Second replica. (**D**) Third replica. (**E**) Fourth replica. (**F**) Fifth replica. (**G**) Sixth replica. As in Figure 3, decay in surface resolution was found from the fourth successive replica obtained.

colloidal silver solution (Silver Conductive Adhesive 416, Electron Microscopy Science) was applied to improve conductivity and the preparations were mounted on SEM aluminum stubs. The original tooth and the molds were examined with a Hitachi S3000N SEM. The buccal enamel surface of the original tooth was observed without gold layer coating, using back-scattered electrons (BSE) at 20 kV accelerating voltage in a low-vacuum, variable-pressure mode (Romero et al., 2004), whereas the tooth impressions were sputter-coated with 15 nm of gold-palladium and observed using secondary electrons (standard error (SE)) and 15 kV accelerating voltage that recorded SEM images at the same location areas and with identical resolution as for the original teeth. All enamel surfaces were placed perpendicular to the electron beam and SEM images (1,280 × 960 pixels resolution) were obtained at 100× magnification on the medial third of the buccal surface under the protoconid cusp.

Preparation and Analysis of Positive Replicas

Negative molds of buccal surfaces were obtained for the four teeth from the La Olmeda site. Only Regular Body President microSystem impression material was used, and six consecutive high-quality polyurethane positive casts were obtained for each mold with Feropur power ratio (PR)-55 (Feroca SL, Spain). A total of 24 casts were obtained and mounted on a brass disc with fusible glue (Ceys™). A colloidal argent belt (Electrodag 1415M-Acheson Colloiden) solution was applied to prevent electrostatic charges from accumulating during the SEM observation (Rose, 1983). Finally, the samples were sputter-coated with a gold layer and kept clean, dry, and dark inside a dust-free collection cabinet. Three SEM pictures at 100×, 500×, and 1000× magnifications were obtained for each cast with a Cambridge Stereoscan 120 SEM. In all cases, casts were placed in a horizontal position, with zero degree of tilt, 12 kV acceleration voltage, and 10–20 mm

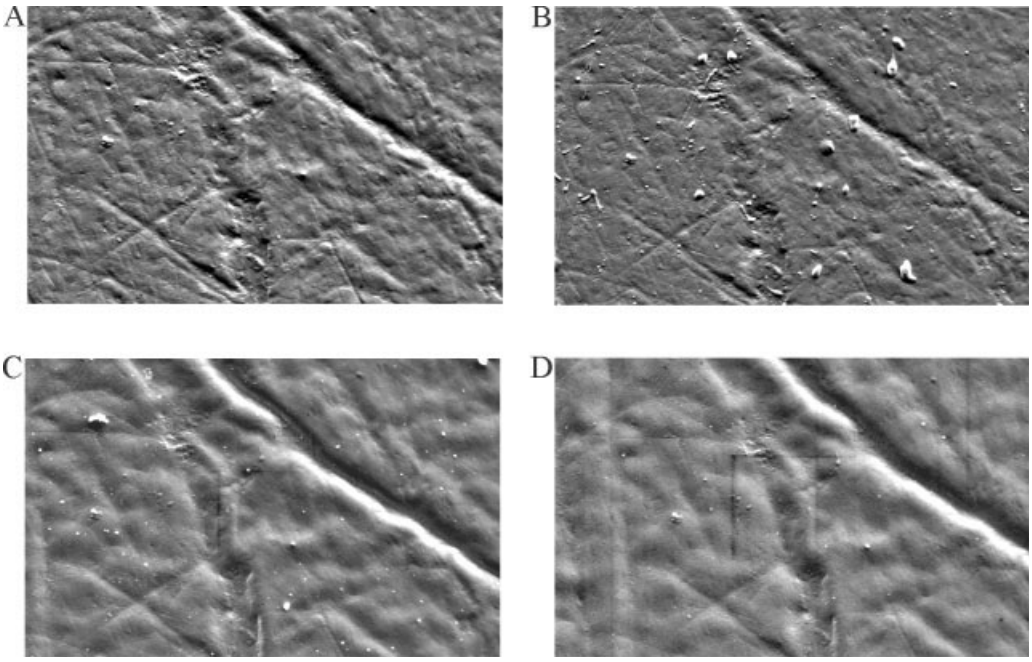

Fig. 5. SEM images at 1000× magnification of the same buccal enamel surface of a left M^1 tooth replica from La Olmeda. The mold was obtained with President microSystem (Coltène) polyvinylsiloxane Regular Body. (**A**) Third replica. (**B**) Fourth replica. (**C**) Fifth replica. (**D**) Sixth replica. The first two replicas of the original tooth are not included, since no differences in cast resolution were found until the fifth replica was studied.

working distance. Digital images (1024 × 832 pixel resolution) were obtained with the SEM Image Slave software. Prior to analysis, all images were enhanced with Adobe Photoshop™ v 7.0 using a 50-pixel, high-pass filter and automatic gray-level adjustment (Galbany et al., 2004a, 2005).

RESULTS

Some differences in surface quality were observed between high- and low-viscosity 3M materials (Fig. 1). Areas of low casting resolution tended to form when high-viscosity (Heavy Body) materials were used (Figs. 1B and 1D), which gave lower detail resolution than when low-viscosity materials (Light and Regular Body) were used (Figs. 1A and 1C).

However, direct observation of the low-viscosity, negative polyvinylsiloxane impressions under SEM showed no damage caused by handling or by anything methodologically related to handling. Negative molds offered good stability and image resolution (SE mode), when compared to the actual enamel surfaces (BSE mode), for the two impression materials used (Fig. 2). Although longitudinal cracks or stress features may form if pressure is applied to the negative molds after coating (Hillson, 1992), vacuum in SEM chamber during observation seems not to have affected the mold stability or shape in this case.

Coltène polyvinylsiloxane Regular Body impression materials always produced high-quality tooth molds of enamel surfaces, and the positive epoxy tooth casts obtained from them showed good feature resolution when observed at 10× to 40× magnifications, with a VMT binocular magnifying glass. SEM observation of these dental casts at 100× magnification yielded great image fidelity of both the original tooth surfaces (Fig. 3A) and the first four consecutive replicas. Comparisons were made at the same enamel spot (Figs. 3B–3E). However, the fifth (Fig. 3F) and sixth (Fig. 3G) replicas showed a noticeable reduction in surface detail and some degree of background noise due to mold deterioration after repeated casting. At 500× magnification, loss of resolution in replicating enamel surfaces is evident from the fourth replica (Figs. 4E–4G), where striation borders appear increasingly less defined and enamel surface roughness is increasingly evident. Finally, micrographs of dental casts obtained at 1000× magnification showed a loss in resolution from the fifth replica onwards (Fig. 5).

DISCUSSION

The comparisons of enamel resolution details in all the SEM images obtained clearly show that low-viscosity silicones are excellent materials for making impressions of buccal tooth surfaces (bubbles tend to appear only when heavy bodies are used), even though the viscosity of the replicating material may vary, depending on the surface to which it is applied (Chee and Donovan, 1992) or the shearing forces placed on it (Mandikos, 1998).

Although obtaining impressions with silicone materials may not always be a predictable process (Gordon, 1984b), in the present study all the low-viscosity, Regular Body polyvinylsiloxane dental molds provided high-quality casts for SEM analysis up to at least the fourth consecutive replica obtained from the same mold, at 100×, 500×, and 1000× magnification. From the fifth cast on, feature definition decreased in all casts: tooth

striations became thicker, with less defined borders, and enamel surfaces appeared rough and irregular. Feature modification after several consecutive casts may be caused by pull-out forces affecting negative molds when the positive cast is removed. Microwear analyses, essentially based on statistical methods, are greatly affected by the methodological approach used (Gordon, 1988) and extra-casting from a single mold needs to be carefully considered. Only a few, most probably from 1 to 4, good-quality casts can be obtained from the same original mold, with enough fidelity to replace original specimens in SEM research. Thus, molds themselves should be considered valuable specimens for use instead of original teeth. Some precautions need to be taken during cast preparation and handling, such as not using latex gloves with sulfur compounds (Browning et al., 1993) or hand lotions (Goodwin and Chaney, 1994) that inhibit polyvinylsiloxane setting. However, if they are properly curated, successive positive casts are a reliable alternative to original specimen analysis, since SEM feature resolution after consecutive casting is independent of magnification level, at least up to 1000×. Cast deterioration shows up after the fourth replica at all magnifications.

In conclusion, SEM observation of tooth enamel surfaces at various magnification levels indicates that both negative, low-viscosity polyvinylsiloxane molds and positive epoxy casts can be used for SEM research instead of original specimens. In addition, at least four consecutive positive replicas can be obtained from a single mold, ensuring that several researchers can study a tooth's morphology without loss of detail resolution.

It would be of general benefit to researchers to produce digital 3D models of tooth crowns from valuable collections. This would give permanent access to virtual tooth replicas without any need of the original tooth. It is not clear yet whether such "replicas" could be used in all kinds of research. SEM analyses of both negative and positive replicas are reliable at resolutions lower than a fraction of a micron, whereas 3D surface topography shows resolution levels between 0.05 mm (mesh-point height-sensing 3D scanner) and 0.2 mm (laser 3D scanning). Unfortunately, as microcomputer tomography 3D models (MCT) are still difficult and expensive to obtain, 3D digital replicas of tooth crown surfaces cannot yet replace high-quality tooth casts in SEM research. Detailed analyses of MCT 3D models are still needed to determine whether they can be used instead of original teeth, replacing traditional high-resolution SEM microwear research using tooth casts. In addition, automatic procedures for dental microwear research, based on roughness and anisotropy measurements of surface topography, still need to be proved significant in determining dietary-related behavior in Primates rather than postmortem enamel abrasion processes (Estebaranz et al., 2005).

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4.3 La mesura i l'error associat

Galbany J, Martínez LM, López-Amor HM, Espurz V, Hiraldo O, Romero A, De Juan J & Pérez-Pérez A (2005) Error rates in dental buccal microwear quantification using Scanning Electron Microscopy.

Scanning 27: 23-29.

L'anàlisi del microdesgast dental mitjançant la utilització del Microscopi Electrònic d'Escombrat (SEM) és un bon indicador de l'abrasió de la dieta i les adaptacions ecològiques dels primats actuals i fòssils, incloent-hi els homínids (Grine 1981; Puech & Albertini 1984; Teaford & Oyen 1989a; Ungar, 1996, 1998; Daegling & Grine 1999; M'Kirera & Ungar 2003).

Els electrons secundaris ofereixen imatges excel·lents dels patrons de microestriació dental que es poden analitzar quantitativament i permeten l'estudi de la seva variabilitat. La mesura de les microestries es realitza de forma semi-automàtica mitjançant la definició de dos punts de cada microestriació (punts inicial i final) que defineixen la longitud total, amb el programari *Sigma Scan Pro 5*, i s'obtenen dades de la longitud i orientació de cada microestriació, així com el nombre total a cada imatge analitzada. El recompte i mesura de les microestriacions està associat a un error de mesura inevitable (Grine et al., 2002) que depèn de molts factors intrínsecs de la imatge, com ara la intensitat de llum de les imatges SEM, el seu focus o la superposició dels trets microscòpics a mesurar, i també altres factors relacionats amb l'observador, com la seva experiència, concentració i fatiga o les condicions ambientals durant la mesura (llum, temperatura, etc.). La mesura del microdesgast dental estarà sotmesa a totes aquestes variables fins que no es desenvolupi un sistema de mesura automàtic, ben estandarditzat metodològicament, per minimitzar l'error de mesura (Grine et al., 2002).

A partir d'imatges del patró de microdesgast dental oclusal, Grine et al. (2002) van realitzar un estudi per determinar la taxa d'error de mesura, tant d'un mateix observador com entre observadors. Aquest capítol analitza quin és l'error associat a la mesura de les microestriacions de la superfície bucal, tant en longitud com en nombre, per tal de comparar-ne els resultats i determinar l'abast de l'error de mesura d'aquesta metodologia i possibilitar l'implementació de solucions per minimitzar-lo. S'ha considerat l'error intraobservador i l'error interobservador.

S'han mesurat les microestriacions de quatre micrografies SEM de l'esmalt dental tant de primats com d'homínids fòssils (Figura 1), per part de set investigadors que pertanyen a dos grups de recerca independents. Entre els investigadors, n'hi ha d'experimentats i d'altres de novells.

Els resultats de l'error de mesura intraobservador calculat com la diferència en percentatge entre les medicions repetides per un sol observador (MAPD), obtinguda seguint la metodologia de mesura i anàlisi descrita per Grine et al. (2002), mostren errors de mesura d'entre el 4.26% i el 15.33%, pel que fa al nombre d'estries comptades en el total d'imatges analitzades, i entre 3,63% i 19,41% d'error per la llargada de les microestriacions (Figura 2 i Taula 1). Els errors de mesura més elevats apareixen en aquells observadors menys experimentats o en els de grups de recerca separats amb els que no hi ha un contacte directe continuat, probablement perquè segueixen tècniques de mesura diferents. En ambdós casos, doncs, hi ha hagut menys possibilitats de consensuar una estandardització metodològica. Els resultats també indiquen que aquells investigadors que tenen, com a mínim, tres anys d'experiència en el grup de recerca, presenten errors inferiors al 6%, tal i com succeeix en l'estudi de Grine et al (2002).

Pel que fa l'error entre els diferents observadors, tot i el gran esforç d'estandardització i consens previ, presenta valors més elevats que l'error intraobservador si considerem totes les variables de totes les imatges mesurades (Taula 2). Aquestes diferències també es poden apreciar al gràfic de dispersió de la variable NT (nombre total d'estries) i XT (longitud promig del nombre total d'estries) per tots els investigadors, imatges i rèpliques analitzades (Figura 3).

L'anàlisi del patró de microestriació dental a partir de les mesures manuals comporta un error inevitable. En observadors experimentats aquest error suposa 9 microestriacions i 10 μm de longitud promig a cada imatge. És per aquesta raó que hauria de ser considerat, sobretot quan es comparen mesures de patrons de microestriació de diversos investigadors.

La minimització de l'error, però, és possible. Una primera alternativa consisteix en que el mateix investigador mesuri totes les microestriacions d'un mateix treball. Cal una millor estandardització sobre la metodologia de mesura entre els investigadors. Finalment, el desenvolupament de noves metodologies quantitatives automàtiques per a la mesura del patró de microdesgast dental podrien evitar o minimitzar els errors. Aquesta conclusió final coincideix plenament amb el treball de Grine et al. (2002).

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Error Rates in Buccal-Dental Microwear Quantification Using Scanning Electron Microscopy

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Summary: Dental microwear, usually analyzed using scanning electron microscopy (SEM) techniques, is a good indicator of the abrasive potential of past human population diets. Scanning electron microscopy secondary electrons provide excellent images of dental enamel relief for characterizing striation density, average length, and orientation. However, methodological standardization is required for interobserver comparisons since semiautomatic counting procedures are still used for micrograph characterization. The analysis of normally distributed variables allows the characterization of small interpopulation differences. However, the interobserver error rates associated with SEM experience and the degree of expertise in measuring striations are critical to population dietary interpretation. The interobserver comparisons made here clearly indicate that the precision of SEM buccal microwear measurements depends heavily on variable definition and the researcher's expertise. Moreover, error rates are not the only concern for dental microwear research. Low error rates do not guarantee that all researchers are measuring the same magnitudes of the variables considered. The results obtained show that researchers tend to maintain high intrapopulation homogeneity and low measurement error rates, whereas significant interobserver differences appear. Such differences are due to a differential interpretation of SEM microwear features and variable definitions that require detailed and precise agreement among researchers. The substitution of semiautomatic with fully automated procedures will completely avoid interobserver error rate differences.

Key words: dental microwear, error rates, scanning electron microscopy, quantification

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Introduction

Phytolyths are abundant not only in plant foods, such as leaves, shoots, fruits, or medullas, but also in dust and ashes that can be incorporated into food items during food handling and processing. The siliceous nature of phytolyths means that they are able to produce microscopic damage, in the form of scratches and pits, on the enamel surfaces of teeth during food chewing. Such damage can be observed using scanning electron microscopy (SEM), and the analysis of dental microwear patterns can be correlated to food consumption and dietary habits (Teaford 1994). Dental microwear research has proved to be a good indicator of the ecological adaptations of extant and extinct primates, including fossil Hominin species, both on occlusal tooth surfaces (Daegling and Grine 1999, Dennis *et al.* 2004, Grine 1981, 1986; M'Kirera and Ungar 2003; Teaford 1985, 1994; Teaford and Oyen 1989; Teaford *et al.* 1996; Ungar 1990, 1992, 1996, 1998; Ungar and Kay 1995; Ungar and Spencer 1999; Ungar and Williamson 2000) and on buccal ones (Galbany and Pérez-Pérez 2004, Lalueza and Pérez-Pérez 1993, Pérez-Pérez *et al.* 1994, 1999; Puech 1981, 1984; Puech and Albertini 1984; Puech *et al.* 1983, 1989). However, the semi-automatic procedures (Pérez-Pérez 1999, Ungar 1995) most frequently employed in counting and measuring microwear features (i.e., pit and scratch widths and lengths) are accompanied by unavoidable interobserver error rates (Grine *et al.* 2002) which depend heavily on a number of factors: SEM brightness and focus, precision in variable definition, the overlap of microwear features, the researcher's expertise and fatigue during the analysis, and observation conditions (room lighting, temperature, quietness, etc.). Until highly automated microwear measuring procedures are developed (Grine *et al.* 2002), detailed analyses of intra- and interobserved error are required for methodological standardization and reliability. Recently, error rate estimations were provided for occlusal microwear analyses (Grine *et al.* 2002), showing that both intra- and interobserver errors should not be neglected and that a certain bias can be as-

sociated with the semiautomatic characterization of microwear patterns on teeth. The present paper seeks to determine the magnitude of error rates associated with buccal microwear analyses in order to compare them with those reported for occlusal tooth surfaces. The methodological procedures for characterizing buccal and occlusal microwear are significantly different. Occlusal microwear research requires characterization of both pits—of various shapes and sizes, and frequently overlapping—and scratches, usually at 500× magnification; in contrast, buccal microwear analysis involves the characterization at 100× magnification of striations only, since no pits are observed on the buccal surfaces of teeth. The typical field width of a 100× image is 1196×972 μm, whereas the 500× image field is much narrower, 240×196 μm.

The analysis of both occlusal and buccal microwear error rates is relevant for the methodological standardization of microwear measuring techniques and for making interobserver comparisons of dental microwear research.

Material and Methods

Four different SEM micrographs of buccal-dental enamel surfaces were selected from the collection of primate and hominid photographs previously obtained by our research group (Galbany *et al.* 2004a). None of the seven researchers involved knew in advance which specimen or species was being analyzed. Two of the selected SEM images belonged to a baboon (*Papio anubis*), a Cercopithecoidea primate, and both were lower left second molars (LM_2) of adult females from the National Museums of Kenya (NMK om6992 and om7288). The other two SEM micrographs were obtained from *Hominidae* teeth: an upper left first molar (LM^1) of OH-13, assigned to an immature female hominin of *Homo habilis* from Olduvai (Tobias 1991), and a lower right third premolar (RPm_3) of LH-4, assigned to a hominin of *Australopithecus afarensis* from Laetoli (White 1978) (Fig. 1). The dental casts of the analyzed teeth were obtained from the original museum collection specimens using the regular-body polyvinylsiloxane President MicroSystem™ (Coltène® AG, Altstätten, Switzerland). Positive casts were made using the epoxy resin Epo-Tek #301 (QdA). The tooth replicas were mounted on aluminium stubs and a colloidal argent belt (Electrodag 1415M, Acheson Colloiden Co., Ontario, Calif., USA) solution was applied to allow electron dispersal and prevent the accumulation of electrostatic charges during SEM observation (Rose 1983). Finally, the samples were sputtercoated with a thin, nonobliterating 400 Å gold layer to allow observation by SEM.

All SEM images were obtained at 100× magnification on the middle third of well-preserved buccal surfaces of tooth crowns, avoiding the occlusal and cervical thirds, and using secondary electrons in a Cambridge Stereoscan S-120 scanning electron microscope. The electron acceleration used was relatively low, around 10–15 kV, and each image was obtained at 72 ppi digitalization resolution with the Image Slave software, 1024×832 pixel images being obtained (Galbany *et al*. 2004b). Each SEM micrograph was cut off to include a 0.56 mm^2 square surface area (748.33 μm of field width), in which scratches were counted manually following standard methodological procedures for buccal microwear research (Galbany *et al.* 2004b, Pérez-Pérez *et al.* 1999). Microwear features were quantified with the Sigma Scan Pro V Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill., USA). All objects longer than 15 μm and with a minimum length-to-breadth ratio of 3:1 on the enamel surface of teeth were measured without considering curvature. Objects with smaller length-to-breadth ratios were considered as pits and were not counted. Each of the four selected images was characterized four times by each of the seven researchers, who showed various degrees of expertise in measuring buccal microwear: five of them had more than 3 years of experience (R1 to R5), one was a fairly inexperienced researcher (R6), and one (R7) had some experience using Ungar's Microwear software (Ungar 2001). Six researchers (R1 to R6) used SigmaScan Pro 5.0 by SPSS for microwear feature characterization, while researcher R7 used Ungar's Microwear software. Each image was measured only once by a single researcher in the same measuring session, and a minimum

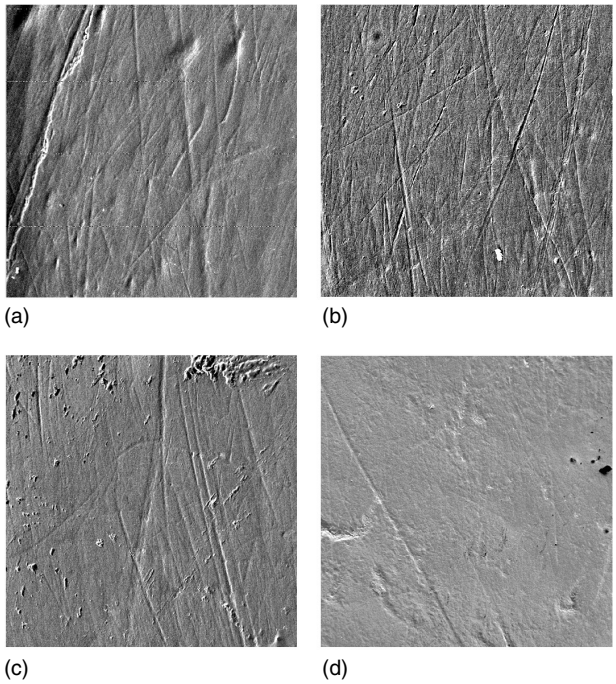

FIG. 1 Micrographs repeatedly measured in the intra- and interobserver error analysis, belonging to (a) *Australopithecus afarensis* hominin from Laetoli–LH-4 (513×513 pixels); (b) *Papio anubis* (baboon) from National Museums of Kenya—om6962 (600×600 pixels); (c) *Papio anubis* (baboon) from National Museums of Kenya—om7288 (600×600 pixels); and (d) *Homo habilis* hominin from Olduvai—OH-13 (513×513 pixels). All four analyzed images included a 0.56 mm^2 square area with a field width of 748.33 μm.

delay of 2 days was required for repeating the characterization of an image. Thus, each observer counted scratches on a total of 16 images and invested a minimum of 8 days for completing the measurements.

All researchers were required to distinguish between natural, antemortem microwear and postmortem enamel damage based on previous knowledge of buccal microwear research (Martínez *et al.* 2001). All observed scratches on the enamel surface >15 μm were measured by defining their initial and final points and without considering curvature. Scratch lengths were then automatically recorded and striations shorter than 15 μm were removed from the database before measures of scratch density and average length (in μm) were derived. All statistical analyses of intra- and interobserver error rates were made with the SPSS v.11 statistical package.

Results

In the first instance, the interobserver error rates were computed as the mean absolute percentage difference (MAPD), as described in Grine *et al.* (2002). The error rate values obtained for the seven researchers, measuring four images four times, ranged between 4.26 (R2) and 15.33% (R6) for the density of striations on the buccal surfaces, and between 3.63 (R2) and 19.41% (R7) for the average length of the striations. Grine *et al.* (2002), analyzing four replicas of two micrographs by one researcher, report MAPD values of 4.1 and 12.9% for the density of scratches on occlusal surfaces, and 4.6 and 7.0% for the length of scratches, similar to those found in the present study.

However, the MAPD is not a precise measure of the dispersion of repeated measurements. Rather, the standard error of the repetitions (Jamison and Zegura 1974, Page 1976, Sokal 1995, Utermohle and Zegura 1982) may better reflect a researcher's reliability in characterizing metric features (Pérez-Pérez *et al.* 1990). Thus, the standard errors of the repeated measurements were computed. Table I shows the average density and length of scratches, along with their standard error (e_x) and variance (V_x), for each analyzed micrograph (M1, M2, M3, M4) and by researcher (R1 through R7). The mean standard error and variance values range, respectively, from 3.52 striations and 2.77% (R2) to 16.01 striations (R7) and 10.21% (R6) for striation density; and from 3.21 μm (R5) and 2.39% (R2) to 12.51 μm and 13.07% (R7) for striation length. Researchers with standard error values below five striations and 5 μm were R2 and R5, while those with variance values < 5% are R1, R2, and R5. Researchers with standard error values for both variables between 5–10 striations and 5–10 μm were R1 and R3, while R3 and R4 showed variance values in the range 5–10%. Researchers R6 and R7 showed the highest standard error and variance values for all variables measured (Table I, Fig. 2). If the least experienced researchers are excluded from the analysis, the maximum standard error and variance values obtained are 10.30 striations and 6.09% (R4) for striation density, and 5.97 μm and 5.66% (also R4) for striation length.

If the repeated measurements reported by Grine *et al.* (2002) are used to compute the standard errors (e_x) and variances (V_x) of the repetitions, the calculations yield a standard error of 5.33 striations with a variance of 5.34% for striation density, a standard error of 0.60 μm, and a variance of 3.99% for striation length (these values are included in Fig. 2 for comparison). Note that the magnitudes of the striation lengths differ greatly between our 100× magnification research and the report of Grine *et al.* (2002) with 500× magnification.

To test whether all researchers were measuring the same magnitudes in each micrograph considered, a multiple analysis of variance (MANOVA) designed for repeated measurements was performed, using SPSS v. 11 and considering two repetition factors (seven researchers and four replicas for each image considered). The MANOVA tests for homogeneity of means showed highly significant differences (F test) among researchers, whereas the replica factor had no effect (Table II). The univariate intersubject effect comparisons (for each of the 15 variables involved in the buccal striation pattern analysis) showed the same patterns: no significant differences among the replicas and highly significant differences among researchers. In fact, a tendency of researcher R7 to measure longer striations was evident (Fig. 3) for all images, since striation fragmentation was seldom considered. However, researcher R1 paid great attention to striation fragmentation and showed the smallest average striation lengths, also for all images; this researcher was followed by R6, R2, R3, R4, and R5. It is significant that those researchers who were conservative in measuring long striations within each image also showed smaller measurement errors. There was also a tendency of researcher R7 to measure a high density of striations in all images, whereas R1, R2 and R3 generally measured low striation densities (Fig. 3).

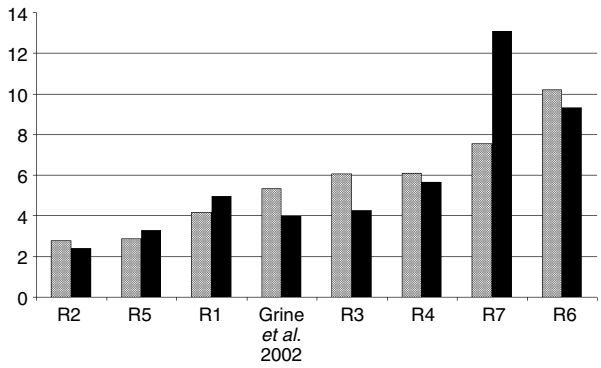

FIG. 2 Bar-plot of the repeated measurements variances in percent as estimation of error rates of striation density and average length for all researchers and including values from Grine *et al.* (2002) for comparison. ▒=density, ■=length.

