In Vivo Rates of Dental Microwear Formation in Laboratory Primates Fed Different Food Items

Mark F. Teaford  
*Touro University California, mteaford@touro.edu*

Peter S. Ungar

Andrea B. Taylor  
*Touro University California, andrea.taylor3@tu.edu*

Callum F. Ross

Christopher J. Vinyard

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Mark F. Teaford\textsuperscript{a,*}, Peter S. Ungar\textsuperscript{b}, Andrea B. Taylor\textsuperscript{a}, Callum F. Ross\textsuperscript{c}, Christopher J. Vinyard\textsuperscript{d}

\textsuperscript{a}Department of Basic Science, Touro University, California, United States
\textsuperscript{b}Department of Anthropology, University of Arkansas, United States
\textsuperscript{c}Department of Organismal Biology & Anatomy, University of Chicago, United States
\textsuperscript{d}Department of Anatomy and Neurobiology, NEOMED, United States

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1. Introduction

Dental microwear analyses have become a standard part of paleobiological research (see [1–6] for reviews). While informative, many microwear interpretations are ultimately based on correlations between patterns of dental microwear in extant taxa and published differences in diet for those taxa. There have been very few studies of dental microwear in living animals [7–16] and even fewer longitudinal, in vivo, studies of dental microwear formation [15–18]. This paucity of in vivo studies has been alleviated to some degree by in vitro studies of microscopic wear on enamel [19–25], as these have allowed researchers to demonstrate relationships between enamel microwear and the processing of specific foods with the application of specific loads, and variations in the approach angles at which those loads are applied. Such work should ultimately shed light on the age-old questions of whether food particles are able to scratch enamel and how variation in microwear patterns might result from controlled and measurably different diets.

Questions about enamel fracture mechanics go back to some of the earliest papers on dental microwear [26–28]. Dental enamel is one of the hardest biomaterials [29,30] leading some researchers to argue that most foods cannot actually scratch it. Some have argued that “diet is not the most important influence on microwear patterns” [31] but that instead, most abrasive wear of enamel is caused by exogenous grit. However, recent nano-scale work [32,33] has demonstrated that the protein bonds that hold hydroxyapatite crystals together are susceptible to breakage by materials traditionally thought to be softer than enamel so long as contact pressure is sufficient to break the “glue” between adjacent nanospheres. This opens the possibility that a vast range of materials, from exogenous grit to endogenous silicates and beyond, can cause the microscopic removal of pieces of enamel.

In order to augment the scant in vivo