TABLE I Summary statistics by researcher of error rates for the density and length of measured striations in each measured micrograph

	R1			R2			R3			R4		
	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x
P1												
Density	112.25	2.39	2.13	111.00	2.48	2.23	112.75	2.95	2.62	155.50	5.61	3.61
Length	112.83	4.29	3.80	146.81	3.90	2.66	119.54	3.15	2.64	109.15	5.19	4.75
P 2												
Density	196.75	2.78	1.41	209.00	6.10	2.92	172.50	8.91	5.17	253.75	25.28	9.96
Length	115.45	7.13	6.18	149.24	2.07	1.39	131.10	6.16	4.70	118.72	8.26	6.96
P3												
Density	146.50	9.54	6.51	124.25	2.95	2.37	86.25	10.81	12.53	173.00	7.54	4.36
Length	93.86	3.53	3.76	134.22	4.40	3.28	124.40	4.09	3.29	97.62	5.41	5.54
P4												
Density	90.50	8.09	8.94	64.50	2.53	3.92	50.75	2.93	5.77	93.25	2.75	2.95
Length	94.68	5.69	6.01	146.15	3.37	2.31	130.18	8.09	6.21	96.72	5.03	5.20
Mean												
Density		5.70	4.18		3.52	2.77		6.40	6.06		10.30	6.09
Length		5.16	4.95		3.44	2.39		5.37	4.25		5.97	5.66

	R5			R6			R7			Mean of group		
	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x
P1												
Density	132.25	8.48	6.41	133.00	6.38	4.80	193.00	8.69	4.50	135.68	5.28	3.89
Length	93.75	3.69	3.94	112.72	13.99	12.41	105.59	11.32	10.72	114.34	6.36	5.56
P2												
Density	326.75	7.05	2.16	219.50	25.44	11.59	312.75	30.08	9.62	241.57	15.09	6.25
Length	109.98	2.84	2.58	125.75	12.67	10.08	100.68	13.17	13.08	121.56	7.47	6.15
P3												
Density	140.75	2.32	1.65	111.75	13.21	11.82	204.00	6.47	3.17	140.93	7.55	5.36
Length	97.62	5.41	5.54	119.19	6.65	5.58	85.18	8.94	10.50	108.49	5.38	4.96
P4												
Density	75.00	1.58	2.11	78.25	10.38	13.27	136.50	18.81	13.78	84.11	6.72	7.99
Length	83.85	1.67	1.99	96.91	10.07	10.39	91.43	16.60	18.16	105.70	7.22	6.83
Mean												
Density		4.86	2.88		13.85	10.21		16.01	7.57		8.66	5.75
Length		3.21	3.27		10.60	9.33		12.51	13.07		6.60	5.87

Symbols are indicative of: x = mean values of the repeated measurements; e_x = standard error of the repeated measurements, and V_x = variance of of the repeated measurements.

Discussion

It is evident that any observational science involving the measurement of continuous variables, such as characterizing tooth microwear patterns, implies a certain degree of measurement error that needs to be controlled for, or at least minimized. Device error is frequently small since modern measuring equipment shows great precision, while clerical error is seldom a problem since the measurements can be directly inputted into a computer database without the need for data handling. However, intra- and interobserver errors still need to be carefully considered. As measuring procedures become more and more sophisticated, researchers' expertise and objectivity are of major concern. The variables to be measured need to be clearly and comprehensively defined so that different observers may replicate the measurements. Fully automatic measuring procedures would eliminate interobserver error, but the characterization of microwear patterns is still far from becoming automatic, at least where the aim is to measure microwear patterns as a combination of individual feature density, size, and orientation. Efforts should therefore focus more on automatic measures of surface roughness and relief.

The standard error of a series of repeated measurements is the best measure of intraobserver error because it determines a confidence interval around the actual variable value. Also, the standard deviation of a sample may be significantly reduced if the average of at least four repetitions is used as the actual variable measurement (Pérez-Pérez *et al.* 1990). Thus, the smaller the standard error the smaller the between-population differences that one can discriminate significantly between populations. The standard error of repeated measurements can be directly compared among researchers, given a significant number of repetitions, and their variance can be used as indicative of a researcher's measure of dispersion, with lower values expected for more experienced and precise researchers. However, the mean absolute percentage difference (MAPD), computed

TABLE II Multivariate and univariate general lineal model contrasts comparing the repeated measurements for the two factors considered (four replicas and seven researchers) of each image analyzed

	Multivariate effects					
	Replica (4 repetitions)			Researcher (7 repetitions)		
Image	Wilks λ	F	p value	Wilks λ	F	p value
1	0.133	3.273	0.060	0.000	7.729	***0.000***
2	0.335	0.994	0.533	0.000	5.353	***0.000***
3	0.213	1.850	0.210	0.000	5.975	***0.000***
4	0.243	1.560	0.284	0.000	5.222	***0.000***

	Intersubjects effects							
	Image 1				Image 2			
	Replica		Researcher		Replica		Researcher	
Variable	F	p value	F	p value	F	p value	F	p value
NH	0.208	0.653	11.146	***0.000***	0.786	0.386	12.379	***0.000***
NV	0.053	0.821	21.523	***0.000***	0.459	0.506	12.182	***0.000***
NMD	1.789	0.196	8.446	***0.000***	2.527	0.128	5.421	***0.002***
NDM	0.634	0.435	9.789	***0.000***	1.357	0.258	2.512	0.056
NT	0.365	0.553	25.356	***0.000***	1.460	0.241	10.484	***0.000***
XH	0.010	0.921	4.560	***0.005***	1.690	0.208	5.231	***0.002***
XV	0.790	0.385	4.274	***0.006***	0.582	0.454	2.437	0.062
XMD	0.210	0.652	6.165	***0.001***	0.404	0.532	3.250	***0.021***
XDM	0.002	0.964	3.781	***0.011***	1.089	0.309	7.155	***0.000***
XT	0.369	0.550	4.791	***0.004***	0.506	0.485	3.373	***0.018***
SH	0.051	0.824	1.290	0.306	2.336	0.142	1.598	0.200
SV	0.343	0.565	8.958	***0.000***	0.059	0.811	13.037	***0.000***
SMD	0.025	0.875	5.030	***0.003***	0.061	0.807	8.560	***0.000***
SDM	0.101	0.754	1.798	0.151	0.015	0.905	8.010	***0.000***
ST	0.052	0.822	8.013	***0.000***	0.028	0.869	13.573	***0.000***

	Image 3				Image 4			
	Replica		Researcher		Replica		Researcher	
Variable	F	p value	F	p value	F	p value	F	p value
NH	0.338	0.567	28.089	***0.000***	3.786	0.066	16.495	***0.000***
NV	0.141	0.711	13.548	***0.000***	0.787	0.386	13.392	***0.000***
NMD	0.170	0.685	4.763	***0.004***	2.518	0.128	4.423	***0.005***
NDM	0.245	0.626	13.069	***0.000***	5.098	0.035	5.313	***0.002***
NT	0.028	0.869	20.569	***0.000***	2.906	0.104	10.338	***0.000***
XH	1.701	0.207	13.527	***0.000***	0.004	0.947	2.451	0.061
XV	0.989	0.332	8.063	***0.000***	0.418	0.525	5.266	***0.002***
XMD	0.443	0.513	5.333	***0.002***	0.509	0.484	2.853	***0.036***
XDM	0.209	0.652	3.783	***0.011***	0.172	0.683	9.519	***0.000***
XT	0.932	0.346	9.973	***0.000***	0.001	0.980	6.905	***0.000***
SH	0.045	0.833	2.295	0.075	0.830	0.373	0.823	0.566
SV	0.015	0.904	26.376	***0.000***	0.594	0.450	7.200	***0.000***
SMD	0.200	0.660	11.683	***0.000***	0.690	0.416	2.414	0.064
SDM	2.082	0.165	0.479	0.816	0.864	0.364	17.311	***0.000***
ST	0.575	0.457	24.894	***0.000***	0.117	0.736	8.938	***0.000***

N= density of striations, X= average length of striations, S= standard deviation of the striation lengths, V= vertical striations, H= horizontal striations, MD= mesio-distal oblique striations, DM= disto-mesial oblique striations.

as the observed value minus the sample mean divided by the sample mean (Grine *et al.* 2002), can only be considered to be a measure of the maximum amount by which a given set of researchers under- or overestimate the mean value (Grine *et al.* 2002); for a reduced number of observers this may not even approximate the actual measurement. The MAPD statistic is highly sensitive to reduced numbers of repetitions and researchers, and varies greatly with the magnitude of the measured variable (for identical dispersion ranges, different MAPD values are obtained if the sam-

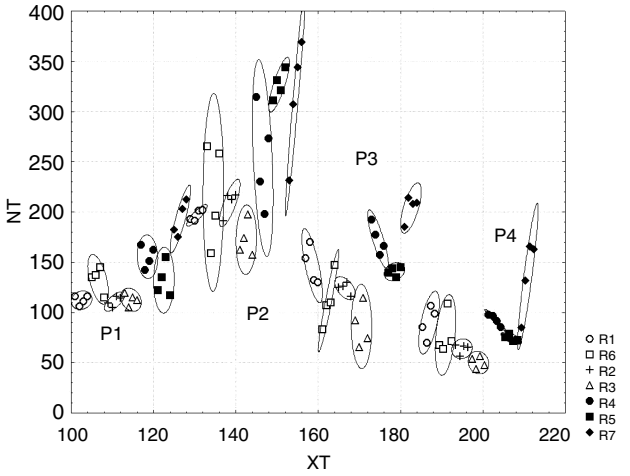

FIG. 3 Plot of striation density (NT) versus striation average length (XT) of all the repeated measurements for each picture (P1, P2, P3, and P4) and each researcher (R1, R2, R3, R4, R5, R6, R7). The ellipses include 50% confidence intervals [x±aσ] of all samples compared.

ple means differ). This makes comparisons between researchers difficult, and a set of only two repetitions is unlikely to be representative of overall measurement error.

The standard errors and coefficients of variance obtained for the buccal microwear analyses performed here (Table I, Fig. 2) show that error rates do indeed vary among researchers, with the least experienced ones (R6 and R7) showing the highest values. The variances among the most experienced researchers (with at least 3 years in buccal microwear research) do not exceed 6%, and this may represent a deviation of about nine striations in density and 10 μm in average length for the images studied here. Therefore, between-group differences in buccal microwear patterns can only be discriminated if such interpopulation differences exceed the error estimations by a large amount. In addition to these error rate estimations, the analysis of the interobserver variability also needs to consider whether all researchers are in fact measuring the same magnitudes of the variables, especially if comparisons between two independent researchers are to be made. From our analysis, it seems clear that although high intraobserver homogeneity is observed for some researchers, they are in fact measuring different things. Despite standardization of measurement procedures, the semiautomatic characterization of dental microwear involves considerable degrees of interobserver error, as well as differences in variable magnitudes (Grine *et al*. 2002), not least if different techniques are also used. Certainly, these results seem discouraging as they suggest that only one experienced observer should make all microwear measurements, thus avoiding interobserver comparisons. However, the results also provide clear guidelines for further methodological standardization among researchers, at least until more precise, automatic surface characterization procedures become widely used in dental microwear characterization.

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4.4 La mida dental en primats *Hominoidea*

Galbany J, Martínez LM, Estebaranz F & Pérez-Pérez A (2006). Tamaño dental, dimorfismo sexual y desgaste oclusal en primates *Hominoidea* a partir de nuevas metodologías semiautomáticas.

Congreso Sociedad Española de Antropología Física (en premsa).

(no s'inclou resum perquè la publicació és en castellà)

Tamaño dental, dimorfismo sexual y desgaste oclusal en primates Hominoidea a partir de nuevas metodologías cuantitativas semiautomáticas.

GALBANY J, MARTÍNEZ LM, ESTEBARANZ F & PÉREZ-PÉREZ A

Diversidad biológica y salud humana

pp. 103-110 (en prensa)

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Palabras clave: Hominoidea, primates, dimorfismo sexual, tamaño dental, molar

Aunque la mayoría de estudios sobre dimorfismo sexual en la dentición de primates Hominoidea se centra en la morfología de los caninos, algunos estudios apuntan un cierto grado de dimorfismo también en la dentición post-canina en diversas especies. El presente estudio se centra en el estudio de la variabilidad del área oclusal de los molares de primates Hominoidea a nivel subespecífico. También se analiza el desgaste oclusal del esmalte en las mismas especies, aportando datos cuantitativos del porcentaje real de exposición de dentina respecto al área oclusal total de la corona. Se ha analizado un total de 598 piezas dentales de molares mandibulares. Los resultados indican que la metodología utilizada permite obtener valores reales del perímetro y área oclusal de la corona dental, que establecen una nueva odontometría de referencia. El dimorfismo sexual observado en los molares es muy evidente en *Pongo* y *Gorilla*, y prácticamente inexistente en *Pan*. Por otro lado, los gibones presentan niveles altos de dimorfismo sexual en molares, aunque tradicionalmente se les ha considerado especies poco dimórficas.

Introducción y Objetivos

Numerosos estudios de morfología craneal en primates han demostrado la existencia de diferencias morfológicas específicas y subespecíficas (Taylor, 1992; O'Higgins & Dryden, 1993; Morgan et al., 2003). Asimismo, la morfología de las piezas dentales también presenta diferencias a nivel de dimorfismo sexual. Los dientes, sobretodo los caninos, permiten diferenciar entre machos y hembras. Aun así, el dimorfismo sexual no es exclusivo de la dentición canina. Numerosos estudios apuntan un cierto grado de dimorfismo sexual en el tamaño de la dentición post-canina en diversas especies de primates, sobretodo en *Gorilla* y *Pongo*, aunque todas ellas presentan niveles parecidos, cercanos al 10% (Garn et al., 1966; Cochard, 1985; Oxnard et al., 1985; Oxnard, 1987).

El presente trabajo se centra en el estudio de la variabilidad del tamaño dental de los molares de primates Hominoidea mediante una nueva metodología que permite estimar el área oclusal de los molares como indicador de tamaño dental real. Asimismo se han estudiado las diferencias sexuales de los tamaños de los molares inferiores de diversas subespecies de todos los géneros de la Super Familia Hominoidea, estableciendo un nuevo concepto de dimorfismo sexual. Al mismo tiempo, se presentan datos sobre el desgaste oclusal del esmalte en las mismas especies, aportando datos cuantitativos del porcentaje real de exposición de dentina, a diferencia de los estudios clásicos que tienden a clasificar en categorías discretas el desgaste oclusal de los dientes (Brothwell, 1981; Chimenos et al., 1999; Hillson, 2002). Los objetivos específicos del presente estudio son aplicar una nueva metodología semiautomática en la cuantificación del tamaño dental, a partir del Área Oclusal Total (AOT) de la corona dental, determinar la variabilidad del tamaño de los molares de las especies Hominoidea actuales, así como las diferencias entre ellas, determinar la existencia de dimorfismo sexual en el tamaño dental de los molares y aplicar la misma metodología semiautomática a la cuantificación de la exposición de dentina debida al desgaste oclusal del esmalte.

Material y método

Los especimenes estudiados pertenecen a diversas colecciones osteológicas: *Royal Museum for Central Africa* (MRAC Tervoren, Bélgica), *Staatssammlung fuer Anthropologie und Palaeoanatomie* (SAPM Munchen, Alemania) y *National Museum of Natural History, Smithsonian Institution* (NMHH Washington, Estados Unidos de América). Los especimenes analizados representan la totalidad de especies de Hominoidea y numerosas subespecies: *Gorilla gorilla graueri*, *Gorilla gorilla beringei*, *Pan troglodytes schweinfurthii*, *Pan paniscus, Pongo pygmaeus pygmaeus* y *Hylobates moloch*. En total se han analizado 598 piezas dentales mandibulares, tanto M1, M2 y M3, pertenecientes a individuos adultos (Tabla 1).

Tabla 1. Especies y número de dientes molares mandibulares analizados (M1: primer molar, M2: segundo molar y M3: tercer molar).

	M1		M2		M3		
Especie	M	H	M	H	M	H	**Total**
Gorilla gorilla graueri	23	18	23	18	18	15	**115**
Gorilla gorilla beringei	13	14	14	14	13	11	**79**
Pan troglodytes schweinfurthii	21	28	21	29	20	25	**144**
Pan paniscus	19	23	19	25	14	19	**119**
Pongo pygmaeus pygmaeus	9	21	9	20	5	16	**80**
Hylobates moloch	9	13	9	13	8	9	**61**
TOTAL	**94**	**117**	**95**	**119**	**78**	**95**	**598**

A partir de moldes negativos de alta resolución obtenidas por el Dr. Peter S. Ungar de la University of Arkansas, se obtuvieron réplicas de todas las piezas dentarias analizadas siguiendo protocolos estandarizados (Pérez-Pérez et al., 1999; Galbany et al., 2004) y a partir de ellas se obtuvieron fotografías digitales en norma superior de la superficie oclusal de toda la corona dentaria, a partir de las que se cuantificó el perímetro oclusal de cada diente en la Unidad de Tratamiento de Imagen y Soporte Informático de los Servicios Científicotécnicos de la Universitat de Barcelona. La utilización del software *Imat* de los SCT de la UB permite obtener información del perímetro oclusal total de cada diente y diversas variables derivadas, como el área oclusal total o el radio medio, entre otras. También se midieron el perímetro y el área oclusal total de todas las áreas de exposición de dentina observadas (Figura 1).

La metodología utilizada en el presente estudio ya había sido aplicada por nuestro equipo de investigación en algunas especies de homininos (Estebaranz *et al.*, 2004; Pérez-Pérez *et al.*, 2003); en el presente estudio, sin embargo, se han desarrollado nuevas aplicaciones y perspectivas de futuro.

Resultados

En primer lugar se realizaron análisis de normalidad (test no paramétrico Kolmogorov-Smirnov) de la variable AOT (Área Oclusal Total) por especie, sexo y pieza dental, comprobando que en todos los casos las variables analizadas siguen distribuciones normales con un error máximo del 5%. Un análisis descriptivo a partir de los diagramas de cajas (Figura 2) muestra que el Área Oclusal Total presenta grandes diferencias entre los grupos considerados en ambos sexos. Los gorilas son los que tienen mayor AOT, seguidos de

orangutanes, chimpancés y gibones. Para todos los grupos, y en ambos sexos, el segundo molar (M2) es el diente con mayor AOT, seguida mayoritariamente por el M3, o el M1, según las especies.

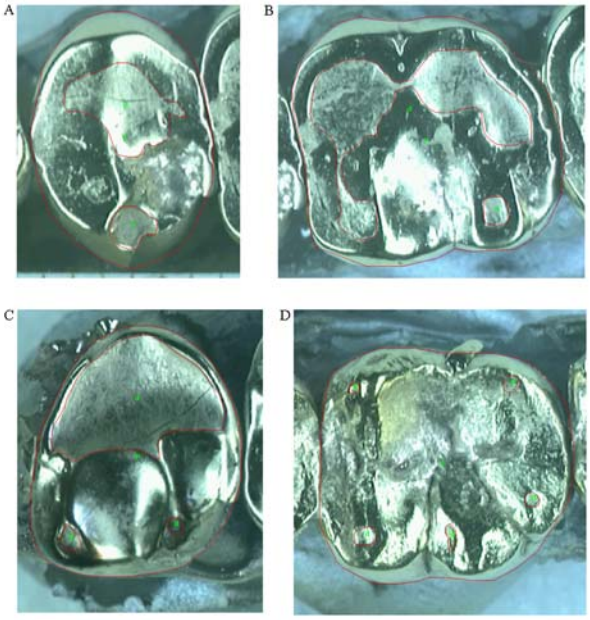

Figura 1. Coronas dentales en norma superior captadas con la cámara de video digital de la Unidad de Tratamiento de Imagen y Soporte Informático de los Servicios Científicotécnicos de la Universitat de Barcelona. Los perímetros en rojo fueron marcados manualmente con el software *Imat* (UB) e identifican el perímetro oclusal total y las áreas de exposición de dentina. A) P4 – *Pan paniscus*, B) M1 – *Pan paniscus*, C) M1 – *Hylobates moloch*, D) M2 – *Pan troglodytes schweinfurthii*.

Asimismo, el AOT también presenta diferencias sexuales en algunas especies. Un análisis de la varianza –Anova de un Factor– (Tabla 2) muestra que *Pongo p. pygmaeus* es el único grupo con diferencias significativas entre sexos para los tres molares considerados, seguido por *Gorilla g. beringei* y *Hylobates moloch*, con diferencias en dos molares. *Gorilla g. graueri* solo presenta diferencias significativas en un único molar y las dos especies del género *Pan* no presentan diferencias significativas. Para un mejor análisis del dimorfismo sexual se calculó el porcentaje del dimorfismo sexual (PDS) para todos los dientes de cada especie mediante la expresión aritmética: PDS = [(AOT Macho – AOT Hembra) / AOT Hembra] * 100.

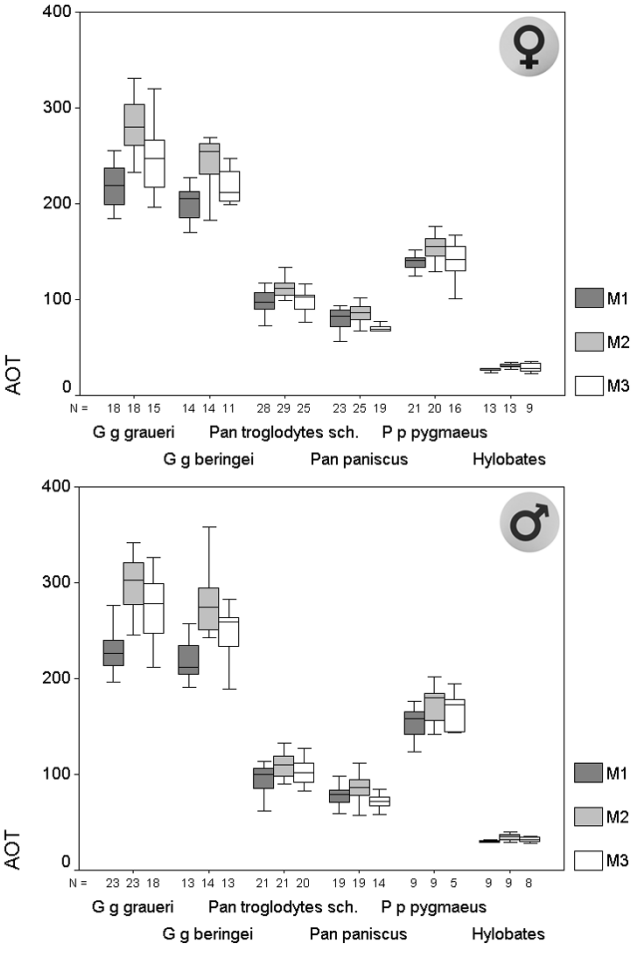

Figura 2. Diagramas de cajas del área oclusal total de los molares mandibulares (M1, M2 y M3) de todas las especies analizadas.

La Figura 3 muestra el porcentaje de dimorfismo sexual (PDS) para los tres molares y todos los grupos analizados. *Gorilla g. beringei* y *Pongo p. pygmaeus* son las dos especies que presentan mayores valores de PDS, entre el 10% y más del 20%; seguidos de *Hylobates moloch* con valores cercanos al 10% para todos los dientes y *Gorilla g. Grau*eri con valores entre el 5% y el 10%. Finalmente, *Pan paniscus* y *Pan troglodytes* presentan un ligero dimorfismo sexual para el M3 (5%) y un ligero dimorfismo sexual a favor de las hembras para el M1. En todos los casos, excepto *Hylobates*, el M3 es la pieza dentaria que presenta un PDS más elevado, siempre a favor de los machos. Esta misma

técnica de medición se aplicó a la estimación del área de exposición de dentina (AED) de cada diente. A partir de AOT y AED se calculó el porcentaje de exposición de dentina (PED) mediante la expresión PED = (AED/AOT) * 100. La Figura 4 muestra el PED para todos los dientes de ambos sexos de los grupos analizados. En general es el M1 el diente que presenta un mayor PED en todos los casos, seguido por M2 y M3.

Tabla 2. Análisis de la varianza –ANOVA 1 factor– del Área Oclusal Total para los molares mandibulares y ambos sexos. Los (*) marcan las diferencias significativas entre sexos con un error de 0,05.

Subespecie	Diente	F	Sig.	
Gorilla g. graueri	M1	2.664	0.111	
	M2	3.856	0.057	
	M3	4.692	0.038	*
Gorilla g. beringei	M1	3.886	0.060	
	M2	10.242	0.004	*
	M3	9.918	0.005	*
Pan troglodytes sch.	M1	0.453	0.504	
	M2	0.000	0.994	
	M3	1.500	0.227	
Pan paniscus	M1	0.759	0.389	
	M2	0.004	0.947	
	M3	1.486	0.232	
Pongo p. pygmaeus	M1	10.482	0.003	*
	M2	10.554	0.003	*
	M3	6.796	0.017	*
Hylobates moloch	M1	9.529	0.006	*
	M2	10.638	0.004	*
	M3	2.994	0.104	

Discusión y Conclusiones

El estudio del tamaño dental y el dimorfismo sexual en primates Hominoidea se ha basado siempre en la odontometría clásica (Garn et al., 1966; Cochard, 1985; Oxnard et al., 1985; Oxnard, 1987; Plavcan, 2001; Swindler, 2002). Sin embargo, el presente estudio ha utilizado una nueva metodología semiautomática para conseguir mediciones muy precisas del tamaño dental en primates, tal y como se realizó en un estudios preliminares en Homininos (Pérez-Pérez et al., 2003; Estebaranz et al., 2004). La variable analizada es el Área Oclusal Total (AOT), que se calcula automáticamente a partir del Perímetro Oclusal Total (POT) marcado manualmente en la imagen mediante el software específico *Imat*. El AOT presenta grandes diferencias entre los grupos considerados, siendo los gorilas las especies con mayor AOT, seguidos por orangutanes, chimpancé, bonobo y gibones. Estas diferencias generales (Figura 2 y Tabla 2) coinciden claramente con numerosos estudios clásicos (Warwick James, 1960; Garn et al., 1966; Cochard, 1985; Oxnard et al., 1985; Oxnard, 1987; Plavcan, 2001; Swindler, 2002).

El dimorfismo sexual en el tamaño de los molares existe en mayor o menor medida según el grupo considerado (Figura 3). *Pongo p. pygmaeus* es la especie que presenta el mayor número de diferencias significativas en el tamaño de los molares entre machos y hembras y elevados valores del Porcentaje de Dimorfismo Sexual (PDS), un 14% en promedio entre los tres molares, y el gorila de montaña le sigue con valores de PDS también elevados. Estos resultados coinciden con Oxnard et al. (1985) y Oxnard (1987), para *Gorilla*

g. beringei, pero no para los orangutanes, que no presentan en este trabajo niveles tan elevados de dimorfismo sexual en los molares. Las dos especies del género *Pan* no presentan diferencias significativas en el tamaño de los molares entre ambos sexos y el PDS promedio es cercano a cero, tal y como aparece en estudios de odontometría clásicos (Oxnard et al., 1985; Oxnard, 1987). Finalmente, *Hylobates moloch* presenta un gran número de diferencias entre ambos sexos y niveles relativamente altos de dimorfismo sexual (PDS), casi el 12% en promedio. Así pues, los gibones, que tradicionalmente han sido considerados monomórficos, quizá no lo sean en su totalidad. *H. moloch* presenta además un ligero dimorfismo sexual cromático (Geissmann, 2004). El dimorfismo sexual, pues, responde a múltiples factores y afecta de manera distinta a diversas estructuras, tal como es el caso de los gibones, que pueden presentar dimorfismo sexual cromático muy acusado (Plavcan, 2001) o en el tamaño de los molares, tal como muestra el presente trabajo, aunque sean consideradas especies no dimórficas.

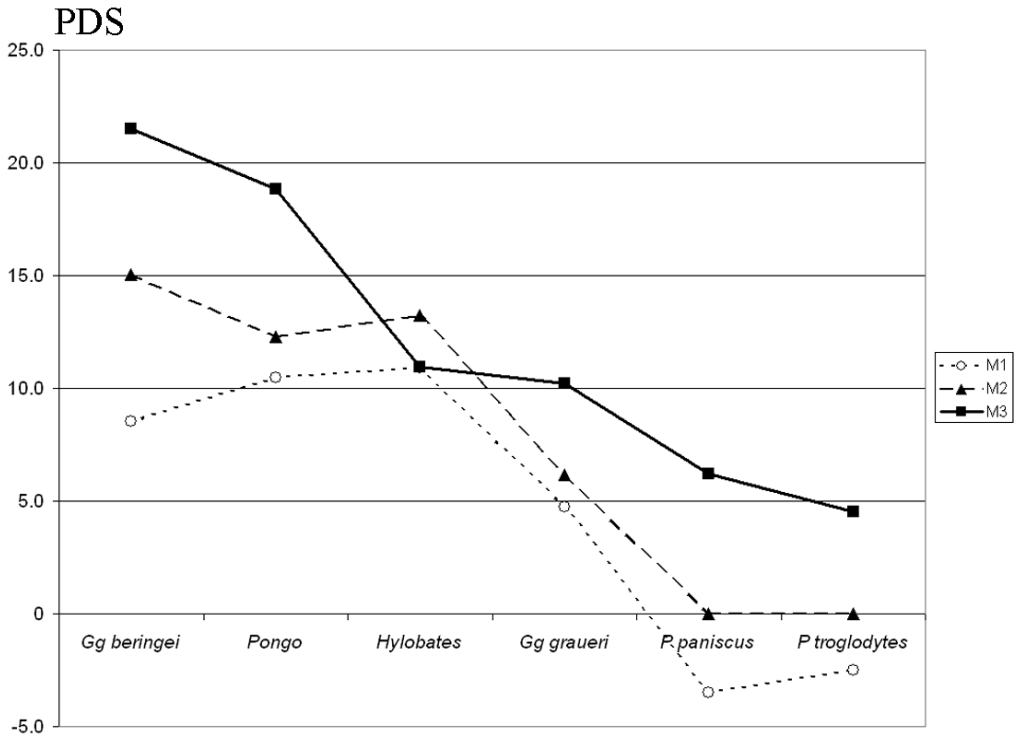

Figura 3. Porcentaje de dimorfismo sexual del área oclusal total de todos los molares mandibulares (M1, M2 y M3) de las especies analizadas.

Asimismo, el dimorfismo sexual en el tamaño de los molares de los primates no muestra niveles similares para todas las especies, cercanos al 10%, como afirman numerosos autores (Garn et al., 1966; Cochard, 1985; Plavcan, 2001), sino que presenta una gran variabilidad, siendo inexistente en algunas especies como en el género *Pan*. Por estas razones, no se deberían considerar las diferencias de tamaño en la dentición post-canina exclusivamente como adaptaciones alimentarias, por ser variables afectadas por dimorfismo sexual, aunque en menor medida que el tamaño de los caninos, pero presente en los primates Hominoidea. El AOT en los molares muestra niveles de dimorfismo sexual, en algunas ocasiones muy superiores a las mediciones clásicas (buco-lingual o mesio-distal) de estos dientes (Swindler, 2002).

Finalmente, el Porcentaje de Exposición de Dentina (PED) presenta una gran variabilidad interespecífica (Figura 4), pero todos los grupos analizados siguen el mismo patrón de desgaste oclusal desde el M1 a M3, igual que en otros estudios (Hillson, 2002; Estebaranz et al., 2004), debido a que este tipo de desgaste está causado por la acción

mecánica de masticación y es acumulativo a lo largo de la vida del individuo (Campillo, 2001). Esta nueva metodología permite cuantificar la exposición de dentina real, a diferencia de las metodologías habitualmente utilizadas que únicamente presentan resultados cualitativos o de categorización discreta (Brothwell, 1981; Chimenos et al, 1999; Hillson, 2002). En el presente trabajo se ha aplicado únicamente a primates no humanos, pero se puede aplicar igualmente a homininos (Bermúdez de Castro et al, 2001; Estebaranz et al, 2004).

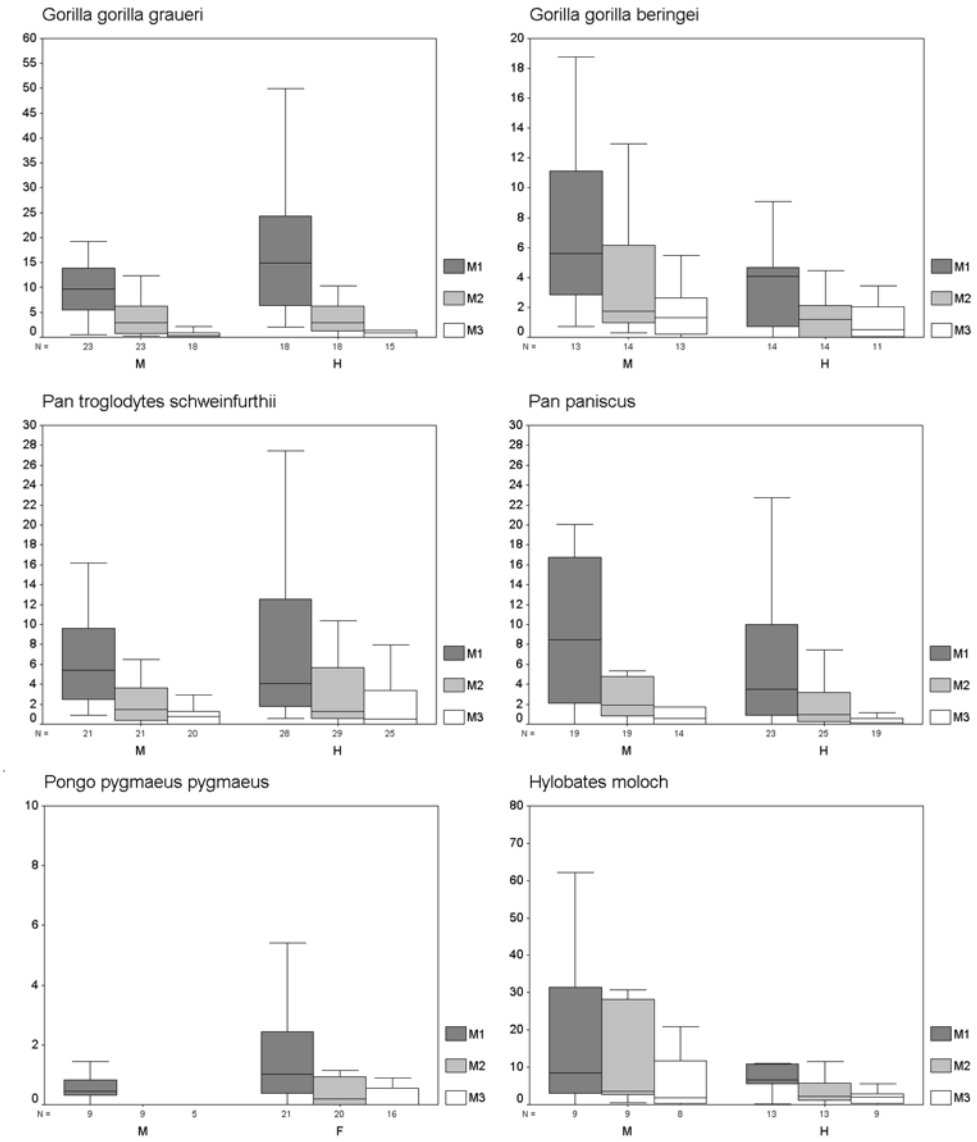

Figura 4. Diagramas de cajas del porcentaje de exposición de dentina de los molares mandibulares (M1, M2 y M3) por especie y sexo.

En conclusión, la variable AOT presenta una gran variabilidad entre las especies estudiadas, por lo que es posible establecer una nueva odontometría de referencia a partir de ella, observándose que el dimorfismo sexual en los molares es muy evidente para esta variable en orangutanes y gorilas, y prácticamente inexistente en bonobos y chimpancés. Es significativa la presencia en los gibones de niveles altos de dimorfismo sexual en molares, aunque tradicionalmente se les ha considerado especies monomórficas.

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4.5 El patró de microestriació, la mida dental i el desgast oclusal

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Tamaño dental, desgaste oclusal y microestriación dentaria en primates *Hominoidea*

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Palabras clave: microestriación dentaria, tamaño dental, desgaste oclusal, primates, *Hominoidea*

El patrón de microestriación dentaria depende no solo de la composición de los alimentos ingeridos, sino también de la presencia en ellos de polvo, cenizas u otras partículas abrasivas de origen externo, generalmente incorporadas al alimento durante su preparación. El presente estudio analiza cómo se ve afectado el patrón de microestriación de la superficie vestibular de los dientes por factores independientes a la estructura y composición alimentaria, como son el tamaño de la pieza dentaria y el desgaste oclusal de la misma. Se han analizado moldes dentarios obtenidos de ejemplares originales de colecciones osteológicas de molares de primates Hominoideos, en los que se ha medido el patrón de microestriación dentaria de todos los individuos, así como el tamaño dental (área oclusal total – AOT) y el desgaste oclusal (porcentaje de exposición de dentina – PED) mediante técnicas semiautomáticas de análisis cuantitativo. Los resultados obtenidos indican que el tamaño dentario y el grado de desgaste oclusal de los molares analizados no están correlacionados con el patrón de microdesgaste dental de sus superficies vestibulares. Ello sugiere que las actividades masticatorias asociadas con las superficies oclusales de los molares no están directamente relacionadas con la capacidad abrasiva del alimento, sino con utilización de la superficie occlusal de la dentición en actividades que no repercuten en el patrón de microestriación de las superficies vestibulares de los dientes.

Introducción

El patrón de microestriación dentaria de los primates no humanos, presente sobre las superficies de esmalte, está directamente relacionado con las condiciones ecológicas y composición de la dieta (Teaford, 1994; Ungar,1998; Ungar & Teaford, 1996; Galbany & Pérez-Pérez, 2004; Galbany *et al.*, 2005a). Lo mismo sucede con los humanos y los homininos fósiles (Pérez-Pérez *et al*., 1994; 1999; 2003a; Romero *et al.*, 2003/4). El patrón de microestriación dental producido *ante-mortem* durante la alimentación es fácilmente distinguible de aquellas alteraciones de origen tafonómico, producidas *post-mortem* sobre el esmalte dental (King *et al.*, 1999; Martínez *et al*., 2001; Pérez-Pérez *et al.*, 2003a; Martínez LM & Pérez-Pérez A, 2004; Galbany *et al.*, 2005b). Las partículas duras, como los fitolitos silícicos presentes en los vegetales o el sílice del polvo acumulado en los alimentos, pueden producir microestriaciones en la superficie del esmalte de las piezas dentarias durante la masticación. Aunque el mecanismo que las produce no está bien estudiado, existe una fuerte relación entre el patrón de microdesgaste dental y las condiciones ecológicas, la composición de la dieta o el modo tecnológico de explotación de los recursos tróficos utilizados por los humanos. El conocimiento de los patrones de microestriación en primates está sirviendo como modelo de referencia para la interpretación de la composición de su dieta, así como también de los Homininos fósiles (Galbany *et al.*, 2002; 2005a). Está ampliamente aceptado que existe una estrecha relación entre tamaño dental, grosor del esmalte y desgaste occlusal con el procesado y fragmentación de los alimentos y la abrasividad de la dieta en primates y humanos actuales (Hannam & Word, 1989; Agrawal *et al.,* 1997, 2000; Bourdiol & Minoche, 2000), sin embargo no se ha determinado el efecto que sobre el patrón de microestriación dentaria en la superficie vestibular

puede tener la utilización de la superficie oclusal del diente en actividades paramasticatorias y masticatorias.

El presente estudio se centra en establecer la relación entre el patrón de microestriación dentaria de los primates Hominoidea y diversas variables biológicas, como son el tamaño de los molares y el grado de desgaste oclusal y exposición de dentina.

Material y métodos

Se ha analizado una muestra de moldes dentales de primates Hominoidea actuales que presentaban superficies de esmalte vestibular bien conservadas, tomados en diversas colecciones osteológicas: *Royal Museum for Central Africa* (MRAC), Tervoren-Bélgica; *Staatssammlung fuer Anthropologie und Palaeoanatomie* (SAPM), München-Alemania; y *National Museum of Natural History, Smithsonian Institution* (NMHH), Washington (Tabla 1). En total se han seleccionado y analizado 18 piezas dentales mandibulares pertenecientes a 18 individuos adultos que representan 4 subespecies de primates Hominoidea: *Gorilla gorilla graueri*, *Gorilla gorilla beringei*, *Pan troglodytes schweinfurthii* y *Pongo pygmaeus pygmaeus* (Tabla 1). La reducida muestra se debe a la dificultad de disponer de moldes bien conservados de la corona de molares de primates, con patrones de microestriación dentaria no alterados y sin pátinas que recubran el esmalte impidiendo la formación de las estriaciones en el mismo (Galbany *et al.*, 2004a,b).

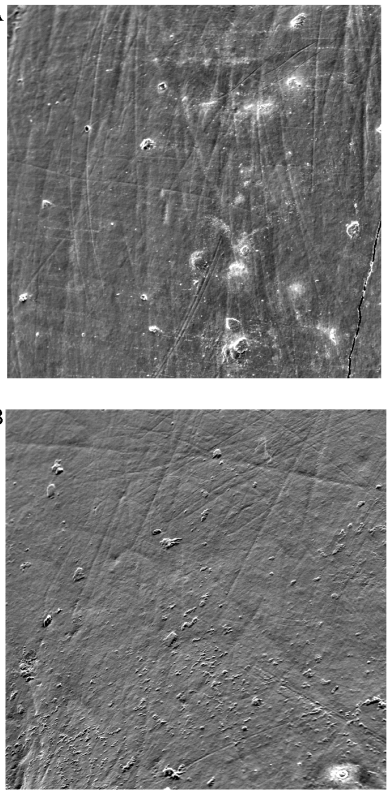

Figura 1. Imágenes a 100 aumentos de superficies de esmalte bien preservado con microestriaciones producidas por partículas abrasivas de los alimentos. A: M1 de macho de *Gorilla gorilla graueri* depositado en el MRAC. B: M1 de hembra de *Pongo pygmaeus pygmaeus* depositado en el SAPM

A partir de los dientes originales se obtuvieron moldes negativos de las coronas en resinas de polivinilsiloxano (*President Microsystemt - Regular body* de *Colténe*®). Sólo se replicó una pieza dentaria por individuo, escogiendo entre el primero y segundo molar inferior izquierdo (M1 y M2), con el fin de estandarizar la metodología de trabajo, lo que redujo considerablemente la muestra disponible. A partir de los moldes negativos, que presentan gran estabilidad temporal y resolución a nivel microscópico (Andritsakis & Vlamis, 1986; Teaford & Oyen, 1989), se realizaron réplicas con resina Epo-Tek 301 de *Química del Aditivo* SL (QdA) o con poliuretano Feropur PR-55, que replica con gran fidelidad los detalles microscópicos (Beynon, 1987; Teaford & Oyen, 1989; Galbany *et al.*, 2004a). Los dos productos ofrecen idénticos resultados y no difieren entre si, ni con el original (Galbany *et al.*, 2004a). Para conocer con más detalles determinados aspectos de la metodología utilizada véase Pérez-Pérez *et al.* (1999) y Galbany *et al.* (2004a).

Con el Microscopio Electrónico de Barrido (SEM) (Cambridge Stereoscan S-120, *Serveis Cientificotècnics* de la Universitat de Barcelona – SCT-UB) se observaron y fotografiaron de forma sistemática las superficies vestibulares de todos los dientes considerados. Los moldes se situaron en posición horizontal y todas las fotografías fueron tomadas a 100X en el tercio medio de la superficie vestibular, bajo una de las cúspides (proximal o distal). Las micrografías del patrón de microestriación se digitalizaron automáticamente con una resolución de 2032 ppp

con el software Image Slave del propio microscopio y se trataron con el programa Photoshop® v. 7.0 de Adobe®, seleccionando la zona mejor conservada de una área estandarizada de 0,56 mm^2. Las imágenes se contrastaron aplicando el filtro "*high pass*" utilizando 50 pixels de radio y se realizó un ajuste automático de los niveles de gris con el mismo software de Adobe®. Este tratamiento se aplicó para maximizar la resolución de la imagen sin alterarla significativamente (Figura 1).

Las microestriaciones fueron contadas manualmente con el programa *Sigmascan Pro V* de *SPSS*, considerando como estría todos aquellos objetos observables sobre el esmalte, producidos por abrasión, que tuvieran una longitud mayor de 15 micras y que como mínimo fueran tres veces más largos que anchos (Pérez-Pérez *et al.*, 1999; Galbany *et al.*, 2004a). Para cada microestriación contada, automáticamente *Sigma Scan* registra su longitud, de ésta manera se obtuvieron el número (NT) y longitud (XT) en micrómetros de todas las microestriaciones de cada fotografía, además de otras variables no analizadas en el presente estudio.

Tabla 1. Especies de *Hominoidea*, colección osteológica de origen (MRAC: *Royal Museum for Central Africa*, SAPM: *Staatssammlung fuer Anthropologie und Palaeoanatomie* NMNH: *National Museum of Natural History – Smithsonian Institution*), sexo (m: macho, h: hembra) y diente analizado (M1: primer molar, M2: segundo molar)

Especie	Colección	Sexo	Diente
Gorilla g. beringei	NMNH	-	M2
Gorilla g. graueri	MRAC	m	M1
Gorilla g. graueri	MRAC	h	M2
Gorilla g. graueri	MRAC	h	M2
Gorilla g. graueri	MRAC	h	M1
Gorilla g. graueri	MRAC	m	M2
Gorilla g. graueri	MRAC	m	M2
Gorilla g. graueri	MRAC	m	M2
Gorilla g. graueri	MRAC	h	M2
Gorilla g. graueri	MRAC	m	M2
Pongo p. pygmaeus	SAPM	m	M2
Pongo p. pygmaeus	SAPM	m	M2
Pongo p. pygmaeus	SAPM	m	M1
Pongo p. pygmaeus	SAPM	h	M2
Pongo p. pygmaeus	SAPM	h	M1
Pongo p. pygmaeus	SAPM	h	M1
Pan troglodytes sch.	MRAC	m	M2
Pan troglodytes sch.	MRAC	h	M2

A partir de los moldes dentales también se obtuvieron fotografías digitales de la superficie oclusal de los dientes estudiados con una cámara de video digital JVC TK-1270 (RGB) conectada a un ordenador mediante una tarjeta gráfica modelo *Comet* de la casa comercial *Matrox*, desarrollado por la Unidad de Tratamiento de Imagen y Soporte Informático de los Servicios Científico-técnicos de la Universitat de Barcelona. De este modo se obtuvieron imágenes de las superficies oclusales de cada diente a una resolución de 635x502 píxeles. A partir de cada imagen se definieron manualmente sobre un monitor el perímetro oclusal total (POT, en milímetros) de cada diente mediante el software *Imat* (SCT-UB). A partir de éste se obtuvo automáticamente el área oclusal real de cada diente o área oclusal total (AOT, en milímetros cuadrados), entre otras variables derivadas indicadoras de tamaño y forma, como la esfericidad. Asimismo, también se obtuvo el perímetro de la corona y el área de exposición de dentina (AED) a partir de la suma de la distintas áreas de exposición de dentina, también delimitadas manualmente (Figura 2). Esta metodología ha sido utilizada para establecer el tamaño

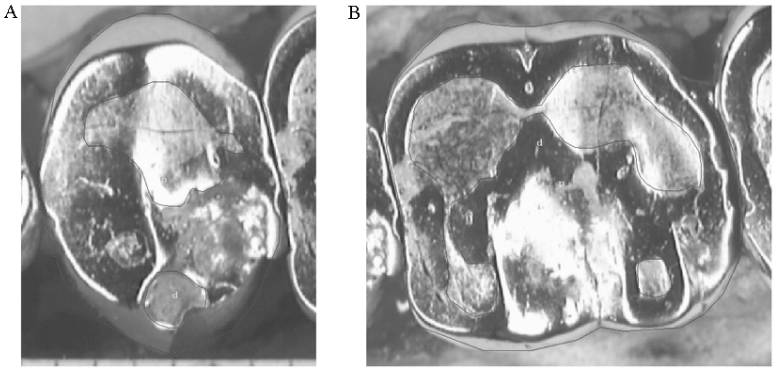

Figura 2. Imágenes en norma superior de la superficie oclusal de dientes de *Hominoidea*

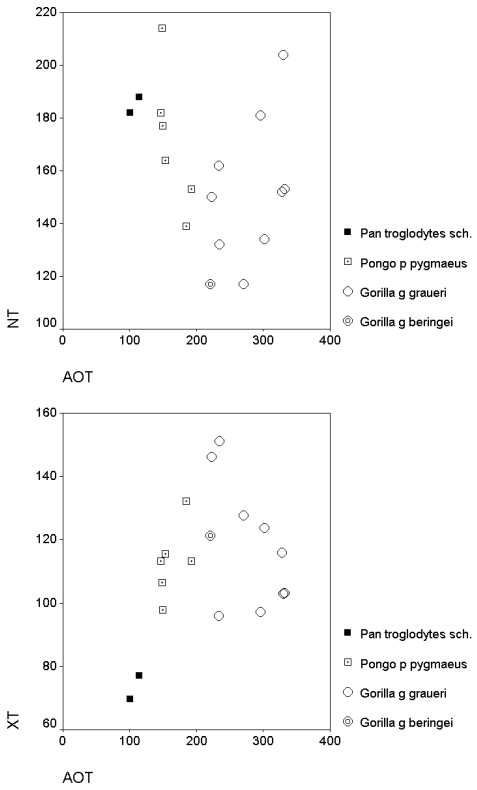

Figura 3. Diagramas de dispersión entre los pares de variable NT-AOT y NT-PED para todos los individuos analizados

dental real en dientes de homininos (Estebaranz *et al.,* 2004; Pérez-Pérez *et al.,* 2003b) y primates Hominoidea (Galbany, 2005; Galbany *et al.*, 2005c). Todos los cálculos y gráficos se realizaron con el paquete estadístico SPSS v.11.

Resultados

A partir del recuento manual de las microestriaciones de las imágenes obtenidas a través del SEM se obtuvieron las variables NT y XT para cada individuo. Asimismo, mediante el programa *Imat* se obtuvo el área oclusal total (AOT, mm^2) de cada diente y se calculó el porcentaje de exposición de dentina (PED) a partir del área de exposició de dentina (AED), mediante la expresión aritmética: PED = (AED/AOT) * 100.

El análisis no paramétrico de normalidad (Kolmogorov-Smirnov) indica que todas las variables siguen distribuciones normales, tanto para la muestra conjunta como también para cada subespecie. Ninguna de las correlaciones bivariadas (correlación de Pearson) realizadas entre las variable de tamaño y la densidad de estrías dentarias resultó significativa, obteniéndose valores r de Pearson muy bajos, que indican que no existe ningún tipo de relación entre la densidad de las microestriaciones (NT) o su longitud media (XT) y el tamaño dental (AOT) o el desgaste oclusal (PED) (Tabla 2). Ésta relación inexistente también se puede visualizar en los gráficos de dispersión bivariados (Figuras 3 y 4) entre las variables indicadas.

Discusión

Al igual que en el estudio preliminar precedente realizado en Homininos (Pérez-Pérez *et al.*, 2003b), los resultados obtenidos para los Hominoidea no muestran ninguna relación entre las variables de tamaño dentario (AOT y POT) y las de caracterización del patrón de microestriación dentaria (NT y XT). Asimismo NT y XT tampoco se correlacionan con el porcentaje de exposición de dentina (PED). El patrón de microestriación dentaria de la superficie vestibular, por tanto, es independiente del tamaño del diente sobre el cual se produce. Este resultado es importante porque los estudios de microestriación dentaria se basan en el número de estrías, así como su longitud y orientaciones, en una superficie estandarizada de 0,56 mm^2 para todos los individuos, independientemente del tamaño del diente que se esté analizando. Así pues, parece que la densidad de estrías y su longitud media en cualquier molar, sea cual sea su tamaño, estarán únicamente relacionadas con el tipo de alimento que las ha causado, y el patrón de microestriación dentaria no se verá

Tabla 2. Correlaciones bivariadas de Pearson (r) y grado de significación (P) entre las variables de caracterización del microdesgaste dentario (NT y XT) y las de tamaño y desgaste oclusal (POT, AOT y PED) para todos los individuos analizados

	r	P
NT-POT	-0.217	0.388
NT-AOT	-0.320	0.196
NT-PED	-0.282	0.257
XT-POT	-0.013	0.959
XT-AOT	0.318	0.199
XT-PED	0.388	0.111

afectado por el tamaño del diente.

Del mismo modo, la no asociación entre NT y XT con el porcentaje de exposición de dentina (Tabla 2 y Figura 4) nos permite concluir que el patrón de microestriación dentaria tampoco se ve alterado por el grado de desgaste oclusal macroscópico, producido por la acción mecánica de masticación de manera acumulativa a lo largo de la vida del individuo (Chimenos *et al.*, 1999; Campillo, 2001).

Esto supone que el patrón de microestriación dentaria no varía cuantitativamente a lo largo de la vida del individuo, sino que se mantiene, dado que distintos individuos con porcentajes de exposición de dentina (PED) distintos, es decir, de edades distintas, presentan valores similares para la variables que cuantifican el patrón de microdesgaste dentario (NT y XT). Esto concuerda con el estudio realizado en la población humana medieval de La Olmeda (Pérez-Pérez *et al.*, 1994), que concluye que la densidad de rasgos en el patrón de microestriación dentaria esta claramente correlacionada con la edad hasta que se estabiliza en los individuos subadultos.

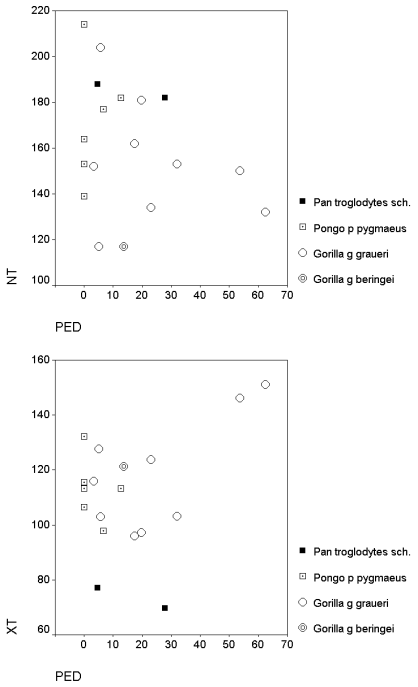

Figura 4. Diagramas de dispersión entre los pares de variables XT-AOT y XT-PED para todos los individuos analizados

El número de microestriaciones se va incrementando en las superficies vestibulares de los molares a lo largo de la vida del individuo desde que incorpora productos alimenticios capaces de rayar el esmalte, a partir del destete, y hasta un punto de saturación en el que deja de aumentar y se mantiene estable durante el resto de la vida del individuo (Pérez-Pérez *et al.*, 1994). La ausencia de diferencias cuantitativas no impide que cualitativamente haya modificaciones en el patrón de microdesgaste, ya que las nuevos rasgos van apareciendo sobre los más antiguos a lo largo del tiempo (Teaford & Oyen, 1989). Nos encontramos delante de un patrón de microestriación dinámico, pero analizable y altamente relacionado con la composición de la dieta, aunque todavía no ha sido explicado por ningún modelo ni mecanismo de formación en relación con la mecánica del proceso de masticación.

Los resultados del presente estudio sugieren que el patrón de microestriación dentaria de la superficie vestibular no está afectado por el uso de la superficie oclusal en actividades masticatorias, que depende también de factores como el contacto interdentario durante la oclusión, sin que esté afectado por variables de tamaño del diente ni cambios en el desgaste oclusal o la edad del individuo una vez estabilizado el patrón de microestriación. Por ello, el análisis del patrón de microestriación de la superficie vestibular del diente es un buen indicador del grado de abrasión del alimento ingerido en los primates, tal y como también han confirmado otros estudios independientes en la superficie occlusal de los dientes (Teaford & Tylenda, 1991; Teaford, 1994; Ungar *et al.*,1995; Ungar & Teaford, 1996).

Agradecimientos

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Abstract

Dental microwear patterns in the human dentition are mainly caused by the composition of the diet, but also by the presence of dust, ash or other abrasive particles on the ingested food items. The present study analyzes how this pattern is affected by other variables independent to the type of foods eaten, such as dental size and gross occlusal wear. Dental moulds of Hominoidea molars were obtained from original specimens curated at osteological collections. The buccal microwear patterns were analyzed in all the teeth, and its crown size (AOT, total occlusal area) and overall occlusal wear (PED, percent of dentine exposure) were measured using a new quantitative semiautomatic methodology. The results obtained suggest that no significant relationships exist between the buccal microwear patterns measured and dental size and occlusal. These indicate that the activities associated to occlusal chewing are independent to diet composition, and that other masticatory activities, not directly related to diet composition, might better explain the evolution of tooth size and enamel thickness.

Key words: dental microwear, tooth size, occlusal wear, Primates, *Hominoidea*

Dental size, occlusal wear and dental microwear in *Hominoidea* Primates

4.6 Variabilitat del patró de microestriació en *Cercopithecoidea*

Galbany J & Pérez-Pérez A (2004) Buccal enamel microwear variability in *Cercopithecoidea* Primates as a reflection of dietary habits in forested and open savanna environments.

Anthropologie 42(1): 13-19.

Els *Cercopithecoidea* habiten una gran varietat d'entorns ecològics i exploten diversos recursos tròfics (Teaford, 1994; Ungar, 1998; Ungar & Teaford, 1996). Es poden considerar un bon grup de primats per veure si les diferències ecològiques i tròfiques determinen o estan relacionades amb el patró de microestriació dentaria vestibular.

Aquest treball presenta l'anàlisi d'una mostra de motlles dentals de primats *Cercopithecoidea* de tres gèneres diferents (*Papio*, *Cercopithecus* i *Colobus*), obtinguts a la col·lecció osteològica del National Museums of Kenya (Nairobi). Es van estudiar un total de 214 motlles obtinguts a partir de les dents originals amb el polivinilsiloxà *President microSystem™* (*Coltène®*) i es van realitzar rèpliques amb resina epoxy seguint metodologies estandarditzades. A partir de les rèpliques dentals es va analitzar el patró de microestriació dental mitjançant el Microscopi Electrònic d'Escombrat (SEM), fent servir les tècniques estandarditzades habituals (Pérez-Pérez et al., 1999; Galbany et al., 2004a).

Els resultats obtinguts mostren que només 65 motlles (Taula 1) van presentar superfícies aptes per a l'estudi del microdesgast dental a la cara bucal. Aquest baix percentatge de preservació del patró de microestriació (30,4%) és degut a múltiples factors. La principal causa és la presència d'una pàtina que recobreix l'esmalt i impedeix la seva observació directa. Aquesta pàtina no és present de la mateixa manera en tots els primats analitzats. Els papions són l'espècie que presenten menys pàtina (42,2% ben preservats), en segon lloc els *Cercopithecus* (31,1%) i finalment els *Colobus*, on només un 11,4% dels individus analitzats són aptes per a l'estudi del microdesgast dental.

L'anàlisi dels patrons de microestriació dental vestibular mostra que existeixen diferències significatives, a nivell específic i genèric, en 12 de les 15 variables que el caracteritzen (Taula 2). Si es consideren les variables NT (densitat total d'estries) i XT (longitud promig de les estries), també es diferencien els primats a nivell de gènere. Els *Colobus* presenten un nombre reduït d'estries curtes, els papions presenten un nombre intermig d'estries de mida llarga i els *Cercopithecus* presenten moltes estries, però de mida intermitja (Figures 2 i 3).

Una anàlisi discriminant (Figura 4) a nivell específic diferencia molt bé els *Cercopithecus* dels *Papio* per la primera funció, que explica un 41,2% de la variabilitat total i està correlacionat fortament amb algunes variables de longitud de les estries (XT: longitud de les estries totals, XV: longitud de les estries verticals, i XDM: longitud de les estries obliqües disto-mesials), i alhora amb ST (desviació de la longitud de les estries totals), SMD (desviació de la longitud de les estries obliqües mesio-distals) i XV (longitud de les estries verticals). La segona funció discriminant, que explica un 31,8% de la variabilitat total, separa els *Colobus* de la resta de *Cercopithecoidea*. Aquest segon factor està correlacionat únicament amb variables de nombre d'estries (NT: densitat total d'estries, NDM: densitat d'estries obliqües disto-mesials, NV: densitat d'estries verticals i NMD: densitat d'estries obliqües mesio-distals).

Hi ha un gran nombre de diferències entre els patrons de microestriació dental de la cara bucal en els *Cercopithecoidea*, tal com succeeix en altres estudis de microdesgast dental basats tant en l'anàlisi de superfícies bucals com oclusals (Teaford, 1994; Ungar & Teaford, 1996; Ungar, 1998). Els *Colobus*, amb una dieta molt folívora, presenten nivells de microdesgast dental reduïts degut a la presència de pàtina que impedeix que es produeixin moltes microestriacions a l'esmalt. Pel que fa als *Cercopithecus*, consumidors de fruits, presenten un gran nombre de microestriacions, com s'ha vist en altres estudis realitzats. Finalment, els papions presenten un patró de microestriació vestibular indicatiu d'una dieta generalista, on abunden tant els recursos de zones obertes com de sabana, que produeixen microestriacions més llargues i més verticals que en els *Cercopithecus*, tot i que en menor nombre.

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BUCCAL ENAMEL MICROWEAR VARIABILITY IN CERCOPITHECOIDEA PRIMATES AS A REFLECTION OF DIETARY HABITS IN FORESTED AND OPEN SAVANNA ENVIRONMENTS

ABSTRACT: Dental microwear analysis has proved to be a good indicator of diet and dietary related behaviour in modern humans, fossil hominids and primates. The composition of the diet and the presence of dust and other abrasive particles, are related to microwear rates on the buccal enamel surfaces of molar teeth. Plant food materials such as leaves or stems include phitoliths in larger quantities than fruits or meat. These particles may scratch the enamel surface of teeth during mastication producing a microwear pattern that may be indicative of food choice and food preferences within primate species. In this study we present a dental microwear analysis of extant Cercopithecoidea primates, based on the analysis of more than 200 dental casts obtained from the osteological collection of the National Museum of Kenya (NMK). Specific, sub-specific and also ecological differences are shown to underlie the buccal microwear variability observed within the studied sample.

KEYWORDS: Non-occlusal dental microwear – Microstriation – Primates – Cercopithecoidea

INTRODUCTION

A lot of evidence supports the hypothesis that dental microwear features on the occlusal surface of teeth are determined by dietary and ecological conditions of the *Cercopithecoidea* (Teaford 1994, Ungar 1998, Ungar, Teaford 1996). Hard particles, such as phytolyths present in leaves, shoots or medullas, as well as dust deposited on food items before ingestion, can produce microscopic striations on the enamel surfaces during chewing. Although a close relationship between diet composition and ecological conditions is accepted for living primates for the occlusal surface of teeth, there is little knowledge on how the composition of the diet or the ecology affect to the buccal microwear pattern in primates. Little research on buccal microwear patterns of living primates has been done so far, and buccal microwear may serve as a model for diet/microwear interactions in past human populations based on the lateral surfaces of teeth instead of on the occlusal ones, much more affected by general macroscopic tooth wear. The present study focuses on the interpretation of the differences in the microwear patterns on the buccal surfaces of teeth, looking at differences between several *Cercopithecoidea* species, and how such differences relate to their habitat, ecology, or dietary preferences.

MATERIAL AND METHODS

The sample includes 214 *Cercopithecoidea* specimens for which a dental replica was obtained. All the specimens are curated at the osteological collection of the National Museum of Kenya (NMK), Nairobi. Details on the original sample studied are shown in Galbany *et al.* (2004a). All the specimens analysed are adult individuals of several species (*Table 1*), with both sexes represented, that were

TABLE 1. Total number of analysed moulds (TAM) of *Cercopithecoid* primates from the NMK osteological collection, and number of specimens with well-preserved enamel surfaces (N). All the moulds were made from adult wild-caught specimens. Less than one third of dental casts obtained from the collection offered good information on dental microwear, 30.4% on the average. Last column shows the percentage of good-condition teeth by group considered.

Cercopithecoidea species	TAM	N	%	Group %
Papio anubis	64	27	42.2	42.2
Cercopithecus nictitans mitis	37	6	16.2	31.1
Cercopithecus aethiops	41	14	34.1	
Cercopithecus aethiops pygerythrus	21	10	47.6	
Cercopithecus neglectus	7	3	42.8	
Colobus abyssinicus	34	3	8.8	11.4
Colobus angolensis	10	2	20.0	
TOTAL	214	65	30.4	

wild-caught in nature for different purposes and are now stored in the NMK osteological collection. None of the specimens studied were ever in captivity or fed by humans, so they inhabited their original distribution areas. Definitive species attribution of each specimen was done by original notes taken from the osteological collection (Galbany *et al.* 2004a) and from Kingdom (2001), correcting possible taxonomic inconsistencies. The studied species were: *Papio anubis*, *Cercopithecus nictitans mitis* (or *Cercopithecus albogularis* or *Cercopithecus mitis*), *Cercopithecus aethiops*, *Cercopithecus aethiops pygerytrhus*, *Cercopithecus neglectus*, *Colobus abyssinicus* (or *Colobus guereza*) and *Colobus angolensis*. These species show a clear ecological diversification and an extensive geographical distribution (Fleagle 1999, Kingdom 2001)

Dental casts of buccal surfaces of teeth were obtained from the original primate teeth in the osteological collection of the National Museum of Kenya (NMK) in Nairobi, using the surface activated *President microSystem™ Regular body* polyvinylsiloxane (*Coltène*®). Only one tooth was casted per specimen, normally the lower-left, second molar (LM_2) in order to standardize the analysis methods. Resin replicas where obtained from the casts using epoxy resin Epo-Tek #301. Dental molds where mounted on a stub for SEM image digitalization of the enamel surfaces. SEM images where obtained at 100× magnification using the Scanning Electron Microscopes Hitachi-2300 and Cambridge Stereoscan-120 at the *Serveis Cientificotècnics* (SCT) of the University of Barcelona. The images where taken following standard SEM procedures and were then processed with *Adobe Photoshop 5.0* for image enhancement (Pérez-Pérez *et al.* 1999). Microstriations where counted semi-automatically with *SigmaScan Pro* 5.0, measuring the total number of striations, their lengths, in micrometers (μm), and their orientation (Pérez-Pérez *et al.* 1999, Galbany *et al.* 2004b)

All the striations were classified into an orientation category, in relation to their anatomical position with regard to the cemento-enamel junction of the tooth. Thus, striations were classified as horizontal (H), vertical (V), mesiodistal (MD) or distomesial (DM). Finally, every analyzed tooth was characterized by the density of microstriations (N), their average length in micrometers (X) and the standard deviation of their length (S). Considering at the same time all the orientation categories and original variables measures, a total of 15 normally distributed variables, characteristic of each individual, could be derived. The statistical analyses were done with SPSS 11.0. For a more detailed description of these procedures refer to Pérez-Pérez *et al.* (1999).

RESULTS

Only 65 (30.4%) out of the 214 original teeth studied showed fully well-preserved buccal enamel surfaces, with microwear features clearly visible. There was a great amount of teeth in which microwear features could not be observed, despite all the samples belonged to properly curated, modern primate populations. There are several causes that do not allow for a good preservation of enamel surfaces in modern primates. The main one is the presence of patina on the buccal surface of teeth, a mineral cover on the enamel that impedes the observation of the enamel surfaces. Patinas are very common on buccal surfaces, but not so on occlusal ones (*Figure 1*). The presence of patina, a clear handicap that dramatically reduces the sample to be studied, affected all the species studied. The genus mostly affected by this mineral cover was *Colobus*, where only 11.4% of all the specimens showed unaffected, well preserved enamel surfaces (*Table 1*). This could be caused by the mainly leaf-eating habits of this species, which would drive to the presence of this mineral layer in order to protect the enamel surfaces from lateral abrasion. In contrast, *Papio anubis*, shows the highest percentage of well-preserved enamel surfaces (42.2%), with lack of patinas covering it.

There is a clear difference in the density of microstriations among the different genera studied, as shown for variable NT (total number of microstriation). *Colobus* is the genus that shows the lowest density of features on the enamel surfaces, while *Cercopithecus*, in a broad sense, shows the

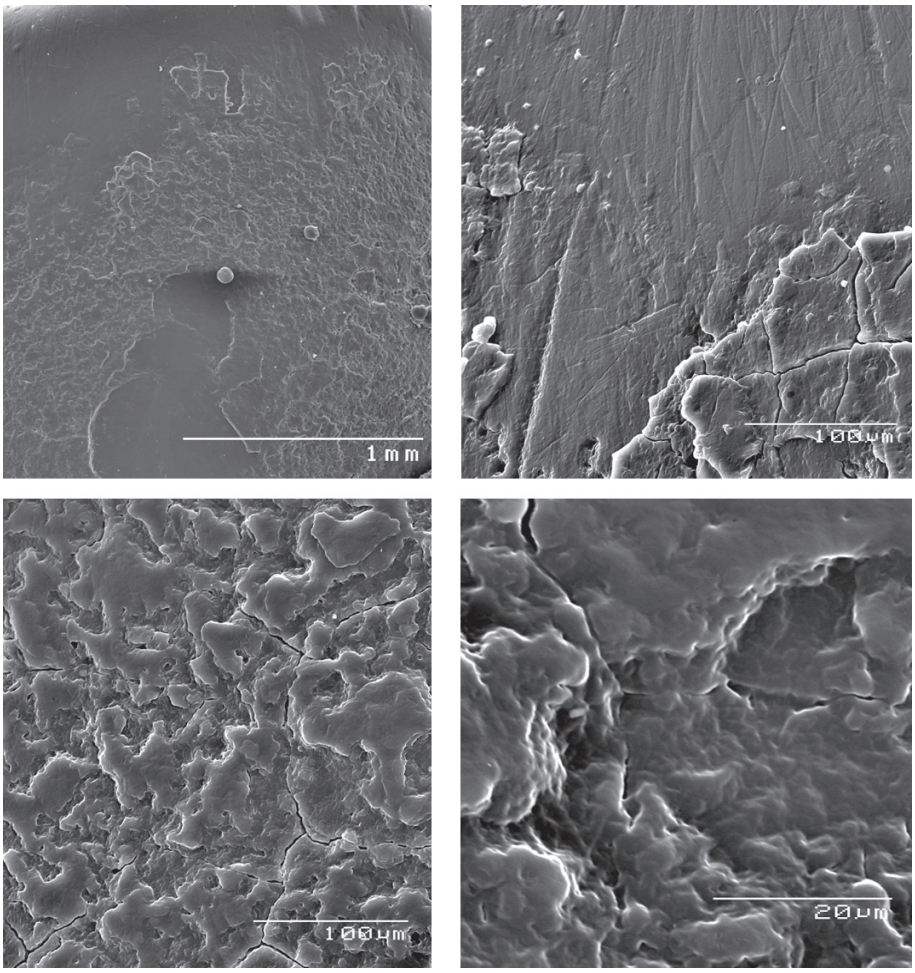


FIGURE 1. Buccal surface of molar teeth showing patina layers at different magnification levels. The pattern shown on the images is very frequent in leaf-eating monkeys (i.e. *Colobus*).

TABLE 2. One-factor ANOVA of the 15 microwear variables considered. Separate analyses were made at the species level (7 groups) and the genus level (3 groups). Almost all variables show significant differences at a 5 % significance level. Only NMD, XDM and SDM show no significant differences for both analyses considered.

Variable	Species		Genus	
	F	p-level	F	p-level
NH	2.808	0.018	6.755	0.002
XH	3.419	0.006	6.310	0.003
SH	2.609	0.026	4.813	0.011
NV	2.724	0.021	4.680	0.013
XV	3.240	0.008	7.326	0.001
SV	4.877	0.000	9.445	0.000
NMD	0.959	0.461	2.662	0.078
XMD	4.517	0.001	9.571	0.000
SMD	3.688	0.004	7.190	0.002
NDM	4.346	0.001	13.564	0.000
XDM	2.038	0.075	2.088	0.133
SDM	1.009	0.429	0.068	0.934
NT	6.833	0.000	17.941	0.000
XT	5.772	0.000	15.570	0.000
ST	4.988	0.000	13.063	0.000

TABLE 3. Bonferroni *post-hoc* test showing the differences between pairs of groups considered at genus level. (Ce: *Cercopithecus*, Co: *Colobus* and Pa: *Papio*). * P=0.05 and ** P=0.01 significance level.

Variables	Ce vs.Pa	Ce vs.Co	Co vs. Pa
NH	**		
XH	*		*
SH			*
NV		*	**
XV		*	**
SV		**	**
NMD			
XMD	**		**
SMD	**		*
NDM	**	**	
XDM			
SDM			
NT	**	**	**
XT	**		**
ST	**	*	**

FIGURE 2. Box-plot showing total number of microstriations (NT) of the *Cercopithecoidea* species analyzed. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).

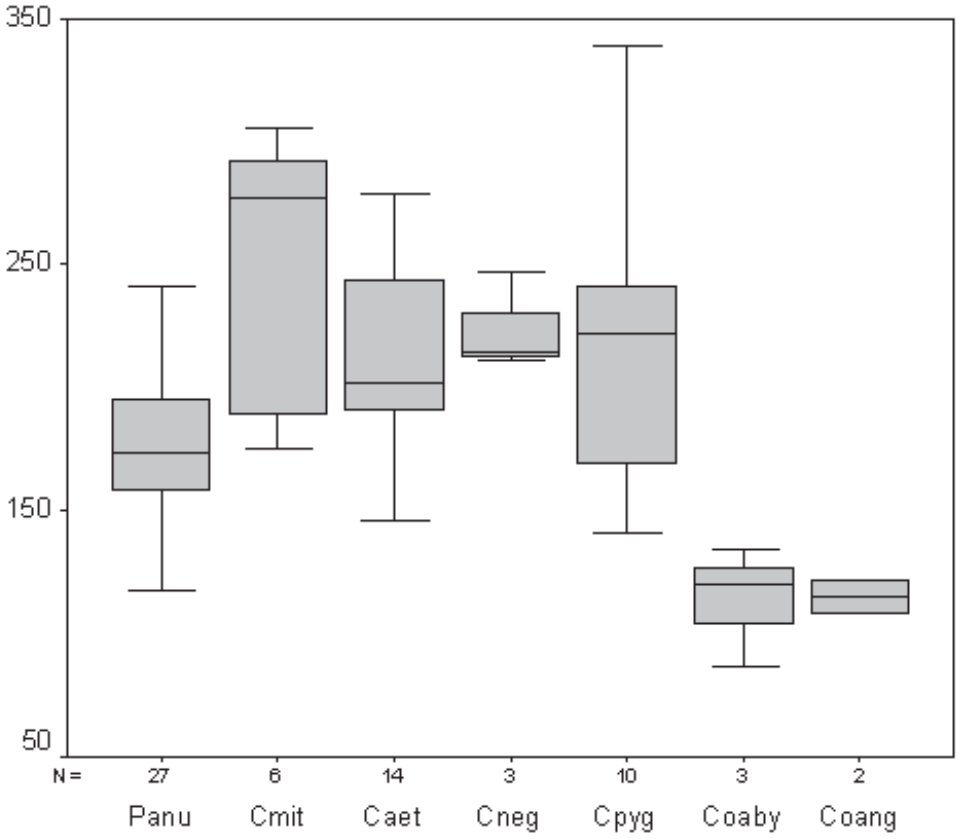


FIGURE 3. Box-plot showing average length of microstriations (XT) in micrometers (?m) in the *Cercopithecoidea* species analyzed. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).

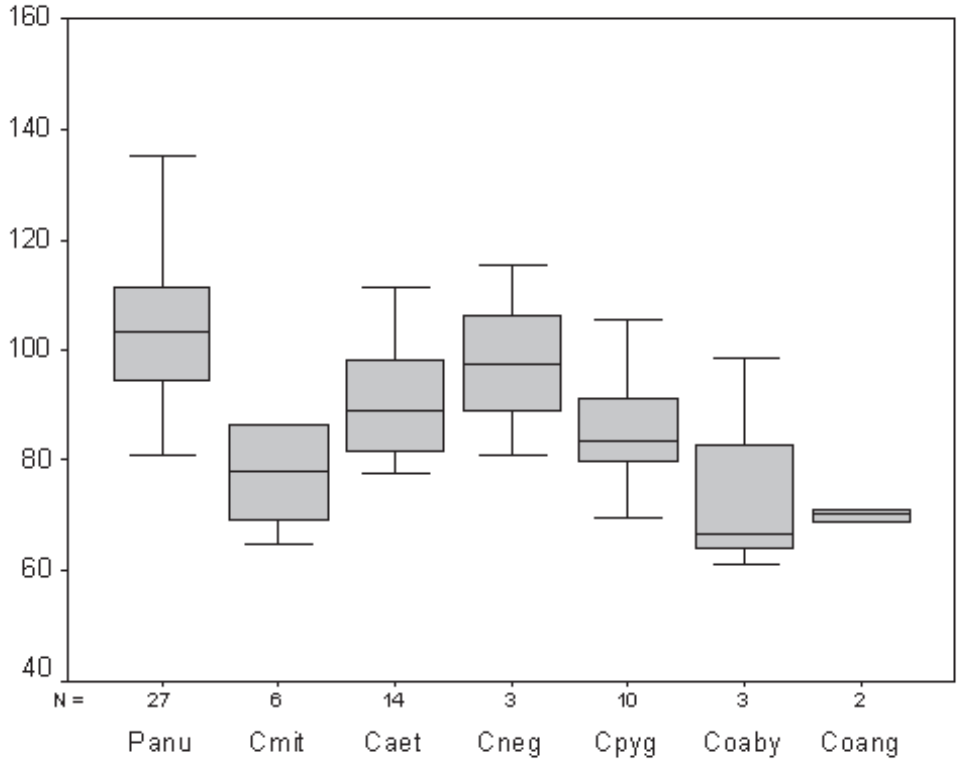
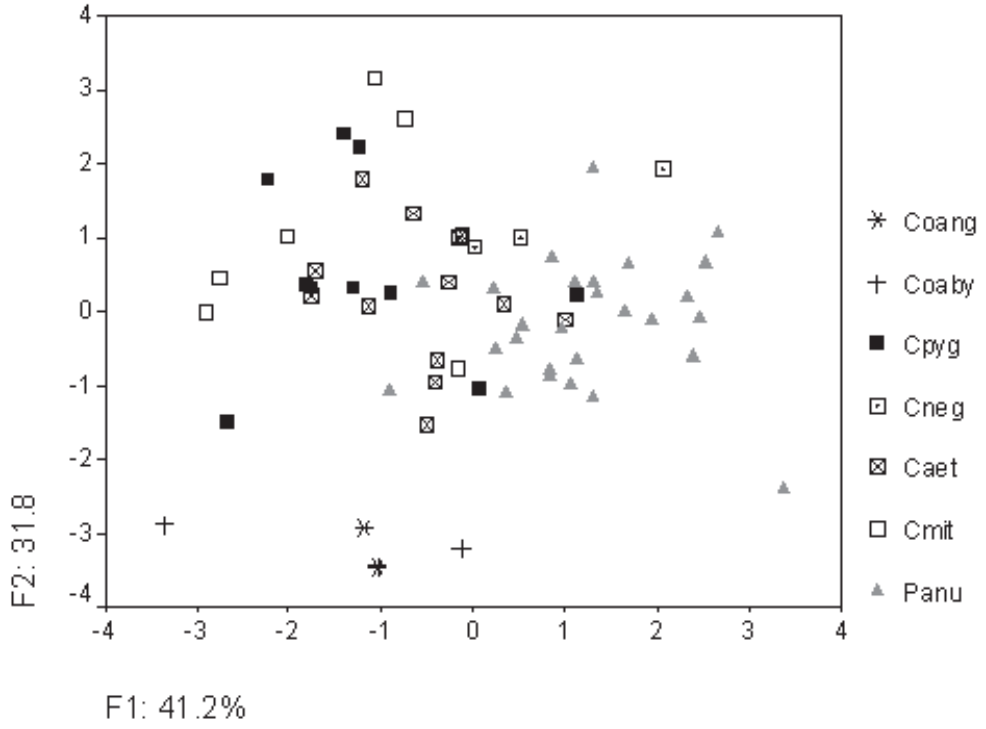


FIGURE 4. Plot of the first two components of the Discriminant Analysis for the *Cercopithecoidea* species studied. The first two functions derived explained 73.0% of total variance, with function 1 explaining 41.2% and function 2 explaining 31.8%. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).



highest NT value. *Papio anubis* is situated between both groups (*Figure 2*). Differences in the lengths of microstriations are also present between the different genera studied. *Papio anubis* shows the longest striations, while *Colobus* shows the shortest ones. One-factor ANOVA analyses show that almost all the analyzed variables significantly differ among the different species studied, as well as when the analyzed species are grouped into genera (*Table 2*). The main significant differences between pairs of genera can be seen with a Bonferroni *post-hoc* test (*Table 3*). In order to analyze the importance of the 15 variables considered, a multivariate Discriminant Analysis was done. The first two derived discriminant factors explain 73.0% of total variability (*Figure 4*). In the first function (explaining 41.2% of total variance) the highest correlations were obtained with variables XT (r=0.60), XDM (r=0.53), ST (r=0.51), SMD (r=0.45), XV (r=0.37) and NH (r=–0.32), while in the second function (31.8% of total variance) the highest correlations were with NT (r=0.72), NDM (r=0.45), NV (r=0.44) and NMD (r=0.27). All the analyzed species show significant between-groups differences. *Colobus* specimens are well grouped and tend to separate from the rest of species for the second function (*Figure 4*). *Cercopithecus* and *Papio* show a gradient for the first component of the discriminant analysis, being *Papio anubis* the species placed on positive values, and *Cercopithecus* that on the left-hand side, where the negative values are. The Discriminant Analysis is not only showing differences in the microwear patterns among the seven species considered, but also between genera. If a step-wise discriminant analysis is done, only variables NT and XT are included. NT strongly correlates with the second function (r=0.96), separating *Colobus* from the rest of species, and XT highly correlates with the first function (r=0.80), showing a clear distinction between *Papio* and the other *Cercopithecus spp.* groups. The descriptive box-plots of NT and XT (*Figures 2* and *3*) show clear between-species and between-genera differences. In general, *Colobus* shows lower densities of microstriations, as well as the shortest ones. *Cercopithecus* has the highest number of microstriations, with an intermediate mean length, and *Papio anubis* has the highest values for the length of the microstriations, but has a moderate mean number of striations. In addition, most of the analysed variables show differences between both species and genera (*Tables 2* and *3*). These results are in accordance to similar studies made for the cercopithecines, both for non-occlusal dental surfaces (Ungar, Teaford 1996), and occlusal ones (Teaford 1994, Ungar 1998).

DISCUSSION

Microwear patterns are clearly related to diet (Teaford 1994, Ungar 1998). Several studies have shown that *Colobus*, that has a mainly folivorous diet (Fleagle 1999, Crissey, Pribyl 1997), has very low wear rates (Teaford, Walker 1984, Teaford 1994, Ungar, Teaford 1996). On the

other hand, widely frugivorous diets are related to high wear rates and a high number of wear incidences (Teaford 1994, Ungar, Teaford 1996). This could be the situation of *Cercopithecus spp*. because fruit is the most important resource in guenons' diet (Beeson *et al.* 1996, Fleagle 1999). In *Cercopithecus mitis* fruit represents 75.5% of total ingestion (Rudran 1978), although generally guenons also eat other items, including foliar material, flowers or animals (Rudran 1978, Beeson, Lea 1994, Beeson *et al.* 1996). *Papio spp.* shows high microwear incidences in buccal surfaces (Ungar, Teaford 1996), and Daegling and Grine (1999) indicate that *Papio ursinus* shows one of the highest values of microwear on occlusal facets, in relation to the mean pit size and percentage, although no *Cercopithecus spp.* species were compared by them.

In the present study, similar values on the buccal microwear patterns between *Papio* and *Cercopithecus* have been found, clearly differing from *Colobus spp*. This may be explained in part by differences in diet composition between the two groups, as well as by differences in the incidences of terrestrial feeding events. As Ungar and Teaford (1996) suggested, the more terrestrial and/or frugivorous cercopithecines show high incidences of microwear on the buccal surfaces, whereas the more arboreal and folivorous colobines show fewer incidences. Moreover, the discriminant analyses made show a clear difference between *Colobus* and the rest of *Cercopithecoidea* for the second function, which strongly correlates with the total number of microwear features, whereas *Cercopithecus* and *Papio* can be discriminated by the first function, highly related to the mean length of the microstriations.

CONCLUSIONS

It is clear that there are some limiting factors to the study of dental microwear of buccal surfaces of non-human primates due to the presence of patina layers, very frequent in some genus as *Colobus*. The *Cercopithecoidea* analyzed in the present study show clear microwear differences on the buccal surface, coinciding with other studies (Teaford 1994, Ungar 1998, Ungar, Teaford 1996). Leaf-eating monkeys, such as *Colobus spp.*, show a low rate of microwear, as well as a very low number, and short microstriations. Guenons, *Cercopithecus spp.* show the highest wear rates, with a high number of microstriations, and longer on average than in *Colobus*. Finally, *Papio anubis* is placed between both groups, but has longer microstriations than *Cercopithecus*. All these results point to the existence of a clear relationship between dental microwear on buccal surfaces, diet composition, and ecological conditions in *Cercopithecoidea*. Leaf-eating monkeys (i.e. *Colobus*), feeding far from the ground, show low rates of dental microwear, despite leaves frequently include large amounts of phytoliths, probably due to a low dust accumulation on the top of trees, but a high presence of patina layers on buccal surfaces, that could be important to prevent excessive enamel abrasion. Guenons, mainly frugivorous, are unexpectedly associated with high wear rates, similar to *Papio anubis*, probably because of their terrestrial foraging, where more dust accumulation can cause longer striations.

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4.7 Variabilitat del patró de microestriació dental en Hominoidea: taxonomia *vs.* ecologia

Galbany J, Martínez LM & Pérez-Pérez A (2002) Variabilidad del patrón de microestriación dentaria en Primates Hominoideos: ¿cuestión de especie o de entorno ecológico?

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Variabilidad del patrón de microestriación dentaria en primates Hominoideos: ¿una cuestión de especie o de entorno ecológico?

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Palabras clave: microestriación dentaria, Primates, Hominoidea, hábitos alimentarios

El análisis del microdesgaste dentario es un buen indicador de la abrasividad de los alimentos consumidos por los Primates, y por los humanos y los Homínidos fósiles en particular. La abrasividad de la dieta es el resultado de la dureza de las partículas de alimento ingeridas, así como de la presencia de polvo y otras partículas externas, incorporadas al alimento durante su preparación, que son las causantes directas de la tipología de desgaste macroscópico y del microdesgaste del esmalte dental. El presente estudio analiza la variabilidad del microdesgaste dentario de las superficies vestibulares de los dientes de las grandes especies de Primates de la Superfamilia Hominoidea (*Gorilla gorilla*, *Pan troglodytes* y *Pongo pygmaeus*) a partir del estudio de moldes dentarios obtenidos de ejemplares originales de colecciones osteológicas de procedencia diversa. Al mismo tiempo se intentan explicar las posibles causas de dichos patrones de microestriación y como se relacionan con el hábitat ocupado por los diversos subgrupos poblacionales. Los resultados obtenidos sugieren que los procesos de adaptación ecológica son básicos en la configuración del patrón de microestriación dentaria, por encima de los modelos de diferenciación alimentaria a nivel de especie.

Introducción

El patrón de microestriación dentaria de los primates está directamente relacionado con las condiciones ecológicas y la composición de la dieta (Teaford 1994, Ungar 1998, Ungar & Teaford 1996). Las partículas duras, como los fitolitos presentes en los vegetales, así como el polvo acumulado en los alimentos, pueden producir estriaciones microscópicas en el esmalte de las piezas dentarias durante la masticación. Aunque la relación entre la naturaleza de los alimentos y la tipología del microdesgaste es clara, no se conoce cual es el mecanismo que las produce o como determinadas condiciones ecológicas y composiciones diversas de la alimentación afectan al patrón de microestriación final formado en la cara bucal de los dientes. Un buen conocimiento de los patrones de microestriación en primates servirá como modelo de referencia para la interpretación de la composición de la dieta, tanto de los primates como de poblaciones humanas de épocas pasadas. El presente estudio se centra en la caracterización de la variabilidad del patrón de microestriación dentaria en la cara bucal de las tres grandes especies actuales de Primates Hominoidea, analizando la variabilidad a nivel específico y geográfico.

Material y métodos

A partir de una gran muestra de moldes de dientes de primates Hominoidea actuales (Galbany *et al.*, 2004a) obtenidos de las colecciones osteológicas del *American Museum of Natural History* (Nueva York) y el *Natural History Museum* (Londres), se seleccionaron ejemplares adultos que nunca habían vivido en condiciones de cautividad y que presentaban superficies

bucales de los molares con el esmalte bien preservado. Además solo se consideraron ejemplares de origen conocido o aquellos que procedían de regiones geográficas claramente identificables y bien representadas. La muestra final analizada consistió en un total de 62 ejemplares, representados cada uno por un solo diente, un molar, pertenecientes a tres especies de Hominoidea actuales (*Gorilla gorilla gorilla*, *Pan troglodytes schweinfurthii* y *Pan troglodytes troglodytes*, y *Pongo pygmaeus pygmaeus*), agrupadas en 6 categorías (Tabla 1). Los grupos así considerados representan modelos ecológicos con dietas claramente diferenciadas procedentes de contextos ecológicos y geográficos bien conocidos.

Tabla 1. Especies analizadas de Hominoidea, lugar de origen, número de muestras analizadas y colección osteológica de procedencia (AMNH: American Museum of Natural History – New York; NHML: Natural History Museum – London)

Especie	Origen	N	Colección osteológica
G. g. gorilla	Camerún	18	AMNH y NHML
	Nigeria	3	NHML
	Congo	5	NHML
P. t. troglodytes	Camerún	6	AMNH y NHML
P. t. schweinfurthii	R.D.Congo (Ex-Zaire)	6	AMNH y NHML
P. p. pygmaeus	Borneo	24	AMNH y NHML
	TOTAL	**62**	

Utilizando los dientes originales *in situ* se obtuvieron moldes dentarios, usando el polivinilsiloxano *President Microsystem, Regular body* de *Colténe*, de las caras bucales de una única pieza dentaria por individuo, principalmente los segundos molares inferiores (M_2) izquierdos, con el fin de estandarizar la metodología de trabajo. A partir de los moldes negativos obtenidos, se realizaron réplicas con resina Epo-Tek 301 de Química del Aditivo S.L. (QdA). Para una revisión detallada de la metodología utilizada véase Pérez-Pérez *et al.* (1999) y Galbany *et al.* (2004b).

Con el microscopio electrónico de barrido se observaron y fotografiaron de manera sistemática las superficies vestibulares de todos los dientes analizados. Los equipos de los *Serveis Científico-Tècnics* de la Universitat de Barcelona (SCT-UB) utilizados fueron el microscopio electrónico de barrido Hitachi H-2300 y el microscopio Cambridge Stereoscan S-120. Los moldes se situaron en posición horizontal, sin inclinación o *tilt*, y todas las fotografías fueron tomadas a 100× aumentos en el tercio medio de la cara vestibular bajo una de las cúspides. Las imágenes se digitalizaron posteriormente con una resolución de 2031×1354 pixels con el *software* Image Slave del propio microscopio y se trataron con el programa Photoshop v. 5.0 de Adobe, seleccionando la zona mejor conservada de un área estandarizada de 0,56 mm^2. A continuación se aplicó el filtro "*high pass*" utilizando 50 pixels de radio y se realizó un ajuste automático de los niveles de gris. Este tratamiento se aplicó para maximizar la resolución de la imagen sin alterarla. El resultado final puede verse en el ejemplo presentado en la Figura 1 que corresponde a una imagen de la cara bucal de un molar de *Pan troglodytes troglodytes* de Camerún (NHML - 24.8.6.1).

Las microestriaciones observables en la superficie de esmalte dentario considerada se contaron manualmente con el programa *Sigma Scan* de *Jandel Scientific*, considerando como definición de estría aquellos objetos observables sobre el esmalte, producidos por abrasión, que tuvieran una longitud de más de 15 μm, sin considerar su curvatura, y que como mínimo fueran cuatro veces más largos que anchos. Para cada microestriación contada, automáticamente *Sigma Scan* registra su longitud y su orientación respecto al plano horizontal representado por la línea amelo-cementaria del diente. En función de la posición anatómica de cada diente, las

estriaciones fueron clasificadas, en base a su orientación, en cuatro categorías: Horizontal (H), Vertical (V), Mesiodistal (MD) y Distomesial (DM). Finalmente, cada diente se caracterizó por el número de estrías presentes o densidad (N), su longitud media en μm (X) y la desviación típica de dicha longitud (S), teniendo en cuente cada orientación. De ésta manera se obtuvieron 15 variables métricas características de cada individuo y que constituye su patrón de microestriación vestibular (véase Pérez-Pérez *et al.* 1999 para una descripción más detallada). Los cálculos se realizaron con el paquete estadístico SPSS v.11.

Figura 1. Imagen a 100 aumentos de una superficie de esmalte bien preservado con microestriaciones producidas por partículas abrasivas de los alimentos. Molar de *Pan troglodytes troglodytes* del Camerún depositado en el Natural History Museum –London (NHML - 24.8.6.1)

Resultados

Se obtuvieron las frecuencias de las 15 variables que caracterizan el patrón de microestriación dentaria de los 62 individuos analizados. Las Figuras 2 y 3 muestran diagramas de cajas de las variables para las variables NT (número total de estrías) y XT (longitud media de las estrías en micrómetros) para cada uno de los grupos considerados. Se observa que para estas dos variables solo existen diferencias entre los grupos para XT (longitud de las estrías totales). Un análisis de la varianza posterior (Tabla 2) muestra diferencias significativas entre los 6 grupos analizados solo para 6 de las 15 variables: XH (longitud de las estrías horizontales), SH (dispersión de las longitudes de las estrías verticales), NV (número de estrías verticales), XV (longitud de la estrías verticales), XT (longitud del número total de estrías y ST (dispersión de las longitudes del número total de estrías).

Finalmente, un análisis discriminante (Figura 4) permite visualizar las diferencias entre los grupos en un gráfico de dos dimensiones con las dos primeras funciones obtenidas, que explican el 95,5 % de la variabilidad total. Dicho análisis se ha limitado únicamente a 5 grupos, excluyendo a *Pongo p. pygmaeus*, para evitar solapamiento excesivo de los grupos en el gráfico. La primera función, que explica el 58,9 % de la variabilidad, esta fuertemente correlacionada con la variable NV (0,373). La segunda función explica el 36,5 % de la variabilidad, y presenta una correlación negativa con varias variables: XH (-0,696), SH (-0,522), XT (-0,488), XDM (-0,350) y XV (-0,300). La variable NT (número total de estrías) presentó una correlación significativa con las otras variables y no entró en el análisis.

El hecho de no haber incorporado al género *Pongo* en la Figura 4 por tener una gran dispersión y solapamiento con las otras especies, permite apreciar una clara separación de los otros tres grupos de *G. gorilla* considerados, aunque pertenecen a la misma subespecie. Contrariamente, los dos grupos de chimpancés, aun siendo de subespecies distintas, quedan cercanos entre ellos y solapados con los gorilas de Camerún.

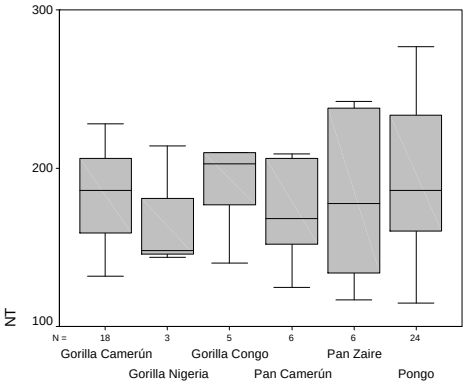

Figura 2. Diagrama de cajas de la variable NT (número total de estrías) para los grupos de Hominoidea analizados

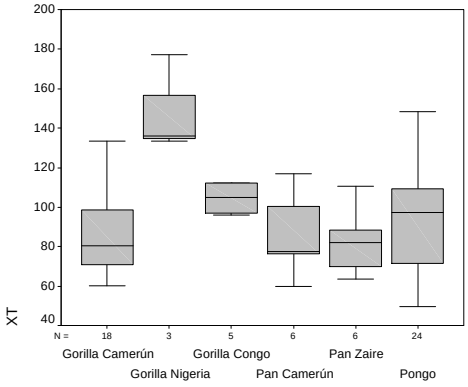

Figura 3. Diagrama de cajas de la variable XT (longitud de las estrías totales) para los grupos de Hominoidea analizados

Discusión

Al igual que en otros estudios que hemos realizado con anterioridad (Galbany *et al.* 2003), los resultados obtenidos no muestran grandes diferencias entre los patrones de microestriación a nivel de especie. Sin embargo, en este análisis mostramos por primera vez diferencias debidas a la variabilidad de hábitats y nichos ecológicos donde se encuentran estas especies. *Gorilla g. gorilla*, que ocupa una gran variedad de hábitats, desde bosques tropicales, formaciones secundarias e incluso formaciones herbáceas (Doran & McNeilage, 1998) refleja distintas especializaciones alimentarias en los grupos analizados debidas mayoritariamente a razones ecológicas.

A diferencia de los distintos grupos de *Gorilla gorilla gorilla* considerados, que presentan una gran variabilidad del patrón de microestriación dentaria a nivel geográfico, en los chimpancés no sucede lo mismo, y muestran una mayor homogeneidad en sus patrones de microestriación dentaria, incluso perteneciendo a subespecies de zonas distintas, tal y como se apunto en el estudio de Galbany *et al.* (2003). Las diferencias encontradas, sobretodo en *Gorilla*, pueden ser debidas a estrategias de aprovechamiento de los recursos alimentarios diferentes entre las distintas poblaciones.

Tabla 2. Análisis de la varianza de los grupos de Hominoidea considerados – Anova 1 factor- para las 15 variables obtenidas. En sombreado se indican las diferencias significativas

Variable	F	P-valor
NH	0.317	0.901
XH	6.516	0.000
SH	3.506	0.008
NV	3.350	0.010
XV	2.690	0.030
SV	1.505	0.203
NMD	0.906	0.484
XMD	1.802	0.127
SMD	1.493	0.207
NDM	0.802	0.553
XDM	2.051	0.085
SDM	1.467	0.215
NT	0.691	0.632
XT	4.298	0.002
ST	3.915	0.004

Por otro lado, las poblaciones de *Gorilla g. gorilla* de Camerún y del Congo se encuentran geográficamente cercanas y, asimismo, los resultados indican un gradiente en la primera función del análisis discriminante (Figura 4) para los patrones de microestriación dentaria entre ambas poblaciones. Contrariamente, la población de Nigeria (realmente ubicada entre Nigeria y Camerún) se encuentra alejada y aislada del resto de poblaciones de *Gorilla g. gorilla* (Doran & McNeilage, 1998). Esta población de gorilas ha sido asignada por parte de varios autores a una subespecie distinta: *Gorilla gorilla dielhi* (Morgan *et al.* 2003; Sarmiento y Oates, 2000). El aisla-

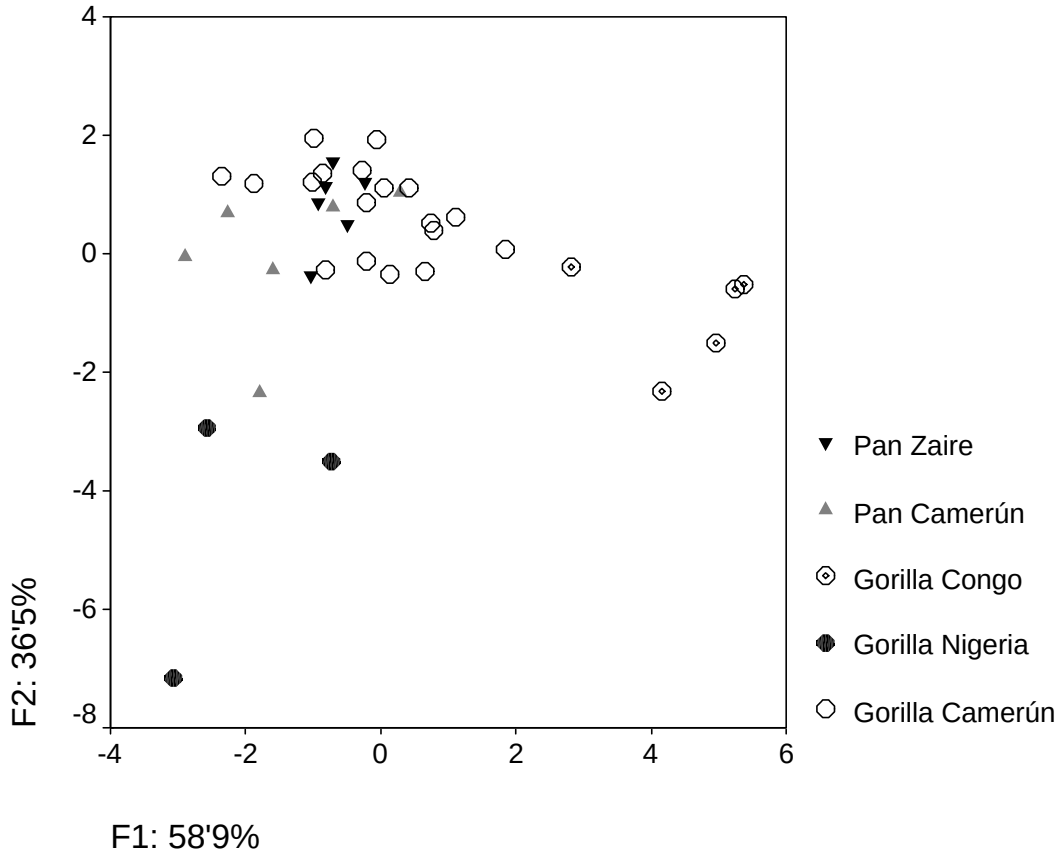

Figura 4. Representación gráfica de las dos primeras funciones del análisis discriminante para los grupos analizados (excepto *Pongo p. pygmaeus*). F1: 58,9 % y F2: 36,5 % de la varianza total

miento geográfico y la diferencia de hábitat podrían ser los causantes de las diferencias de dieta que producirían un patrón de microestriación también distinto en esta población, aunque faltan estudios ecológicos y de alimentación que lo corroboren.

Por lo que respecta a los chimpancés, *Pan t. troglodytes* de Camerún presenta un patrón de microestriación muy parecido al de los gorilas de la misma región geográfica, solapándose en la representación gráfica de las dos primeras funciones. Esta situación puede deberse a que estas dos especies simpátridas presentan un modo similar de explotación de recursos en un mismo hábitat. En este sentido, Tutin y Fernández (1994) apuntan que *Pan t. troglodytes* y *Gorilla g. gorilla* en la Reserva de Lopé, en Gabón central, comparten 127 ítems alimenticios, mayoritariamente frutos, que suponen una superposición del 82 % respecto al total de alimentos ingeridos. Además, el mismo estudio afirma que las técnicas de procesado de los alimentos también son compartidas por ambas especies. Parece claro, pues, que un mismo hábitat y un aprovechamiento de los recursos similar, pueda causar un patrón de microestriación parecido.

Finalmente, el presente estudio muestra que *Pongo p. pygmaeus* presenta una gran dispersión en el patrón de microestriación dentaria, que no se ha considerado en el análisis discrimi-

nante para no enmascarar las pequeñas diferencias entre los otros grupos. Esta gran variabilidad puede ser debida a múltiples causas, entre las más importantes hay considerar la variabilidad debida a las diferencias sexuales (Galbany *et al.* 2003), ya que el orangután es una especie muy dimórfica y con diferencias en la tipología de la alimentación según el sexo (Fleagle, 1999; Sugardjito y Nurhuda, 1981; Utami y Van Hooff, 1997). No ha sido posible separar los especimenes de *Pongo p. pygmaeus* en localidades concretas por falta de información, aunque sean todos de la isla de Borneo. En general, *Pongo* es uno de los grupos de Hominoideos con mayor número de estrías (Figura 2), lo que se asocia a una dieta con un fuerte componente de vegetales duros, como pueden ser hojas, tallos y cortezas (Delgado y Van Schaik, 2000).

Así pues, el presente estudio demuestra que el patrón de microestriación dentaria no solo puede poner en evidencia la variabilidad alimentaria interespecífica, sino que existe una gran componente ecológica que afecta al patrón de microestriación vestibular independiente de las especies consideradas, y que pone de relieve estrategias diversas de aprovechamiento de los recursos. Hay que tener en cuenta, para futuros estudios, nuevas variables causantes de variabilidad como son las diferencias de alimentación relacionadas con la estacionalidad y las épocas de baja disponibilidad de recursos, en las que los primates consumen una mayor proporción de hierba, hojas y cortezas (Rogers *et al.*, 1990; Rogers *et al.*, 1994; Remis *et al.*, 2001; Tutin *et al.*, 1997), al mismo tiempo que tener las posibles diferencias sexuales en la explotación de los recursos.

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Abstract

The analysis of the microwear pattern of teeth is a good indicator of the abrasiveness of food stuffs in the diet in Primates, as well as in extant and fossil Hominids. The abrasive capability of the diet depends on the hardness of the particles included in the ingested foods and on the presence of dust and other exogenous elements incorporated during the process of food processing. These items are responsible for the typology of the microwear that can be observed on the enamel surfaces of Primate teeth. The present study analyses the variability of the buccal microwear pattern on the great Hominoidea Apes (*Gorilla gorilla*, *Pan troglodytes* y *Pongo pygmaeus*) using tooth moulds obtained from the original specimens at different osteological collections. Several explanations for the differences in microwear patterns observed are discussed. The results seem to indicate that the ecological adaptations at a subspecies level may account for such differences in relation to habitat and ecological conditions within populations rather than between species.

Dental microwear pattern variability in Hominoidea Primates: a question of species differentiation or ecological determinants?

Key words: microwear pattern, Primates, Hominoidea, dietary habits

4.8 El patró de microestriació dental i la interpretació de la dieta dels primats fòssils

Galbany J, Moyà-Solà S & Pérez-Pérez A (2005). Dental microwear variability on buccal tooth enamel surfaces of extant Catarrhini and fossil Miocene Hominoidea *Dryopithecus laietanus*.

Folia Primatologica 76(6): 325-341.

El patró de microestriació dental vestibular presenta una gran correlació amb l'abrasivitat de la dieta en els primats actuals, tant *Cercopithecoidea* (Galbany & Pérez-Pérez, 2004) com *Hominoidea* (Galbany et al., 2002).

Aquest treball analitza la variabilitat d'aquest patró en el conjunt de primats *Hominoidea* i *Cercopithecoidea*, comparant-lo alhora amb els patrons de microestriació de les espècies de primats fòssils *Dryopithecus laietanus* i *Oreopithecus bambolli*, per tal d'establir el grau d'abrasió de la dieta d'aquestes espècies fòssils. Aquest tipus d'estudi s'ha realitzat en nombroses ocasions per a diverses espècies de primats fòssils (Teaford & Walker, 1984; Walker et al., 1994; Ungar & Kay, 1995; Teaford et al., 1996; Ungar, 1996, 1998).

S'han replicat amb polivinilsiloxà *President microSystem™* (*Coltène®)* i resina epoxy Epo-Tek #301 (Galbany et al., 2004a) un total de 362 molars d'individus procedents de diverses col·leccions osteològiques (Taula 1). A partir de les rèpliques, i seguint metodologies estàndard amb el Microscopi Electrònic d'Escombrat (SEM), es van obtenir imatges digitals que van ser analitzades amb les metodologies habituals (Pérez-Pérez et al., 1999; Galbany et al., 2004a).

Els resultats mostren que només 111 dents presenten patrons de microestriació dental en bon estat de conservació (Taula 1 i Figura 1). La variabilitat de la microestriació dental en els primats *Catarrhini* és elevada, tal i com mostren els diagrames de caixes de les variables NT (densitat total d'estries) i XT (longitud promig de les estries) (Figura 2 i Taula 3). Una anàlisi de la variança de les 15 variables que caracteritzen el patró de microestriació troba diferències significatives entre les espècies a 9 d'elles (Taula 4).

Els primats *Hominoidea* són els que presenten major homogeneitat del patró de microestriació vestibular en comparació als *Cercopithecoidea*. Les espècies més heterogènies en el seu patró de microestriació són *Pongo pygmaeus* i *Cercopithecus aethiops*.

La comparació del patró de microestriació dels espècimens fòssils de *Dryopithecus laietanus* i *Oreopithecus bambolli* amb el patró general de microestriació dental permet determinar quina és la seva posició dins la variabilitat total. Una anàlisi discriminant de totes les variables mostra que les dues espècies de primats fòssils difereixen en els patrons de microestriació dental. El 80% dels *Dryopithecus laietanus* analitzats (8 de 10) es classifiquen

com a similars a *Gorilla gorilla*, amb una probabilitat de classificació d'entre el 94,2% i el 100%. Per contra, l'únic espècimen d'*Oreopithecus bambolli* que va donar resultats fiables (Bac62) es va classificar com a *Papio anubis* amb una probabilitat del 99,1%.

La Figura 3 mostra la representació gràfica de les dues funcions discriminants. Tot i el solapament d'alguns grups, es pot observar una certa separació entre ells. Els *Cercopithecoidea* es distribueixen al llarg de la segona funció, correlacionada amb les variables NT (densitat total d'estries), XT (longitud promig de les estries) i NMD (densitat de les estries obliqües mesio-distals). Al llarg d'aquest gradient, els *Colobus* presenten els valors més baixos i els *Cercopithecus* els més elevats.

Dryopithecus laietanus presenta valors baixos a la segona funció, similars als goril·les, mentre que *Oreopithecus bambolli* se situa entre *Colobus* i *Papio anubis*. La caracterització de la dieta d'aquests primats fòssils pot contradir les conclusions d'estudis previs basats en morfologia dental (Ungar & Kay, 1995). La gran similitud de *Dryopithecus laietanus* i *Gorilla gorilla* indica que aquest primat podria haver consumit recursos tròfics abrasius, tals com plantes herbàcies, fulles o medul·les, tot i que no es descarta el consum de fruits, com fan els goril·les. *Oreopithecus bambolli*, tot i la reduïda mostra analitzada, sembla que presenta un patró de microdesgast proper al dels papions, és a dir, més omnívor. Aquest resultat també és contradictori amb el d'altres autors que consideren que aquest primat presentava una dieta basada en el consum majoritari de fulles (Carnieri & Mallegni, 2003; Ungar & Kay, 1995; Ungar, 1996).

La interpretació de la dieta de primats fòssils en base als patrons de microestriació dental requereix encara de més anàlisis i un millor coneixement de la variabilitat poblacional dels patrons de microestriació, així com noves estandarditzacions metodològiques. Els patrons de microestriació vestibular, que no es veuen afectats per l'atrició deguda al contacte entre dents, present a les facetes oclusals, sembla un bon indicador de les relacions entre abrasió dental i la dieta en primats, així com en homínids (Pérez-Pérez et al., 1999, 2003b; Galbany et al., 2002; Galbany & Pérez-Pérez, 2004).

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Dental Microwear Variability on Buccal Tooth Enamel Surfaces of Extant Catarrhini and the Miocene Fossil *Dryopithecus laietanus* (Hominoidea)

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Key Words
Buccal microwear · Scanning electron microscopy · Hominoidea · Cercopithecoidea · *Dryopithecus laietanus*

Abstract

Analyses of buccal tooth microwear have been used to trace dietary habits of modern hunter-gatherer populations. In these populations, the average density and length of striations on the buccal surfaces of teeth are significantly correlated with the abrasive potential of food items consumed. In non-human primates, tooth microwear patterns on both occlusal and buccal wear facets have been thoroughly studied and the results applied to the characterization of dietary habits of fossil species. In this paper, we present inter- and intra-specific buccal microwear variability analyses in extant Cercopithecoidea *(Cercopithecus mitis, C. neglectus, Chlorocebus aethiops, Colobus* spp.*, Papio anubis)* and Hominoidea *(Gorilla gorilla, Pan troglodytes, Pongo pygmaeus).* The results are tentatively compared to buccal microwear patterns of the Miocene fossils *Dryopithecus* and *Oreopithecus.* Significant differences in striation density and length are found among the fossil taxa studied and the extant primates, suggesting that buccal microwear can be used to identify dietary differences among taxa. The *Dryopithecus* buccal microwear pattern most closely resembles that of abrasive, tough plant foods consumers, such as the gorilla, in contrast to studies of dental morphology that suggest a softer, frugivorous diet. Results for *Oreopithecus* were equivocal, but suggest a more abrasive diet than that previously thought.

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Introduction

Dental microwear patterns on occlusal tooth surfaces of extant primates [Teaford and Walker, 1984; Ungar, 1990, 1994a, b; Teaford and Glander, 1991; Teaford, 1994] have proved to be very useful in the interpretation of fossil primate dietary habits. Occlusal microwear patterns have been applied to fossil specimens of *Sivapithecus* [Teaford and Walker, 1984] and *Proconsul* [Walker et al., 1994], as well as to Oligocene [Teaford et al., 1996] and Euro-Asiatic Miocene primates, such as *Pliopithecus, Dryopithecus, Anapithecus, Ouranopithecus* and *Oreopithecus* [Ungar and Kay, 1995; Ungar, 1996, 1998]. Using a combination of occlusal microwear and dental morphology, Ungar and Kay [1995] found that the best modern diet analogues for *Oreopithecus* might be the colobines and howler monkeys, with a specialized folivorous diet. By contrast, *Dryopithecus* shows shearing crests similar to the extant frugivorous gibbons and chimpanzees, suggesting it had a soft, fruit-based diet, lacking specializations for both hard-object feeding and extreme folivory [Ungar, 1996]. Ungar also indicates that the differences in mean pit to scratch ratios in the occlusal phase II facets between *Dryopithecus* and *Oreopithecus* are similar to those between *Pan troglodytes* and the folivores *Alouatta palliata* and *Colobus guereza,* respectively [Ungar, 1996].

Microwear research has been extensively applied to fossil hominids, both on occlusal and buccal enamel surfaces [Puech, 1982, 1984, 1986a, b; Grine, 1986, 1987; Lalueza and Pérez-Pérez, 1993; Lalueza et al., 1993, 1996; Pérez-Pérez et al., 1999, 2003]. Buccal microwear studies on modern human hunter-gatherer populations have shown a significant variability of striation patterns in relation to dietary habits and ecological conditions [Pérez-Pérez et al., 1994; Lalueza et al., 1996]. Also, a certain degree of age- and sex-dependent, within-group variability has been shown in the *Homo heidelbergensis* sample from Sima de los Huesos in Atapuerca [Pérez-Pérez et al., 1999]. Although great numbers of microwear studies in extant primates are available for occlusal wear facets [e.g., Teaford, 1985; Teaford and Oyen, 1989; Teaford and Robinson, 1989; Gordon, 1992; Ungar, 1998], analyses of buccal microwear patterns on extant primates are rare [Ungar 1994a; Ungar and Teaford, 1996; Galbany et al., 2002; Galbany and Pérez-Pérez, 2004]. Such studies on the buccal surfaces of teeth could serve as key references for the interpretation of dietary habits of fossil primates, and to test dietary hypotheses derived from microwear of occlusal shearing and grinding tooth surfaces, and from analyses of gross dental morphology. This paper presents the first intra- and inter-species variability analysis of the buccal microwear pattern of extant anthropoids.

Methods

Extant Primates

The extant primate sample studied (table 1) included a total of 100 specimens belonging to eight anthropoid primate species: *Chlorocebus aethiops, Cercopithecus mitis, C. neglectus, Papio anubis, Colobus* spp. (including *C. abyssinicus*, or *C. guereza* and *C. angolensis*), *Gorilla gorilla gorilla, Pan troglodytes troglodytes* and *Pongo pygmaeus pygmaeus.* The skeletal collections studied are at the American Museum of Natural History in New York, the National Museums of Kenya in Nairobi, and the Natural History Museum in London. All the primate specimens studied were captured and killed in the wild, and are as-

Table 1. Sample sizes of extant Hominoidea and Cercopithecoidea and fossil species examined (casts examined) and the number included in the buccal microwear analysis (casts analysed)

Species	Casts examined	Casts analysed	Origin	Osteological collections
Gorilla g. gorilla	32	17	Cameroon	AMNH, NHML
Pan t. troglodytes	38	9	Cameroon, Gabon, Nigeria	AMNH, NHML
Pongo p. pygmaeus	49	24	Borneo	AMNH, NHML
Cercopithecus mitis	37	6	Kenya	NMK
Cercopithecus neglectus	7	3	Kenya	NMK
Chlorocebus aethiops	41	9	Kenya	NMK
Papio anubis	64	27	Kenya and North Tanzania	NMK
Colobus spp.	44	5	Kenya	NMK
Dryopithecus laietanus	35	10	Fossil taxa	MPMC
Oreopithecus bambolli	15	1	Fossil taxa	MPMC
Total	362	111		

Geographical origin and osteological collections. AMNH = American Museum of Natural History; NHML = Natural History Museum of London; NMK = National Museums of Kenya; MPMC = Museu Paleontològic Miquel Crusafont, from Sabadell Barcelona.

sumed to have fed using natural strategies depending on the ecological conditions of the environment. The specimens studied were selected to represent a limited geographical distribution in Africa and Asia (table 1). The eight extant species represent a wide range of ecological and dietary adaptations [Kingdom, 2001].

Cercopithecus spp. use both ripe and unripe fruits as their most important food resource, although some *Cercopithecus* spp. may include leaves, buds, flowers and invertebrates [Beeson et al., 1996; Fleagle, 1999; Strier, 2000; Tweheyo and Obua, 2001; Nakagawa, 2003]. Fairgrieve and Muhumuza [2003] have demonstrated that different populations of the same guenon species may show significant differences in diet. *Colobus* spp. are mainly leaf eaters [Baranga, 1983; Crissey and Pribyl, 1997; Usongo and Amubode, 2001], but show a certain degree of fruit consumption [Davies et al., 1999; Fashing, 2001]. *Papio* sp. is a terrestrial forager from open savannah environments that feeds from various parts of the *Acacia* trees, eating leaves, blossoms, seeds and seed pods, as well as grass seeds, grass blades, tubers and some fruits. Animal foods are sometimes included in the diet of *Papio* in the form of eggs, insects, some birds and small reptiles and mammals [Altmann and Altmann, 1970; Rowel, 1979; Wahungu, 1998; Pochron, 2000; Hill and Dunbar, 2003].

The Hominoidea sample includes three subspecies with distinct geographical provenances: *G. g. gorilla* exclusively from Cameroon, *P. t. troglodytes* from Cameroon, Gabon and Nigeria, and *P. p. pygmaeus* from Borneo. The gorillas show well-developed tooth cusps associated with a mainly folivorous diet [Fleagle, 1999], although they are not strict leaf eaters, except in habitats that allow no alternatives [Rogers et al., 1990]. Western lowland gorillas usually include sweet, succulent fruits, making up to 50% of the food ingested, depending on seasonal fluctuations and availability [Jones and Sabater Pi, 1971; Sabater Pi, 1977; Remis, 1997; Tutin et al., 1997; Doran and McNeilage, 1998], but the diet may vary according to locality and activities [Jones and Sabater Pi, 1971; Tutin and Fernández, 1994], and may also include aquatic, herbaceous vegetation, leaves, bark, cambium and phloem of trees or lianas. Chimpanzees' diets vary greatly from one population to another, not only because of ecological differences in habitats but also because of traditional preferences [Estes, 1997; Malenky et al., 2001]. The chimpanzees feed mainly on fruits and nuts, these making up to about 60% of their total food intake, and on leaves and stems, which make up

Table 2. Fossil specimens analysed from the Museu Paleontològic Miquel Crusafont, Sabadell (Spain)

Reference	Tooth	Side	Jaw
Dryopithecus			
CLL1764	M2	R	M
CLL1780	M1	R	M
CLL1771	M2	L	X
CP1821	M2	L	M
TI1804	M2	L	M
CLL18000	M2	R	X
CLL1796	M2	L	M
CLL1763	M1	R	X
CLL1802	M2	R	M
TI1803	M2	R	M
Oreopithecus			
Bac62	M2	L	X

CLL = Can Llobateres; CP = Can Ponsic; TI = La Tarumba I; M2 = second molar; M1 = first molar; R = right; L = left; M = mandible; X = maxilla.

around 20% of their food intake. Seasonal shifts in diet have also been reported [Sabater Pi, 1979; Fleagle, 1999]. Chimpanzees' diet also includes bark, resin, flowers and seeds, although these may represent only a small proportion of it [Yamakoshi, 1998; Nishida et al., 2000]. Protein, in the form of insects and vertebrates, may form up to 5% of their diet [Wrangham, 1984, Wrangham and Riss, 1990]. Finally, the orangutans have diets with a high diversity of fruits, mainly soft pulp fruits [Delgado and Van Schaik, 2000] but also some hard-seed fruits [Fleagle, 1999]. They also consume young and soft leaves, shoots, seeds, buds, flowers and inner bark [Delgado and Van Schaik, 2000].

Dryopithecus laietanus *and* Oreopithecus bambolli

The fossil *Dryopithecus* teeth sample studied includes all the specimens at the Museu Paleontològic Miquel Crusafont in Sabadell, Spain. The analysed sample consisted of 10 well-preserved teeth of different specimens of *Dryopithecus laietanus* from the site of Can Llobateres [Moyà-Solà and Köhler, 1993], also attributed to *D. fontani* [Ribot et al., 1996] (table 2). *Dryopithecus* lived in a highly mature ecosystem with a high degree of faunal diversity. Can Llobateres is the *Dryopithecus* locality with the highest diversity of European Miocene faunas [Agustí et al., 2003].

The *Oreopithecus* teeth examined include 2 specimens (Bac62 and Bac101) of *Oreopithecus bambolli* from the Museu Paleontològic Miquel Crusafont and 13 specimens from Peter Ungar's cast collection [Ungar, 1998], including teeth from the sites of Baccinello, Ribolla and Montemassi, in Italy. However, only a single specimen was acceptable for buccal microwear analysis.

Specimen Preparation and Microwear Analysis

Casts of occlusal surfaces of most of the extant primate specimens included in this study had already been obtained for occlusal analysis [Ungar, 1996, 1998]. Therefore, to prevent further deterioration of the original museum specimens from additional handling, moulds of these teeth were made of only the buccal surfaces of teeth. The enamel surfaces were gently cleaned with pure acetone and ethanol with a cotton ear bud. Negative casts

were obtained with *President Microsystem* regular body polyvinylsiloxane (Colténe^{TM}). A single tooth, the lower left second molar (LM_2), was consistently selected and moulded as representative of the buccal microwear pattern of each individual. If the LM_2 was not present, or had macroscopic evidence of non-preserved enamel on the buccal surfaces or of tartar deposits or enamel defects, the lower left first molar (LM_1) was used instead. If neither of these 2 teeth was suitable for microwear analysis, a RM_2 or M^1 was used. The impression material was applied from the occlusal border to the tooth roots, including the cemento-enamel junction, and from the mesial to the distal borders [Galbany et al., 2004a]. The teeth of *Dryopithecus* were moulded using the same procedure except that whole crown moulds were obtained. From the negative moulds, a positive cast was obtained with a stable, two-base component epoxy resin (Epo-Tek 301, by QdA), which provided faithful replicas with excellent detail for scientific research [Rose, 1983]. The epoxy resin was thoroughly stirred and then centrifuged for 1 min at 3,000 rpm to eliminate air bubbles. The resin was poured into the mould with a Pasteur pipette and centrifuged for 3 min at 2,500 rpm to remove air bubbles in contact with the buccal surfaces. The replicas were mounted on scanning electron microscopy (SEM) stubs and sputter coated with a 400-ångström gold layer for SEM observation. Casts of *Oreopithecus* teeth were obtained using Ungar's whole crown moulds, except for 1 tooth at the Museu Paleontològic Miquel Crusafont in Spain.

Only well-preserved enamel surfaces, without post-mortem handling or storage erosion or deposits, were selected for SEM analysis. All the teeth were observed at 40× magnification with a VMT binocular magnifying glass and only those lacking any kind of enamel damage, patina, or mineral deposit on large portions of the buccal surfaces were selected. A total of 111 well-preserved teeth (table 1) were selected and observed under SEM, using a Hitachi 2300 and a Cambridge Stereoscan 120 scanning electron microscopes. The moulds were placed at a horizontal position in the SEM chamber, with regard to the cemento-enamel junction, with zero degrees of tilt. Digital pictures (1,024 × 832 pixels) of preserved enamel surfaces were obtained on the middle third of the buccal surface, avoiding both the occlusal and cervical thirds of the tooth. All the images were obtained at 100× magnification and 10–12 kV electron acceleration. Only those SEM images that showed clear microwear features in the form of striations of various lengths and orientations, not affected by microscopic enamel erosion, enamel prisms or perikymata exposure, were considered for further analyses. These strict criteria of analysis were adopted to ensure that no post-mortem damage or tooth preservation treatments were misinterpreted as natural, dietary-related features. A 0.56-mm^2 square area of each SEM image was cut off for methodological standardization, following the usual procedures for buccal microwear research [Pérez-Pérez et al., 1999; Galbany et al., 2004b]. The resulting grey-scale, digital picture was adjusted to enhance contrast with Adobe Photoshop (v. 5.0) using a high-pass, 50-pixel filter and an automatic grey-level adjustment (fig. 1).

All microwear striations (including any that were truncated by the area studied) were counted and measured (length in micrometers and orientation in degrees from 0º to 180º) within the 0.56 mm^2 analysed area, using the Sigma Scan ProV (SPSS^{TM}) package. A striation was defined as a linear mark on the enamel surface, at least three times longer than its width with a minimum length of 15 μm, independent of its curvature. All striation angles were measured in degrees and classified into 45º orientation class groups [Pérez-Pérez et al., 1999] as horizontal (H), vertical (V), mesiodistal (MD) and distomesial (DM). For each orientation category, as well as for the total number of striations (T), the average number (N), length (X) and standard deviation of the length (S) of all observed striations were computed. Thus, a total of 15 variables were derived for each analysed image: NH, XH, SH, NDM, XDM, SDM, NMD, XMD, SMD, NV, XV, SV, NT, XT and ST (number, average length and standard deviation of the length for each orientation category). Kolmogorov-Smirnov normality tests, single-classification ANOVA and discriminant analyses were made with the SPSS v. 11 statistical package. The discriminant analysis was used to ensure maximum discrimination among groups and to classify the fossil samples using the discriminant functions (DFs) derived.

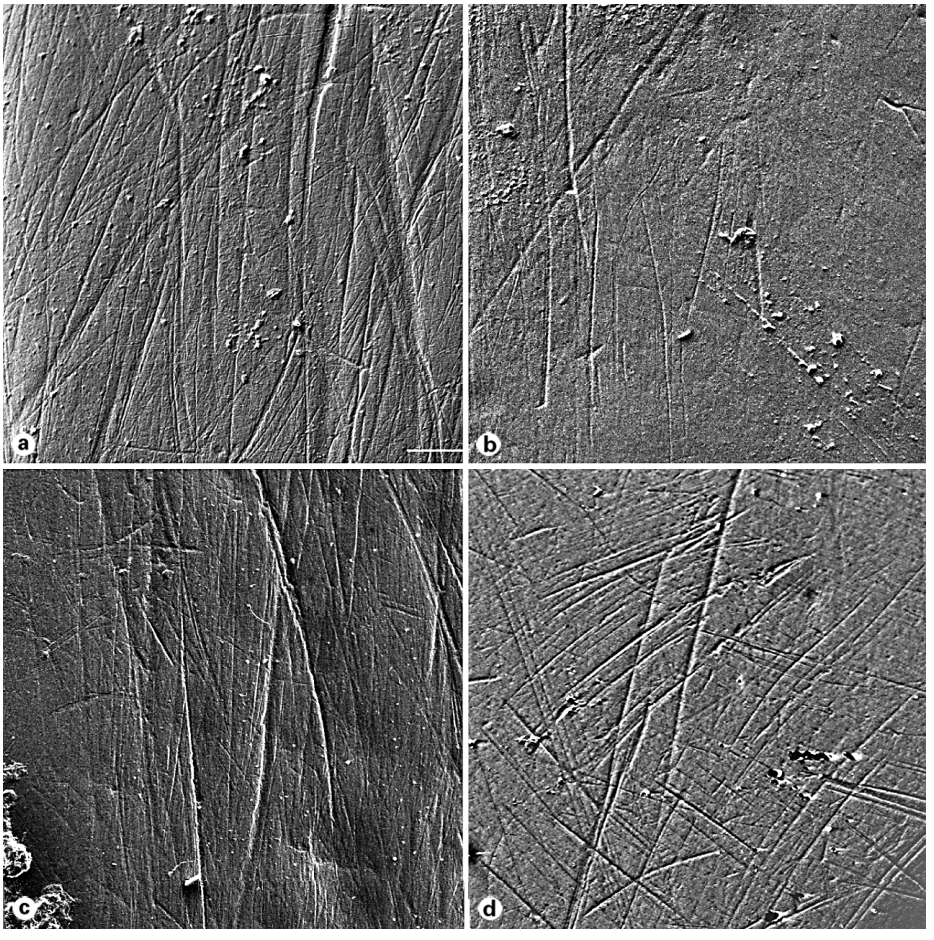

Fig. 1. SEM images of selected specimens studied. **a** *G. g. gorilla* NMHL-36.7.14.1. **b** *C. angolensis* om340 NMK. **c** *P. anubis* om6617 NMK. **d** *D. laietanus* MPMC-CLL9001. Each square surface analysed covers exactly 0.56 mm^2 of enamel surface. Occlusal towards top of micrograph.

Results

All the variables considered passed the normality tests ($p > 0.05$) and, thus, parametric statistics could be used. Mean values, minimums, maximums and standard deviation values for NT and XT variables of all species are shown in table 3. Figure 2 shows the same variables in a more visual way. *Cercopithecus* presents the highest density of striations (NT), followed by *P. anubis* and the Hominoidea taxa, whereas *Colobus* spp. show the smallest values. *D. laietanus* overlaps completely with the Hominoidea and *P. anubis* for the total density of striations (NT). Striation lengths (XT) show great dispersion ranges in most of

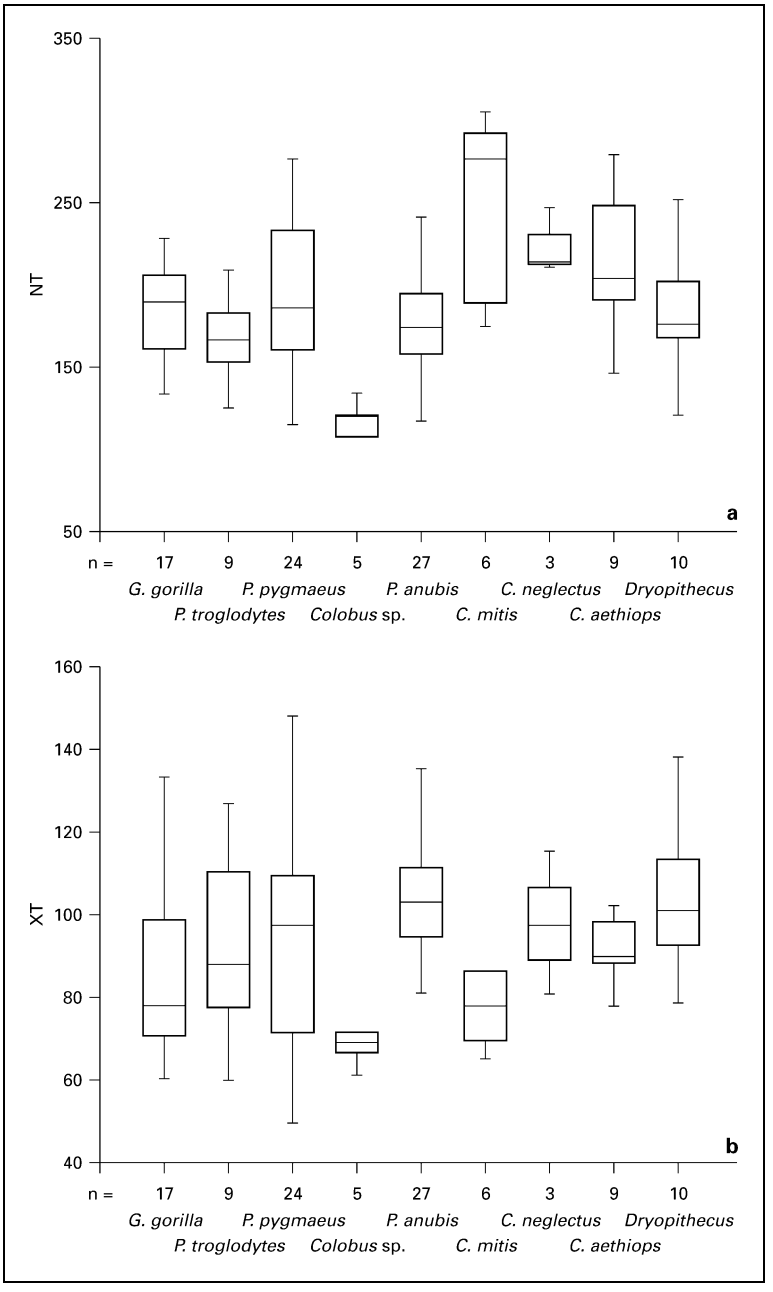

Fig. 2. Boxplot showing the density of microstriations (NT) (**a**) and the length of microstriations (XT) (**b**) observed in the teeth of Hominoidea, Cercopithecoidea and *D. laietanus*. Sample sizes are indicated in the x-axis for each species studied. The central line in the boxes indicates sample median, the boxes include 25–75 percentiles and the whiskers represent minimum and maximum values observed.

Table 3. Descriptive statistics of total number of striations (NT) and total length in micrometers (XT) for all species analysed: minimum, maximum, mean and standard deviation

Species	Variable	n	Minimum	Maximum	Mean	Standard deviation
Gorilla g. gorilla	NT	17	134.0000	228.0000	184.0588	29.2200
	XT	17	60.3063	154.8235	89.4051	26.6541
Pan t. troglodytes	NT	9	125.0000	209.0000	169.8889	26.6526
	XT	9	59.9345	126.7609	92.5763	22.1770
Pongo p. pygmaeus	NT	24	115.0000	277.0000	193.3333	44.8084
	XT	24	49.5367	148.1694	93.8981	24.6813
Colobus spp.	NT	5	87.0000	134.0000	114.0000	17.6777
	XT	5	61.2789	98.6216	73.3979	14.5832
Papio anubis	NT	27	117.0000	263.0000	177.4815	34.3168
	XT	27	81.2065	135.1785	103.7296	14.2914
C. mitis	NT	6	175.0000	305.0000	252.5000	55.8346
	XT	6	65.0823	118.8838	82.6094	19.7900
C. neglectus	NT	3	211.0000	247.0000	224.0000	19.9750
	XT	3	80.7750	115.4094	97.8772	17.3212
Chlorocebus aethiops	NT	9	146.0000	279.0000	211.8889	46.8227
	XT	9	77.8639	102.0914	90.9100	8.3414
Dryopithecus laietanus	NT	10	121.0000	252.0000	183.4000	34.3550
	XT	10	78.5435	138.1769	102.7119	17.5624
Oreopithecus bambolli	NT	1	156.0000	156.0000	156.0000	
	XT	1	87.2382	87.2382	87.2382	

the groups considered. The Hominoidea show the highest values, as well as *P. anubis* and, again, *D. laietanus. C. mitis* and *Colobus* spp. show the lowest values. The chimpanzees and gorillas show highly homogeneous microwear patterns compared to the Cercopithecoidea, most probably because the Hominoidea specimens were selected from restricted geographic distributions, since they show significant geographical differences in diet [Sugardjito and Nurhuda, 1981; Tutin et al., 1997], which may be clearly discriminated with the buccal microwear patterns [Galbany et al., 2002]. On the other hand, despite all the *P. p. pygmaeus* specimens being from the island of Borneo, there is no information about the exact provenance of each specimen, as is also the case for *Chlorocebus aethiops,* and thus geographical and sex-related differences might explain the great variability observed within this group.

The *O. bambolli* specimens showed unfruitful results. None of the casts obtained from Ungar's collection showed well-preserved buccal enamel surfaces. This might be due to the fact that moulds were made for occlusal research. However, buccal surfaces of all *Oreopithecus* spp. studied showed a great deal of enamel damage, including the specimen Bac101 from the Museu Paleontològic Miquel Crusafont. Only specimen Bac62, also from the Museu Paleontològic Miquel Crusafont collection, showed an acceptable level of enamel preservation, although erosion cannot be ruled out for this specimen either.

Table 4. Analysis of variance of the 15 variables studied for all primate groups considered

Variable	F	Significance
NH	5.982	0.000
XH	2.091	0.043
SH	1.647	0.121
NV	6.298	0.000
XV	2.043	0.049
SV	3.374	0.002
NMD	1.568	0.144
XMD	1.555	0.148
SMD	1.134	0.347
NDM	4.678	0.000
XDM	0.931	0.495
SDM	0.474	0.872
NT	6.083	0.000
XT	2.039	0.049
ST	2.159	0.037

Nine of the studied variables show significant between-group differences at a 0.05 level of significance (marked in grey).

Significant between-group differences in the extant primates' buccal microwear patterns were observed; the one-factor ANOVA showed significant differences in 9 of the 15 variables analysed (table 4). The Bonferroni post hoc test within the ANOVA (table 5) shows that *Colobus* is the most distinct group, with at least one significant difference compared with any of the other groups, and the *Gorilla-Papio, Pongo-Colobus, Colobus-Papio* and *Papio-Dryopithecus* are the most divergent species. *D. laietanus* showed significant differences for some of the variables only compared with *Colobus, Papio* and the three *Cercopithecus* spp. The *Cercopithecus* spp., mainly frugivorous [Beeson et al., 1996], show the highest densities of striations, with mean values for NT in all three species of over 210 striations, and mean lengths of over 82.6 μm (97.87 μm in *C. neglectus*), as previous studies also reflected [Teaford, 1994; Ungar and Teaford, 1996; Galbany and Pérez-Pérez, 2004] (table 3). *P. anubis* showed intermediate values, as was expected [Galbany and Pérez-Pérez, 2004], with 177.48 being the average total number of striations (NT) observed. *Colobus* showed the lowest density of striations, with a mean NT value of 114.00 and a mean length (XT) of 73.40 μm, despite being a mainly folivorous primate [Crissey and Prybil, 1997; Fleagle, 1999] (table 3).

A multivariate discriminant analysis of the extant Catarrhini species was performed to discriminate among extant species, based on buccal microwear patterns, and to classify the fossil *Dryopithecus* and *Oreopithecus* specimens. *P. pygmaeus* and *C. aethiops* were the most heterogeneous groups and, thus, the outliers showing correct classification probabilities smaller than 30% were removed from the sample (5 *P. pygmaeus* and 3 *C. aethiops*). Eight groups entered the final discriminant analysis: *G. g. gorilla* (n = 17), *P. t. troglodytes* (n = 9), *P. anubis* (n = 27), *C. mitis*

Table 5. Bonferroni post hoc test showing the significant between-group differences

Variable	*Gorilla* vs. *Colobus*	*Gorilla* vs. *P. anubis*	*Gorilla* vs. *C. mitis*	*Pan* vs. *Colobus*	*Pan* vs. *C. mitis*	*P. pygmaeus* vs. *Colobus*
NH		*				
XH		*				
NV			*		*	
SV	*			*		**
NMD						
NDM		**				*
NT	*		**		**	**
ST						

Variable	*P. pygmaeus* vs. *P. anubis*	*P. pygmaeus* vs. *C. mitis*	*P. pygmaeus* vs. *Dryopithecus*	*Colobus* vs. *P. anubis*	*Colobus* vs. *C. mitis*	*Colobus* vs. *C. neglectus*
NH	**					
XH						
NV			*			
SV		*		*		
NMD						
NDM	**					
NT		*		*	**	**
ST				*		

Variable	*Colobus* vs. *C. aethiops*	*Colobus* vs. *Dryopithecus*	*P. anubis* vs. *C. mitis*	*P. anubis* vs. *Dryopithecus*	*C. mitis* vs. *Dryopithecus*	*C. neglectus* vs. *Dryopithecus*	*C. aethiops* vs. *Dryopithecus*
NH		**		**			
XH							
NV					**	**	**
SV							
NMD				*			
NDM							
NT	**	*	**	*	*		
ST							

* p = 0.05; ** p = 0.01. Only the variables and groups where differences have been detected are shown.

(n = 6), *C. neglectus* (n = 3), *Colobus* spp. (n = 5), *P. pygmaeus* (n = 19), and *C. aethiops* (n = 6). All variables passed the stepwise tolerance test ($F > 2.71$, $p < 0.1$; between-group ANOVA after a new variable entered the analysis), except the total number of striations (NT), which is frequently correlated with the other variables [Galbany et al., 2002; Galbany and Pérez-Pérez, 2004]. Seven DFs were derived, the four main ones explaining 91.7% of total variance (38.4, 28.5, 16.5 and 8.4%, respectively). The first function (DF-1) was significantly correlated ($p < 0.05$) with NDM ($r = 0.525$) and NH ($r = 0.478$), and the second (DF-2) with NV ($r = 0.571$) and NMD ($r = 0.375$). The power of discrimination of the seven combined func-

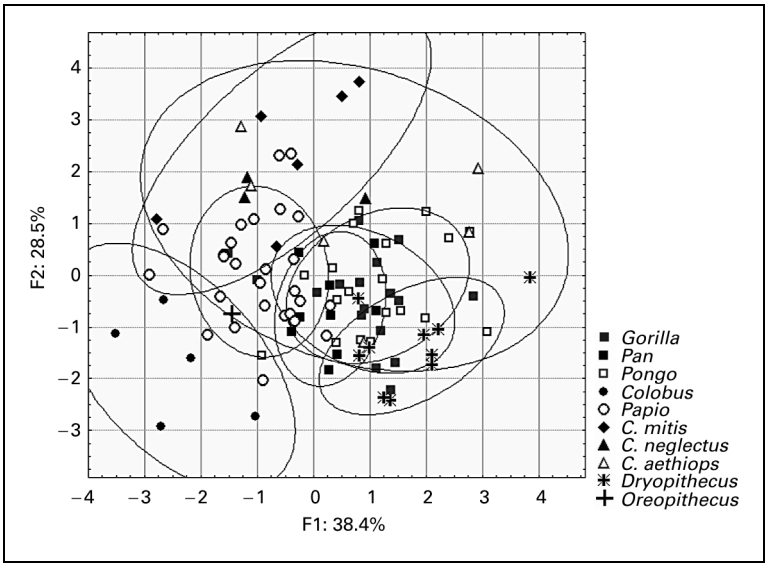

Fig. 3. Plot of the two first functions from the discriminant analysis of the 15 variables studied for the extant Hominoidea, Cercopithecoidea and the fossil specimen of *D. laietanus* and *O. bambolli*.

tions, measured with Wilkes' lambda ($\lambda = 0.100$, $\chi^2 = 181.982$), was highly significant ($p < 0.001$). The percent of correct classification was 90.1%. *Colobus* spp., the three *Cercopithecus* spp. and *Pan* were all correctly classified; the percent of correct classification was 92.6% for *P. anubis*, 83.3% for *P. pygmaeus* and 76.5% for *G. gorilla.*

The classification of *D. laietanus* using the DFs derived was *G. gorilla* in 80% of the cases (8 out of the 10 specimens analysed), with probabilities of correct classification ranging from 100 to 94.2%, *P. t. troglodytes* for 1 specimen with a 99.9% probability, and *P. anubis* for the remaining specimen with a 99.9% probability. The microwear pattern of the single *O. bambolli* specimen (Bac62) was included in the discriminant analysis only for comparative purposes. It was tentatively classified as *P. anubis* with 99.1% probability.

Figure 3 shows the first two DFs obtained. Despite a great overlap occurring among most groups in this two-dimensional plot, significant differences were found for the multivariate space and some associations are evident. The Cercopithecoidea tend to be distributed along the second function (predominantly related to variables NT, NV and NMD), with *Colobus* showing the lowest values and *Cercopithecus* the highest ones. *D. laietanus* shows low DF-2 values, similar to *Gorilla* and *Oreopithecus,* and lies between *Colobus* and *P. anubis.*

To illustrate further the species affinities based on buccal microwear patterns, a single-linkage cluster analysis (square Euclidean distance) was constructed using the average values of the seven DFs obtained as representative of the groups considered in the discriminant analysis. Figure 4 plots the cladogram obtained showing

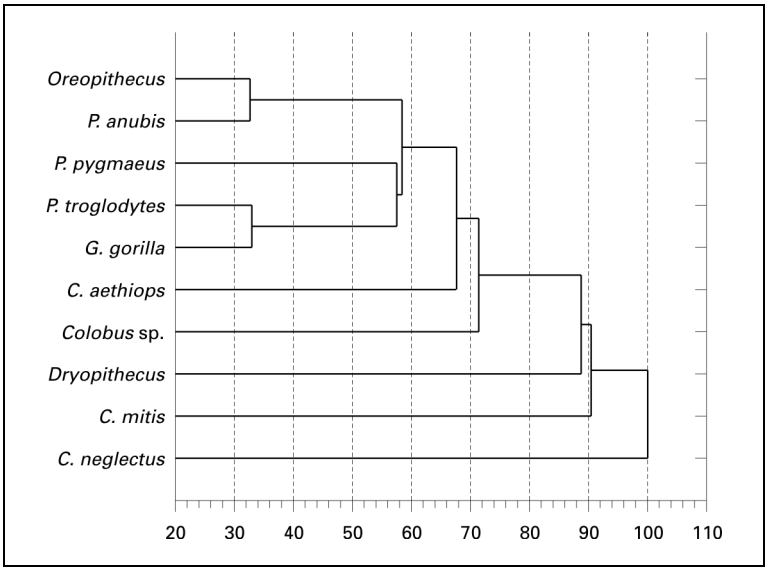

Fig. 4. Dendrogram derived from a single-linkage cluster analysis, using the squared Euclidean distance, calculated with the mean values of the 7 DFs of the discriminant analysis made, representing all groups analysed.

the main microwear affinities between group centroids. If closer clustering of groups is seen as indicative of similarities in their buccal microwear patterns and, hence, in diet abrasiveness and composition, the buccal microwear pattern seems to discriminate Hominoidea diets efficiently from those of the Cercopithecoidea studied. Further, *Oreopithecus* shows microwear similarities with *Papio,* and *Dryopithecus* shows great dissimilarities with the extant Hominoidea.

Discussion

The buccal microwear patterns of the extant primates studied show a clear association with ecological conditions, reflecting not only biological adaptations of species, but also geographic and 'cultural' differences among groups and species. It is of particular interest to note that the buccal microwear analyses of the extant Hominoidea do not show significant between-group differences in the discriminant analysis when compared to the Cercopithecoidea. Similarities in buccal microwear patterns within the Hominoidea could be a reflection of the great dietary diversity of each hominoid species and of the disparity of habitats and ecological conditions in which they live. A more detailed population diversity analysis, rather than a species comparison, has already been shown to be more informative for this primate group [Galbany et al., 2002], since diet is not only the result of strict biological constraints but also of food availability and choice. Clear differences arise, though, between the Hominoidea and the Cercopithecoidea species. These could be related

to the importance of hard fruit ingestion in the Cercopithecoidea, which would cause higher microdamage levels on enamel surfaces [Teaford and Walker, 1984; Teaford, 1994; Ungar and Teaford, 1996; Galbany and Pérez-Pérez, 2004]. However, *Colobus* clearly deviates from the cercopithecoid pattern in showing the lowest density of striations, despite being a largely folivorous primate [Crissey and Prybil, 1997; Fleagle, 1999]. Leaf eating may result in very high abrasion levels, literally removing all microwear objects by polishing the enamel surfaces. The presence of a thick patina layer, or calculus, on the enamel surfaces, very common in *Colobus* teeth, may serve as a protection factor to reduce the abrasive effect of leaf-dependent dietary habits [Galbany and Pérez-Pérez, 2004]. Previous studies have shown that 80% of all *Colobus angolensis* specimens analysed presented patina layer in all buccal surfaces [Galbany et al., 2004b].

Dietary Habits of the Fossil Specimens

Dryopithecus, a Miocene European hominoid that lived between approximately 12 and 9.5 million years, has been considered a primitive member of the *Pongo* clade [Begun et al., 1990; Begun, 1992; Moyà-Solà and Köhler, 1993, 1995, 1996], with climber and suspensory capabilities, well-adapted to below-branch locomotion and arboreal quadrupedalism [Moyà-Solà and Köhler, 1996]. *Dryopithecus* lived along seashores and forested regions in south, western and central Europe [Köhler et al., 1997], where palm trees of the genus *Sabal,* today known from the swamps of Florida, were abundant, as were fig trees and trees of the *Cinnamomum* and *Cassia* groups [Sanz de Siria, 1998], all requiring high humidity and subtropical to tropical temperatures, but there were also more drought-tolerant plants in the hinterland, such as oaks, pines and members of the family Lauraceae.

On the other hand, *Oreopithecus* is an Upper Miocene primate from Italy dating to 9.5–6.5 million years [Moyà-Solà and Köhler, 1997], of approximately 32 kg [Köhler and Moyà-Solà, 1997] that lived on an island in the Tyrrhenian Sea, isolated from the continent for several million years. Fossil remains have been collected from the old lignite mines of Monte Bamboli, Casteani, Ribolla and Baccinello (Tuscany, Italy), and most recently from fluvial deposits of Fiume Santo (Sardinia, Italy). *O. bambolli,* described by Gervais [1872], is considered to be a sister taxon of *Dryopithecus* [Moyà-Solà and Köhler, 1996] and has been described as a biped, with increased manual abilities, adaptated to an insular ecosystem [Köhler and Moyà-Solà, 1997; Moyà-Solà et al., 1999; Rook et al., 1999].

Oreopithecus lived under climatic conditions that differed noticeably from those described for the older *Dryopithecus* sites in Hungary and Spain. The floral composition of Baccinello is comparable to the extant flora from the north-western Mediterranean regions [Harrison and Harrison, 1989], with a mixed mesophytic forest community of broad-leaved deciduous angiosperms and evergreen gymnosperm trees of either European or Eurasian origins. Aquatic seed plants and algae, abundant on moist substrates [Harrison and Harrison, 1989; Benvenuti et al., 1994], as well as Nymphaea, Cyperaceae, *Sparganium,* and moisture-loving reeds and ferns, coexisted with plants, such as those in the family Ericaceae (e.g. *Arbutus*), that are found in upland mountain habitats [Benvenuti et al., 1994].

The characterization of the dietary habits of the fossil specimens based on buccal microwear contradict some previous studies based on dental morphology [Ungar and Kay, 1995; Ungar, 1996, 1998; Carnieri and Mallegni, 2003]. The buc-

cal microwear pattern shown here for *Dryopithecus* closely resembles that of *Gorilla,* suggesting that *D. laietanus* could, indeed, have relied on abrasive resources, such as herbaceous plants, leaves, stems and cambium of terrestrial plants, as well as succulent fruits, as gorillas do, despite the thin enamel and broad, rounded cusps on its cheek teeth that indicate a predominantly frugivorous diet [Fleagle, 1999]. Ungar [1996, 1998] also suggested that *D. laietanus,* with shearing crests similar to the extant, soft-fruit eating Hominoidea, such as the gibbons and chimpanzees, could have been a soft-fruit eater, lacking specializations for either hard-object feeding or extreme folivory [Ungar and Kay, 1995]. However, our results, while not discounting intense fruit consumption, are indicative of ingestion of more abrasive foods as well. This hypothesis is consistent with the environmental reconstruction of *Dryopithecus* habitat, one with both a dense humid forest and more open and drier areas.

Results for *O. bambolli,* though very tentative because of the small sample size, do indicate that this species has a distinct microwear pattern, different from that of *Dryopithecus* and more closely related to that seen in *Papio.* Despite some authors indicating that *O. bambolli* might have had a leaf-based diet, based on occlusal dental microwear studies [Ungar and Kay, 1995; Ungar, 1996; Carnieri and Mallegni, 2003], the results seen here suggest the possibility that *O. bambolli* had a wide-ranging diet or, at least, a diet that included a significant variety of abrasive material, rather than only leaves, showing a microwear pattern clearly distinct from that of *Colobus* spp.

Dietary interpretation of fossil primate specimens based on microwear patterns requires further analyses for a better understanding of population variability, not only species related but also population and locality related. In addition, different microwear patterns on buccal and occlusal enamel surfaces might result from the same diet. Buccal microwear patterns, not affected by tooth-tooth contact, have been shown to be a good indicator of dietary-related abrasion in primates, as well as in hominins [Pérez-Pérez et al., 1999; Galbany et al., 2002; Pérez-Pérez et al., 2003; Galbany and Pérez-Pérez, 2004], although further methodological standardizations are still required.

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4.9 Variabilitat del patró de microestriació dental dels *Hominoidea* africans i interpretació de la dieta d'*Australopithecus afarensis*

Galbany J, Estebaranz F, Martínez LM & Pérez-Pérez A (2006) Buccal dental microwear variability in extant African *Hominoidea* primates: implications to a preliminary interpretation of dietary habits of *Australopithecus afarensis* from Afar localities (Ethiopia) and Laetoli (Tanzania)

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La variabilitat poblacional del patró de microestriació dental en primats ha estat poc analitzada. Aquesta presenta una estreta relació amb les diferències ecològiques i alimentàries a nivell poblacional, i també diferències a nivell subespecífic (Galbany et al., 2002).

Aquest darrer treball aborda l'estudi de la variabilitat poblacional dels patrons de microestriació dental vestibular dels primats *Hominoidea* africans (*Pan troglodytes* i *Gorilla gorilla*) per tal de detectar diferències ecològiques, subespecífiques i geogràfiques, i inclou l'estudi d'algunes poblacions singulars com els *Gorilla gorilla gorilla* del Congo o de Guinea Equatorial, que presenten una gran diversitat ecològica respecte a les altres poblacions de goril·les analitzades (Jones & Sabater Pi, 1971; Rogers et al., 2004).

La variabilitat del patró de microestriació dental dels *Hominoidea* africans actuals es compara amb el patró de microestriació de l'homínid *Australopithecus afarensis* per determinar l'abrasivitat de la seva dieta. Al mateix temps s'analitza *Papio anubis*, considerat un primat d'ecologia de sabana (Galbany et al. 2004a).

La mostra analitzada consisteix en 76 rèpliques dentals d'*Hominoidea* actuals amb localitats d'origen ben conegudes i 10 dents molars d'*Australopithecus afarensis* (Taules 1 i 2). Les dents originals van ser replicades amb polivinilsiloxà *President microSystem™* (*Coltène®)* i resina epoxy Epo-Tek #301 i poliuretà Feropur PR55 (Rose, 1983; Galbany et al., 2004a). Per tal d'estandarditzar al màxim la mostra analitzada es van considerar únicament les segones molars inferiors esquerres.

A partir de les imatges obtingudes amb el Microscopi Electrònic d'Escombrat (SEM) (Figura 1) es van realitzar els recomptes de les microestriacions amb el programari *Sigma Scan* i es van obtenir les variables quantitatives definitòries del patró de microestriació dental vestibular (Pérez-Pérez et al., 1999; Galbany et al., 2004a). Una primera anàlisi amb el test de Kolmogorov-Smirnov indica que totes les variables segueixen distribucions normals. La Taula 3 mostra les mitjanes i la desviació estàndard de totes les variables per cadascuna de les poblacions analitzades. L'anàlisi de la variança (ANOVA) indica que únicament 5 de les 15 variables presenten diferències intergrupals significatives (Taula 4). Aquestes són SH (desviació estàndard de la

longitud de les estries horitzontals), NV (densitat de les estries verticals), SMD (desviació estàndard de la longitud de les estries obliqües mesio-distals), NT (densitat total d'estries) i ST (desviació estàndard de la longitud de les estries totals). El test *post-hoc* Bonferroni de l'ANOVA mostra que la variable NV és la que presenta més diferències significatives entre les poblacions considerades, sobretot entre els *Gorilla gorilla gorilla* del Congo i la resta de poblacions (Taula 5).

En una anàlisi de components principals es van obtenir 5 components que expliquen el 81,18% de la variabilitat total. Aquests components es van utilitzar per realitzar una anàlisi d'agrupacions (*cluster*) i representar un dendrograma basat en la distància Euclídea al quadrat (Figura 2). Aquest mostra que els goril·les del Congo són els més diferenciats. Els goril·les del Camerun i els ximpanzés *Pan troglodytes troglodytes* i *Pan troglodytes schweifurthii* formen un grup molt homogeni i presenten com a grup germà un conjunt format per *Gorilla gorilla graueri* i *Pan troglodytes verus*.

L'anàlisi discriminant de totes les variables i poblacions considerades, incloent els *Papio anubis*, va permetre classificar els *Australopithecus afarensis*. Les quatre primeres funcions derivades expliquen el 91,6% de la variabilitat total. La primera funció, que explica un 44,3% de la variabilitat total, està correlacionada amb NV (densitat de les estries verticals) i NDM (densitat de les estries obliqües disto-mesials), i la segona funció, que explica un 26,3% de la variabilitat total, està correlacionada amb NT (densitat total d'estries). La caracterització del patró de microestriació d'*Australopithecus* afarensis a partir de les funcions va indicar similitud amb *Papio anubis* (50% de probabilitat), amb *Gorilla gorilla gorilla* del Camerun (40% de probabilitat) i amb *Pan troglodytes troglodytes*, amb un 10%. La Figura 3 mostra la representació gràfica de les dues primeres funcions de l'anàlisi discriminant.

Cap dels *Australopithecus afarensis* es va classificar com a similar a *Pan troglodytes verus* o goril·les del Congo o Guinea Equatorial, que habiten entorns ecològics en mosaic formats per boscos i sabanes (Kortlandt, 1983; Tutin & Fernández, 1994) i consumeixen una gran proporció d'aliments fibrosos abrasius com a aliments clau durant els períodes d'escassetat (Rogers *et al.*, 1990; Rogers *et al.*, 1994; Remis *et al.*, 2001; Tutin *et al.*, 1997; Rogers et al,

2004). Això suggereix que aquests recursos no van ser explotats per aquesta espècie d'homínid i que, per contra, presentaria una dieta basada en aliments de baixa qualitat durant tot l'any, obtinguts a la sabana, tal com farien els papions actuals, i als boscos, com farien els goril·les.

Aquest resultat és consistent amb altres estudis que conclouen que *Australopithecus afarensis* tindria una dieta diferent a la dels ximpanzés i més similar a la dels papions, incloent llavors, arrels i rizomes, tot i que hauria preferit recursos rics en sucres, com ara fruits (Picq, 1990; Teaford & Ungar, 2000).

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Buccal dental microwear variability in extant African *Hominoidea* primates: implications to a preliminary interpretation of dietary habits of *Australopithecus afarensis* from Afar localities (Ethiopia) and Laetoli (Tanzania)

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Abstract

Analyses of buccal microwear patterns on teeth have proved to be good indicators of the abrasiveness of food stuffs in the diet and have been used to trace dietary habits of fossil species, both primates and hominin. However, buccal dental microwear variability is not well known yet, and no mechanisms of formation have been proposed yet. The abrasive capability of the diet depends on the hardness of the particles included in the ingested foods, but also in the presence of dust and other exogenous elements incorporated during the process of food processing. These items are responsible for the typology of the microwear that can be observed on the enamel surfaces of primate teeth. The present study analyses the variability of the buccal microwear pattern on the great african Hominoidea Apes (*Gorilla gorilla* and *Pan troglodytes*) using tooth moulds obtained from the original specimens at different osteological collections. The results obtained seem to indicate that the ecological adaptations at a subspecies level may account for such differences in relation to habitat and ecological conditions within populations rather than between species. *Australopithecus afarensis* dental microwear is analyzed and compared with that of the African apes. The fossil specimens analyzed show a highly heterogeneous dental microwear, similar to those of *Papio anubis* and *Gorilla g. gorilla* from Cameroon, suggesting thatt *Australopithecus afarensis* had an heterogeneous diet based on tropical forest foodstuffs as well as savanna resources.

Keywords: dental microwear, SEM, *Pan troglodytes, Gorilla gorilla, Australopithecus afarensis*

INTRODUCTION

Dental microwear patterns on buccal tooth surfaces of extant Primates (Ungar 1994; Ungar & Teaford, 1996; Galbany et al., 2002; Galbany & Pérez-Pérez, 2004), though still scarce, have proved to be very useful in the interpretation of fossil primate dietary habits (Galbany et al., 2005a). Dental microwear research has been extensively applied to fossil hominids and modern humans hunter-gatherer populations, both on occlusal and buccal enamel surfaces (Puech, 1982; 1984; 1986a; 1986b; Grine, 1986; 1987; Ungar & Grine, 1991; Lalueza & Pérez-Pérez, 1993; Lalueza et al., 1993; 1996; Pérez-Pérez et al., 1994; 1999; 2003a). Also, these studies on the buccal surfaces of teeth could serve as a key reference to the interpretation of dietary habits of fossils primates, as comparing microwear of extant primates with fossil ones (Galbany et al., 2005a). This paper presents an intra- and inter-species variability analysis of the buccal microwear pattern of extant African Hominoidea, in relation to the ecological environment and the alimentary items they ingest. Previous studies has proved that Hominoidea primates present an homogeneous dental microwear patterns when are compared with other Cercopithecoidea species belonging to genus *Cercopithecus*, *Colobus* or *Papio* (Galbany & Pérez-Pérez, 2004; Galbany et al., 2005a), but when intraspecific variability is analyzed taking into account only Hominoidea, some differences are detected, despite of all homogeneity (Galbany et al., 2002). Preliminary analyses of dental microwear on different Hominoidea primates have shown that dental microwear pattern is not only capable to discriminate among distinct feeding behaviours at a specific level, but also among ecological adaptations of different populations. Populations that inhabit isolated and singular ecological sites, such as Gorillas from Congo and those from Nigeria, show distinct dental microwear patterns, whereas different species from the same ecological whereabouts, such as chimpanzees and gorillas from Cameroon, show similar dental microwear patterns (Galbany et al., 2002).

From this ecological perspective, the present study tries to make inferences of thedietary adaptations of a preliminary sample of *Australopithecus afarensis* teeth in comparison to other extant Hominoidea samples analyzed, and a *Papio anubis* population as representing open savanna primate. *A. afarensis* inhabited a diversity of biomes (Bonnefille et al., 2004), and could have fed on various kinds of resources, such as ripe fruits from forested environments as well as seeds, roots or rhizomes from more opened savannas (Ryan and Johanson, 1989; Picq, 1990; Teaford and Ungar, 2000; Ungar and Teaford, 2001; Ungar, 2004).

Dietary habits and ecology of African Hominoidea

The gorillas have been considered a mainly folivorous species (Fleagle, 1999), although they are not strict leaf-eaters, except in habitats that allow no alternative diets (Rogers *et al.*, 1990). Western lowland gorillas, *Gorilla gorilla gorilla*, usually include sweet, succulent fruits, up to 50% or more of their diet depending on seasonal fluctuation and availability (Jones & Sabater Pi, 1971; Sabater Pi, 1977; Doran & McNeilage, 1998; Remis, 1997; Tutin *et al.*, 1997). However, Rogers et al. (2004) in a synthetic longitudinal study indicate that fruit is the most consumed item by western gorillas, from 51% at Bai Hokou (Central Africa Republic) to 70% of total food species at Mondika (Central Africa Republic), and it is eaten year round, even when availability is low. Gorillas also consume many other items year round such as leaves, stems, pith, shoots, roots and bark. Dietary overlap among gorillas is considerably high (Rogers et al. 2004). In Rio Muni (Equatorial Guinea), *G. g. gorilla* subsists largely on the profuse growths of the wild ginger *Aframomum*, by eating its leaves, pith, roots and fruits (Jones & Sabater Pi, 1971). In the Lopé Reserve (Gabon), the gorillas obtain more than 83% of their foods directly from the trees (Tutin & Fernández, 1994), although they live in a peculiar mosaic habitat of savannas and Marantaceae forests (Rogers et al. 2004). The diet of the gorillas may also include other resources, such as aquatic, herbaceous vegetation, leaves, bark, cambium, and phloem of trees or lianas (Doran & McNeilage, 1998). Fallback foods constitute an important food resource of western lowland gorillas from Congo basin and Nigeria (Rogers et al. 2004), often including foods of low nutritional quality, such as piths, leaves, barks, and fibrous fruits (Rogers *et al.*, 1990; Rogers *et al.*, 1994; Remis *et al.*, 2001; Tutin *et al.*, 1997), There is no evidence of meat ingestion among gorillas (Sabater Pi, 1977), even though insects, principally *Cubitermes* and several species of Himenoptera are eaten in many localities (Tutin & Fernandez, 1983, 1992; Remis, 1997; Deblauwe et al., 2003). Sabater Pi (1960) has described a gorilla chewing wax from the nest of subterranean bees. Remis (1997) registered sex related differences in diet among the gorillas, being males more frugivorous than females during the dry season, but also consuming more abrasive food stuffs, such as bark and herbs, than females.

Chimpanzees occupy either tropical forests or dry arboreal savannas. This wide range of environments allow for a diversification in their behavioral strategies related to local habitat conditions and resource availability, as well as to family traditions. Thus, chimpanzee's diet varies greatly from one population to another, and even from one community to the next, not only because of botanical differences but also because of traditional preferences (Estes, 1997). The chimpanzees frequently eat on the ground, walking from one site to another and mainly feeding on fruits and nuts, up to 70-80% (Tutin et al., 1997), although this proportion depends on the population analyzed. Basabose (2002) reported a 58% fruit consumption in Kahuzi chimpanzees (*Pan t. schweinfurthii*), in Democratic Republic of Congo, whereas Tweheyo & Obua (2001) reported an 83% of fruit ingestion at Budongo Forest Reserve in Uganda, also for *Pan t. schweinfurthii*. Other studies have reported either 87% of fruits and seeds in *Pan t. troglodytes* (Tutin & Fernández, 1993) or 72% in *Pan troglodytes verus* from Bossou (Sugiyama & Koman, 1992), a value similar to that reported for the Kibale chimpanzees (*Pan t. schweinfurthii*) by Wrangham et al. (1991): 67% of fruit consumption. Other resources, such as leaves and stems, represent around 20% of their total food intake (Estes, 1997), depending on the population considered.

Seasonal shifts in diet have also been described (Fleagle, 1999; Sabater Pi, 1979). The chimpanzees from Gombe consume oil-palm nuts, whereas in Mahale, only 20 Km away, they do not. Chimpanzees from savannah environments are highly dependant on resources from gallery forests or patches of evergreen forests (Nishida, 1968). Chimpanzees' diet also includes bark, resins, flowers and seeds, although these represent only a small proportion of it (Nishida *et al.*, 2000; Yamakoshi, 1998), as well as social insects, basically termites, and some species of mammals, generally small artiodactyls and some primate species, such as *Colubus* or *Papio*, which are frequently consumed. Up to 5% of a chimpanzees' food procurement activities are spent obtaining and eating animal foods, mainly in the form of insects (termites, ants, bees and other insect larvae and eggs, caterpillars, leaf galls, etc.), and vertebrates, notably young antelopes and bushpigs, monkeys, bushbabies, nestling birds, eggs (Wrangham, 1984) and bushbucks, duikers, young baboons as well as smaller items (Wrangham & Riss, 1990). Predation and

hunting behaviors in chimpanzees have been observed in many localities (Stanford *et al.*, 1994; Newton-Fisher, 1999; Boesch, 2001).

Australopithecus afarensis

These hominins would probably have preferred soft, sugar-rich fruits, but would have been able to make better use of hard resources as fallback foods given seasonal availability of favored items (Picq, 1990; Teaford & Ungar, 2000). Ryan & Johansson (1989) have suggested a consumption of both forest and savanna plants, such as roots, seeds and rhizomes, based on the study of dental microwear features on their anterior teeth. This results is also supported by Bonnefille et al. (2004) that suggest that there was no preference by *A. afarensis* for any single biome, including forest.

Microwear studies on the teeth of upper Pliocene hominids suggest both nonabrasive frugivory and consumption of hard, gritty foods, such as roots, seeds, and rhizomes (Wolpoff, 1999). These conclusions are coincident with Puech & Albertini (1984), that showed that the dental microwear of early hominids indicates adaptation to a very powerful mastication, with meat-eating adaptations coming later, over last 1-2 million years, based on occlusal microwear of LH-5 and Hadar (AL-199 and AL-200). Ungar (2004), based on dental topography, concluded that extant ape diets differ mostly in fallback foods, and it is reasonably to suggest that the *A. afarensis* diet may also have differed from that of chimpanzees largely also in fallback resources, which could have included more hard, brittle items. This is consistent with the dietary reconstructions made by Ryan and Johanson (1989) and Picq (1990). Thus, *Australopithecus afarensis* was well suited to crush hard, brittle foods.

Dietary interpretation of fossil primate specimens based on dental microwear patterns requires analyses of the intra and interpopulation variability, both between species and population from different localities. Buccal microwear patterns, not affected by tooth-tooth contact, have shown to be a good indicator of dietary-related abrasion in primates, as well as in hominins (Pérez-Pérez et al., 1999; Galbany et al., 2002; Pérez-Pérez et al., 2003a; Galbany & Pérez-Pérez, 2004). This paper focuses on the variability of the buccal microwear patterns of extant *Hominoidea* primates and analyzes the implications of the results obtained to the interpretation of dietary habits derived from a small, preliminary sample of the fossil hominin species *Australopithecus afarensis*.

Table 1. Sample sizes of extant and fossil species examined, geographical origin and osteological collections: American Museum of Natural History (AMNH), National Museums of Kenya (NMK), Natural History Museum (NHML), Harvard Museum of Comparative Zoology (HMCZ), Peabody Museum of Archaeology and Ethnology (PMAE), Royal Museum for Central Africa (MRAC), Museo Nacional de Ciencias Naturales (MNCN), Universität Zürich -Anthropological Institute & Museum (IMAZ), National Museums of Kenya (NMK) and National Museums of Ethiopia (NME).

Subspecies	Origin	Casts analyzed	Osteological collections
Gorilla g. gorilla	Cameroon	31	AMNH, NHML, IMAZ, HMCZ
	Congo	4	NHML
	Eq.Guinea & Gabon	8	IMAZ, MNCN
Gorilla g. graueri	Democratic Republic of Congo	7	MRAC
Pan t. troglodytes	Cameroon, Equatorial Guinea, Gabon, Nigeria	10	AMNH, NHML, IMAZ, MNCN
Pan t. schweintfurthii	Republic of Congo, Tanzania, Uganda	9	AMNH, NMK, NHML, MRAC
Pan t. verus	Liberia	7	IMAZ, PMAE
Australopithecus afarensis	Fossil taxa from Afar localities and Laetoli	10	NMK, NME
TOTAL		**86**	

MATERIALS AND METHODS

Extant *Hominoidea*

The extant primate sample studied (Table 1) included a total of 86 specimens belonging to three *Pan troglodytes* [*Pan troglodytes troglodytes* (n=10), *Pan troglodytes schweintfurthii* (n=9), and *Pan troglodytes verus* (n=7)], and four *Gorilla gorilla* [*Gorilla gorilla gorilla* from Cameroon n=(31), *Gorilla gorilla gorilla* from Congo n=(4), *Gorilla gorilla gorilla* from Equatorial Guinea and Gabon n=(8) and *Gorilla gorilla graueri* n=(7)] species geographically diverse. The skeletal collections considered are curated at the American Museum of Natural History (AMNH) in New York, the National Museums of Kenya (NMK) in Nairobi, the Natural History Museum (NHML) in London, the Harvard Museum of Comparative Zoology (HMCZ) and the Peabody Museum of Archaeology and Ethnology (PMAE) in Cambridge – MA, the Royal Museum for Central Africa (MRAC) in Tervoren (Belgium), the Museo Nacional de Ciencias Naturales (MNCN-CSIC) in Madrid, and the Universität Zürich -Anthropological Institute & Museum (IMAZ) in Zürich. All the primate specimens studied were wild-captured, so it is assumed that they fed using natural strategies and depending on environmental conditions. The groups analyzed were selected to represent a limited geographical distribution of the subspecies considered (Table 1), covering a wide range of ecological and dietary adaptations of the African *Hominoidea* (Kingdom, 2001). All the studied subspecies share a mainly herbivorous diet, but distinct proportions of leaves and fruits are eaten by each of them.

Table 2. Fossils specimens analyzed of *Australopithecus afarensis* from Afar localities (Ethiopia) and Laetoli (Tanzania).

Species	Site	Specimen
Australopithecus afarensis	Afar Localities (Ethiopia)	AL-417-1A
		AL-333-59
		AL-400-1a
		AL-288-1i
		AL-333W-32
		AL-188-1
		AL-333W1b
		AL-145-35
		AL-333W-1a
	Laetoli (Tanzania)	LH-4

Australopithecus afarensis

The *Australopithecus afarensis* specimens analyzed in the present study belong to two different localities: Afar, in Ethiopia, and Laetoli, in Tanzania, (Table 2). Hadar formation, located in the Awash River Valley within Afar depression (Ethiopia), is a Pliocene site dated older than 3 my (Bonnefille et al., 1987). The sediments accumulated from river streams in a basin that was periodically inundated from a large lake (Johanson et al., 1982; Kimbel et al., 1996). Early hominids have been discovered there since 1973 (Johanson & Taieb, 1976) in the lower part of Kada Hadar Member and the three underlying members, that have yielded more than 320 fossils of *A. afarensis*, which represent from 40 to 100 individuals dating back to 3.4-2.9 BP (Kimbel et al., 1994). Laetoli, a site located 45 km South of Olduvai, was the first to bring australopithecine remains, as in 1935 a lower canine, and in 1939 a small fragment of a maxilla with premolars and M3, were found. Since then, up to 33 individuals have been unearthed. The paleoenvironmental reconstruction of the site indicated that Laetoli was a seasonally savannah woodland where most of the water fell in a single season (Wolpoff, 1999). It is not clear whether the hominids that inhabited this area were perfectly adapted to such a limiting environment, or if they only occurred it seasonally. However, microwear has yielded a somewhat interesting support to the second possibility as it has been shown that their diet might have bennmainly frugivorous, with an important intake of abrasive, gritty and hard items, such as roots, seeds and rhizomes. LH-4, the first Laetoli fossil described (White, 1977), shows postcanine teeth larger and with thicker enamel than those of aramis, and premolars are larger than the molars. The anterior portion of their mandible is broad and squared off, with large anterior teeth, as in chimpanzees, which could be indicative of fruit processing activities. However, the study of Hadar maxilla's incisors suggests that they were used in for stripping leaves. *Australopithecus afarensis* has played a key role in early human evolution, leading the line to the

genus *Homo* (Johanson & White, 1979; Strait et al., 1997). The preliminary analysis of a small sample of *A. afarensis* specimens would serve to check buccal microwear research as a tool for inferring dietary habits of fossil himinins.

Specimen preparation and microwear analysis

Because of the difficulty to study unique original primate teeth, casts of all the specimens studied were made. The enamel surfaces were cleaned with pure acetone and ethanol with a cotton ear-cube, in order to remove consolidants, as well as dust. Negative casts were obtained with *President Microsystem* regular body polyvinylsiloxane (Colténe^{TM}). A single tooth, the lower left second molar (LM_2), was consistently selected and molded as representative of the buccal microwear pattern of each individual, in order to standardize the methods. The impression material, that shows an excellent dimensional stability and reproduction detail (Andritsakis & Vlamis, 1986; Teaford & Oyen, 1989), was applied from the occlusal border to the tooth roots, including the cemento-enamel junction, and from the mesial to the distal borders (Galbany et al., 2004a). From the negative molds a positive cast was obtained with a stable, two-base component epoxy resin (Epo-Tek 301, by Química del Aditivo), which provides faithful replicas with excellent detail for scientific research (Rose, 1983), or the also bicomponent polyurethane Feropur PR-55, that offers the same microscopic detail (Galbany et al., 2004a). The epoxy resin or polyurethane were gently stirred and poured into the molds with a Pasteur pipette and centrifuged during 3 minutes at 2,500 rpm to remove air bubbles in contact with the buccal surfaces. The replicas were mounted on SEM stubs and sputtered coated with a 400 Å gold layer for SEM observation, and were stored in a dust-free cupboard as a part of a larger collection (Galbany et al., 2004a, b). Some of analyzed moulds, which were originally from MRAC - Royal Museum for Central Africa, and were provided by Peter Ungar, who applied the same procedure to obtained them. Positive casts were done using the same procedures. Obtaining successive replicas from the same mould is not a methodological problem because they have been proved to be as good as the original teeth (Galbany et al., 2006).

All the teeth were observed at 40× magnification with a VMT binocular magnifying glass. Only well preserved teeth, those lacking any kind of enamel damage, patina, or mineral deposit on large portions of the buccal surfaces, were selected. A total of 86 well preserved teeth (Table 1) were selected and observed under SEM, using a Hitachi 2300 and a Cambridge Stereoscan 120 Scanning Electron Microscopes. The molds were placed in a horizontal position in the SEM chamber, with regard to the cemento-enamel junction, with zero degrees of tilt. Digital pictures (1024×832 pixels) of preserved enamel surfaces were obtained on the middle third of the buccal surface, avoiding both the occlusal and cervical thirds of the tooth. All the SEM pictures were taken at 100× magnification and 10-12 KV of electron acceleration. Only those SEM images that showed clear microwear features, in the form of striations of various lengths and orientations, not affected by microscopic enamel erosion, enamel prisms or perikymata exposure, were considered for further analyses. These strict criteria of analysis were adopted to ensure that no *post-mortem* damage or tooth preservation treatments were misinterpreted as natural, dietary related features. A 0.56 mm^2 square area of each SEM image was cut off for methodological standardization, following usual procedures for buccal microwear research (Pérez-Pérez, et al., 1999; Galbany et al., 2004a). The resulting gray-scale, digital picture was adjusted to enhance contrast with Adobe Photoshop (v. 7.0) using a high-pass, 50 pixel, filter and an automatic gray-level adjustment (Figure 1).

All microwear striations were counted and measured (length in µm and orientation in degrees from 0º to 180º) within the 0.56 mm^2 analyzed area using the Sigma Scan ProV (SPSS^{TM}) package. All measures were done by the same researcher in order to minimize the error rate by eliminating the inter-observer error, only considering the intra-observer error, which is less determinant in microwear research (Galbany et al., 2005b). A striation was defined as a linear mark on the enamel surface, at least three times longer than its width with a minimum length of 15 µm, independently of its curvature. All striations angles were measured in degrees and classified into 45º orientation class-groups (Pérez-Pérez et al., 1999) as horizontal (H), vertical (V), mesio-distal (MD), and disto-mesial (DM). For each orientation category, as well as for the total number of striations (T), the average number (N), length (X), and standard deviation of the length (S) of all observed striations were computed. Thus, a total of 15 variables were derived for each analyzed image: NH, XH, SH, NDM, XDM, SDM, NMD, XMD, SMD, NV, XV, SV, NT, XT, and ST (number, average length and standard deviation of the length for each orientation category). Kolmogorov-Smirnov Normality tests, single classification ANOVA and Discriminant analyses were made with the SPSS v.11 statistical package.

Table 3. Descriptive statistics of all variables for all subspecies analyzed: number (N), mean (X) and standard deviation (S)

	G g gorilla (Cameroon)		*G g gorilla* (Congo)		*G g gorilla* (Eq. Guinea)		*G g graueri*		*Pan t troglodytes*		*Pan t schweintfurthii*		*Pan t verus*	
	n=31		n=4		n=8		n=7		n=10		n=9		n=7	
	X	S	X	S	X	S	X	S	X	S	X	S	X	S
NH	40.10	22.61	37.50	21.99	46.75	23.30	34.29	15.77	52.80	33.15	37.56	21.32	28.43	6.95
XH	83.14	38.39	74.17	11.32	93.40	15.81	87.34	21.66	92.34	36.10	66.01	24.45	87.32	19.57
SH	64.90	45.19	55.18	16.76	111.00	17.49	71.22	32.02	77.80	46.14	42.78	32.66	81.74	39.86
NV	53.26	29.58	123.25	32.71	56.13	30.30	44.29	9.95	40.40	23.82	50.78	23.17	20.14	8.45
XV	128.93	34.51	148.70	33.70	137.50	46.36	162.64	36.60	126.89	16.62	113.05	34.88	153.81	44.89
SV	117.24	42.34	149.24	27.41	143.84	60.06	153.13	47.56	119.50	30.42	112.13	43.50	138.77	44.85
NMD	44.32	22.21	36.00	26.42	56.75	33.68	33.71	11.28	38.80	11.04	32.89	13.64	50.14	17.07
XMD	83.56	36.85	87.96	47.10	108.69	42.26	82.09	29.07	79.01	26.72	67.95	13.95	98.68	43.29
SMD	76.97	50.08	60.59	36.32	130.90	61.69	77.34	41.56	68.68	37.71	52.70	32.10	102.96	56.71
NDM	47.03	21.16	26.25	18.06	52.25	37.23	45.29	27.52	39.90	13.54	52.44	18.66	42.00	9.52
XDM	90.99	26.79	69.21	27.83	93.03	35.96	85.67	26.76	94.31	38.24	73.17	19.56	106.58	23.26
SDM	84.97	45.10	65.97	40.42	81.52	51.02	79.66	37.52	84.60	51.36	64.09	27.81	111.73	22.32
NT	184.71	32.39	223.00	54.55	211.88	79.92	157.57	28.80	171.90	31.81	173.67	37.26	140.71	18.07
XT	100.81	26.63	120.06	32.04	115.51	29.86	109.60	12.84	98.31	28.24	82.62	17.55	105.94	26.93
ST	101.74	33.11	124.56	25.02	134.56	29.73	121.37	22.22	94.63	35.94	82.72	27.58	111.80	26.68

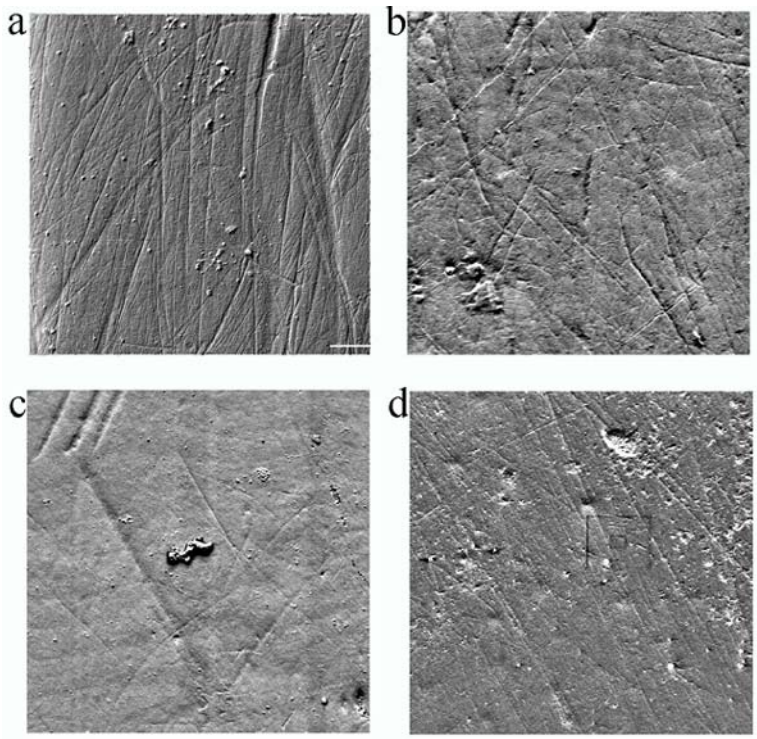

Figure 1. Scanning Electron Microscopy images of selected specimens studied: a) male *Gorilla gorilla gorilla* from Cameroon NHML-36.7.14.1, b) *Pan troglodytes troglodytes* IMAZ-7419, c) *Pan troglodytes verus* PMAE-7553, and d) *Australopithecus afarensis* AL-188-1. Each square surfaced analyzed covers exactly 0.56 mm^2 of enamel surface. Occlusal towards top of micrograph.

RESULTS

Extant Hominoidea dental microwear variability

All the variables considered for each group passed the Kolmogorov-Smirnov normality test ($P>0.05$) and, thus, parametric statistics could be used. Number of specimens, mean values and standard deviation for all variables of all groups are shown in Table 3. The one-factor ANOVAs show significant differences in only 5 of the 15 variables considered between the subspecies considered, $p<0.05$ (Table 4): SH (Standard deviation of horizontal microstriations), NV (Number of vertical microstriations), SMD (Standard deviation of mesio-distal microstriations), NT (Number of total microstriations) and ST (Standard deviation of total microstriations). The Bonferroni *post-hoc* test shows that NV (number of vertical microstriations) is the variable that has most differences between the analyzed subspecies. *Gorilla g. gorilla* from Congo shows significant differences for this variable with almost all other subspecies (Table 5).

A Principal Component Analysis (PCA) of the extant *Hominoidea* groups was performed to analyze the variability among the extant subspecies, based on their buccal microwear patterns. All groups were considered in this analysis and five principal components were obtained explaining 81.18% of total variability (CP1: 37,51%, CP2: 15.13%, CP3: 11.92%, CP4: 8.90% and CP5: 7.71%). The factors of the first five components for each subspecies considered were used in a single-linkage cluster analysis (Square Euclidean Distance). The cladogram obtained (Figure 2) shows that the *Gorilla gorilla gorilla* from Congo is the most differring of all samples. The gorillas from Cameroon, *Pan t troglodytes* and *Pan t. schweifurthii* group together, close to a twin group formed by *Gorilla g. graueri* and *Pan t verus*.

Australopithecus afarensis

In order to infer dietary habits of *Australopithecus afarensis* from dental microwear, a savanna primate species, *Papio anubis*, was added to other subspecies considered. They were also molded from originals curated at the osteological collections at National Museums of Kenya (Galbany & Pérez-Pérez, 2004).

Table 4. Analysis of variance (ANOVA) of the 15 variables studied for all groups considered. Significant differences (*) at 0.05 confidence interval.

	F	P
NH	1.036	0.4096
XH	0.825	0.5548
SH	2.497	**0.0303**
NV	7.286	**0.0000**
XV	1.954	0.0843
SV	1.366	0.2405
NMD	1.465	0.2033
XMD	1.219	0.3070
SMD	2.515	**0.0293**
NDM	0.941	0.4715
XDM	1.337	0.2527
SDM	0.953	0.4638
NT	3.232	**0.0074**
XT	1.725	0.1281
ST	2.884	**0.0144**

Table 5. Bonferroni post-hoc test of 15 variables studies for all groups considered. Significant differences (*) at 0.05 confidence interval.

		Variable	STD	P
Ggg Congo	Ggg Cameroon	NV	13.764	0.0001
	Ggg Eq.Guinea	NV	15.864	0.0015
	G g graueri	NV	16.238	0.0001
	Pan t troglodytes	NV	15.327	0.0000
	Pan t schweinfurthii	NV	15.568	0.0003
	Pan t verus	NV	16.238	0.0000
	Pan t verus	NT	25.282	0.0370
Ggg Eq.Guinea & Gabon	*Pan t schweinfurthii*	ST	15.016	0.0200
	Pan t schweinfurthii	SH	19.190	0.0144
	Pan t schweinfurthii	SMD	23.144	0.0252
	Pan t verus	NT	20.876	0.0230

A Discriminant Analysis of all variables for all subspecies, including *Papio anubis*, were done in order to classify *Australopithecus afarensis.* All variables passed the step-wise tolerance test, except the total number of striations (NT), which is frequently correlated with the other variables (Galbany et al., 2002, 2005a; Galbany & Pérez-Pérez, 2004). Seven discriminant functions (DF) were derived, the four main ones explaining 91.6% of total variance (44.3%, 26.3%, 13.8% and 7.3% respectively). The first function (DF-1) was significantly correlated ($P<0.05$) with NV (r=-0.594) and NDM (r=0.444), and the second (DF-2) present high correlations with NT (r=0.432), despite of not being significant. The power of discrimination of the seven combined functions, measured with Wilks lambda ($\lambda=0.073$, $\chi^2=238.424$) was highly significant ($P<0.001$).

The classification of *Australopithecus afarensis* using the discriminant functions derived was *Papio anubis* in 50% of the cases (5 out of the 10 specimens analyzed), *Gorilla gorilla gorilla* from Cameroon for 4 specimen (40%), and *Pan troglodytes troglodytes* for one specimen (10%). The total well-classified specimens were 67.0%, and by subspecies: *Gorilla g gorilla* Cameroon (54.8%), *Gorilla g gorilla* Congo (75.0%), *Gorilla g gorilla* Eq.Guinea and Gabon (75.0%), *Gorilla g graueri* (42.9%), *Pan t troglodytes* (60.0%), *Pan t shweinfurthii* (33.3%), *Pan t verus* (71.4%), and *Papio anubis* (96.3%).

Figure 3 shows the first two discriminate functions obtained with ellipses of 70% of total variability. Despite great overlap occurs among most groups in this two-dimensional plot, some associations are evident, mainly occurs between Cameroon gorillas, *Gorilla g graueri*, *Pan t schweinfurthii* and *Pan t verus*. Gorillas from Congo and Equatorial Guinea and Gabon have higher values for DF-1, and *Pan t troglodytes* lower ones. *Papio anubis* present lower values for DF-2, similar to *Australopithecus afarensis*, that are clustered between *Papio anubis* and *Pan t troglodytes* for DF-2.

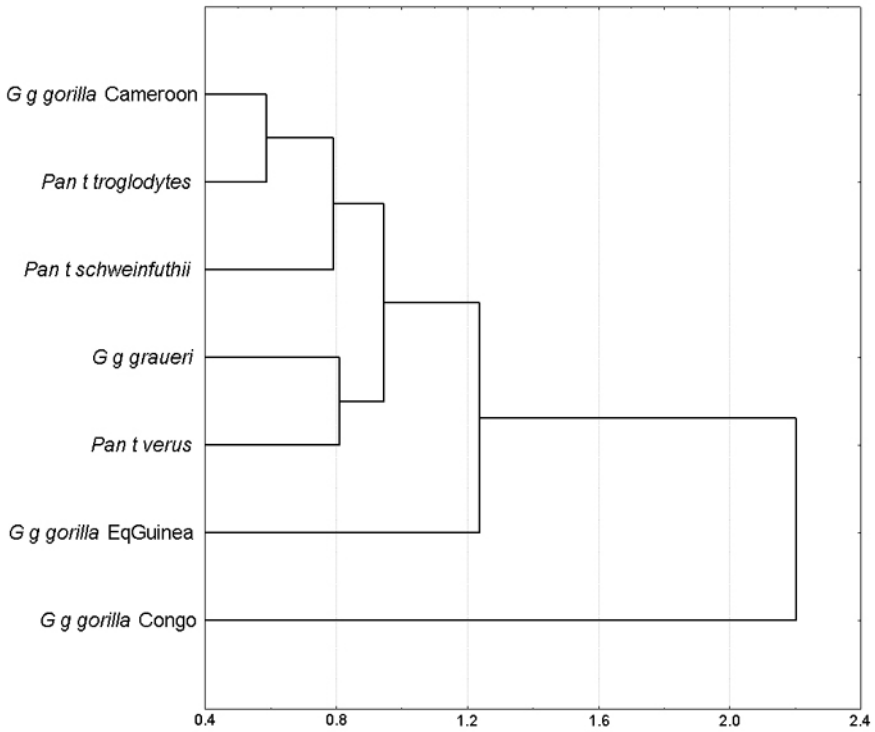

Figure 2. Plot representing the percentage of classification of each group derived from Discriminant Analysis of 15 variables studied for extant Hominoidea and *Australopithecus afarensis*.

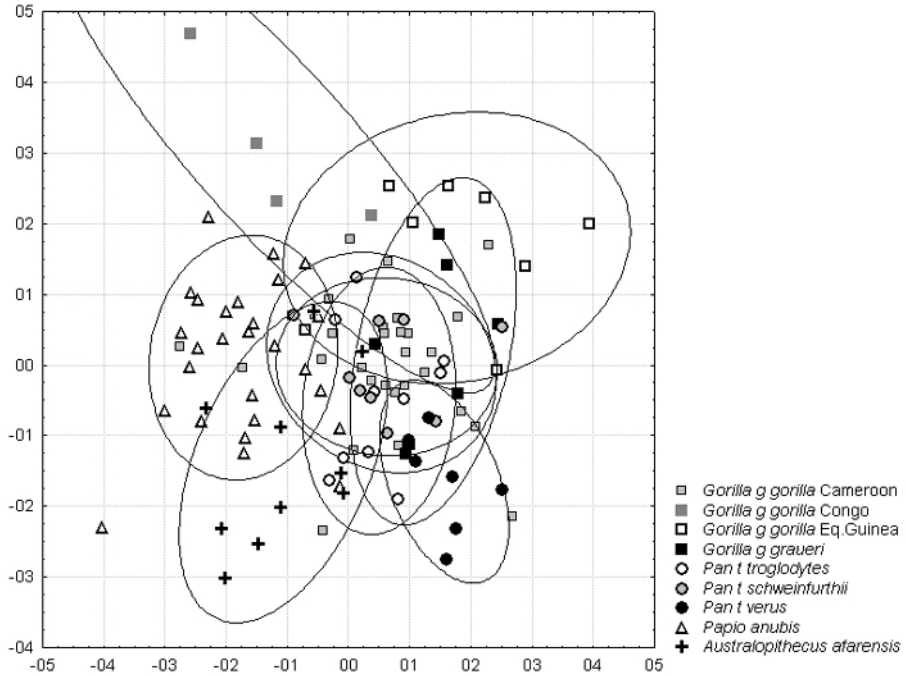

Figure 3. Plot of the two first functions from the Discriminant Analysis of the 15 variables studied for the extant Hominoidea and *Australopithecus afarensis*. First function explains 44.3% of total variability, and the second 26.3%.

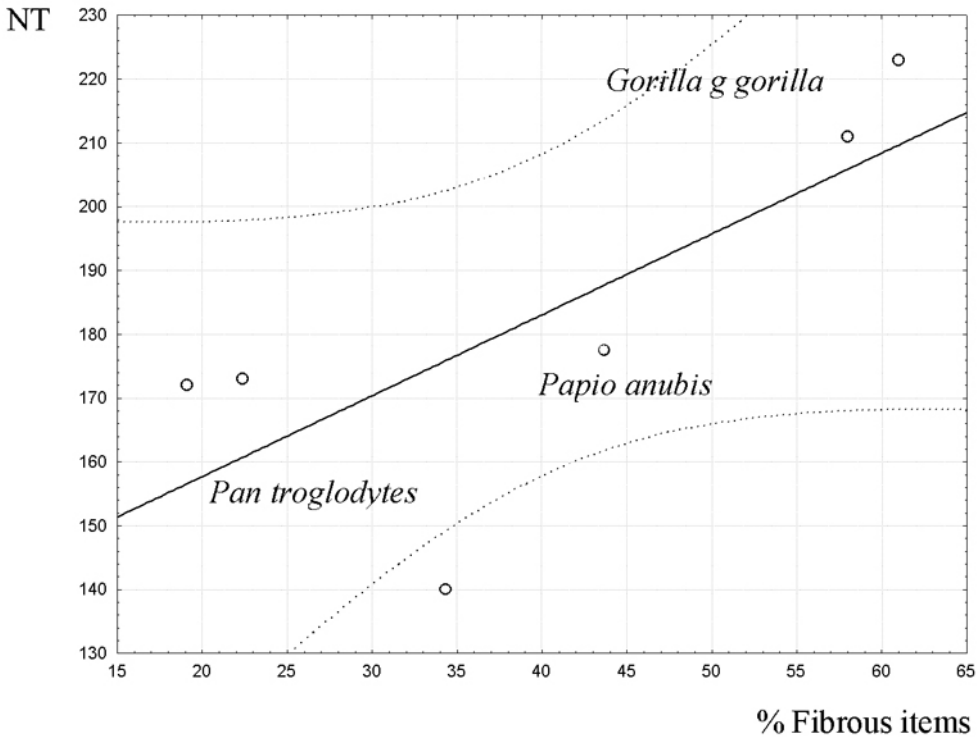

Figure 4. Dispersion graphic between mean values of percentage of fruit consumed and total number of microstriations (NT) for different subspecies of *Pan troglodytes* and *Gorilla gorilla*.

DISCUSSION

The buccal microwear patterns of the extant *Hominoidea* studied show a high homogeneity, despite of being selected from restricted geographic distributions determinant of geographical differences in diet (Wrangham et al., 1991; Sugiyama & Koman, 1992; Tutin & Fernandez, 1993; Tutin et al., 1997; Remis, 1997; Yamakoshi, 1998; Newton-Fisher, 1999; Tutin, 1999; Tweheyo & Obua, 2001; Basabose, 2002; Doran et al., 2002; Stanford & Nkurungungi, 2003; Rogers et al., 2004). ANOVA analyses between subspecies (Table 4) reflect that only 5 of 15 variables show significant differences between the subspecies analyzed. These results are consistent with other studies that considered all catarrhini buccal microwear patterns and do not show great differences between Hominoidea (Galbany et al., 2002, 2005a).

The cladogram (Figure 2) derived from the Principal Component Analyses shows the similarities in buccal dental microwear between the subspecies analyzed. Closer clustering of groups are indicative of similarities in buccal microwear pattern between groups, indicative of similarities in diet abrasivity and composition. The close relationship between Cameroon gorillas and *Pan t. troglodytes* and *Pan t. schweintfurthii* in the single-linkage cluster analysis is consistent with previous studies that showed a high similarity of dental microwear between these groups (Galbany et al, 2002). Cameroon gorillas and *Pan t. troglodytes* are species that inhabit the same areas and present similar ways to exploit the alimentary resources in the same habitat. Gorillas from Congo, Equatorial Guinea and Gabon show higher differences in their dental microwear patterns, and are the most remote groups in the plot. Tutin & Fernández (1994) noted down that *Pan t. troglodytes* and *Gorilla g. gorilla* of Lopé reserve in Gabon share 127 alimentary items, mainly fruits, which means an 82% of total ingested foods. This small difference in diet might be responsible their distinct microwear patterns, mainly for the Gabon gorillas that show a wider range of intraspecific variability.

The relative importance of leaves, pith, barks and other abrasive food items in gorilla's and chimpanzee's diets could be directly related to the microwear pattern. The abundance of these items in the diet of some gorilla or chimpanzee populations is less documented than amount of fruit (Table 6). Figure 4 shows a direct relationship between total number of microstriations (NT) and the mean percent of fibrous items ingested in the diet, such as leaves, pith and barks, by subspecies in different or geographic areas (Table 6). Gorillas show a lower percent of fruit consumption, and a high consumption of herbs, pith or barks, that correlates with a high value for the total number of microstriations. Chimpanzees, in contrast, show lower NT values and higher fruit ingestion in the diet, in detriment of fibrous items. *Papio*

anubis populations, although consuming highly variable amounts of fibrous items in relation to their geographic origin, they show an intermediate NT value between gorillas and chimpanzees. The correlation between NT and the percent of fibrous items is not significant, though (P=0.088), despite the high correlation value observed (R= 0,747) (Figure 4).

All the results obtained point to the existence of a close relationship between the number of microstriations, as an indicator of wear rates on buccal enamel surfaces caused by both fibrous and herbaceous food items.

Dietary habits of *Australopithecus afarensis*

The dental microwear of *Australopithecus afarensis* shows a certain homogeneity, as Puech et al. (1983) concluded by studying wear facets, and is mostly related to that of *Papio anubis* and Gorilla g. gorilla from Cameroon. For this reason, this fossil species might have had a similar diet to these primates, based primarily in fibrous items throughout the whole year, though also consuming ripe fruits. Microwear results show that *Australopithecus afarensis* diet shows microwear patterns of a typical savanna primate and, at the same time, similar to that of the gorillas that inhabit dense rainforests.

No *Australopithecus* specimens were classified in the present study as having a microwear pattern similar to that of *Pan troglodytes verus* or the gorillas from Congo, Equatorial Guinea and Gabon, which inhabit mosaic habitats with savannas and grassland (Kortlandt, 1983; Tutin & Fernández, 1994), consuming more fibrous and abrasive fallback foods (Rogers *et al.*, 1990; Rogers *et al.*, 1994; Remis *et al.*, 2001; Tutin *et al.*, 1997; Rogers et al, 2004). Thus, it may suggest that very fibrous fallback resources were not, or at least not intensely, exploited by this fossils species, for which food items from savannas and rainforests would constitute the main diet resource throughout the whole year, such as leaves, pith and ripe fruits. Sept (1986) has concluded that seasonal differences in fruit availability, though not highly significant, could account for a fruit-eating hypothesis for *Australopithecus* year round. However, the microwear data obtained in this research do not support it, and it is possible that *Australopithecus afarensis* had to fed on several resources from different typologies, as well as keystone foods during scarcity periods.

These results are consistent with previous ones by P Ungar (2004), who concluded that *Australopithecus afarensis* diet might have differed from that of chimpanzees largely in fallback resources, which could have included larger amounts of hard brittle items, as noted also by Ryan and Johanson (1989) based on dental microwear features supporting the consumption of both closed forest plants and open savanna foods. Other studies concluded that *A. afarensis* would probably have had a forest-savanna resource adaptation rather than a hard-object specialization. So, these hominins would probably still have preferred soft, sugar-rich fruits, but would have been able to make better use of hard, brittle resources as fallback foods (Picq, 1990; Teaford and Ungar, 2000).

Table 6. Percentage of fruit and leaves, pith and bark consumed by several *Gorilla gorilla*, *Pan troglodytes* and *Papio anubis* populations.

Especie	Site	% fruit	% fibrous	citation
Pan t troglodytes	Lopé Reserve (Gabon)	69.2	19.1	Tutin et al. (1997)
		87.0	-	Tutin & Fernández (1993)
		77.0	-	Tutin (1999)
Pan t verus	Bossou (Republic of Guinea)	72.0	-	Sugiyama & Koman (1992)
		60.7	34.3	Yamakoshi (1998)
Pan t schweinfurthii	Kahuzi (Democratic Rep. Congo)	58.0	-	Basabose (2002)
	Kibale National Park (Uganda)	67.0	-	Wrangham et al. (1991)
	Bwindi Impenetrable NP (Uganda)	64.6	27.1	Stanford & Nkurununngi (2003)
	Budongo Forest Reserve (Uganda)	64.5	23.0	Newton-Fisher (1999)
		83.0	17.0	Tweheyo & Obua (2001)

Gorilla g gorilla	Lopé Reserve (Gabon)	40.9	58.0	Tutin et al. (1997)
		47.0	-	Tutin (1999)
	Mondika (Central African Republic – Democratic Rep. Congo)	37.0	61.0	Doran et al. (2002)
	Bai Hokou (Central African Republic)	51.0	48.0	Remis (1997)
Papio anubis	Bole (Etiopía)	41.0	42.0	Dunbar & Dunbar (1974)
	Chololo (Kenya)	23.0	42.0	Hill & Dunbar (2002)
	Gilgil (Kenya)	10.0	80.0	Harding (1976)
	Gombe (Tanzania)	49.0	21.0	Hill & Dunbar (2002)
	Masai Mara (Kenya)	46.0	52.0	Hill & Dunbar (2002)
	Shai Hills (Ghana)	59.0	25.0	Hill & Dunbar (2002)

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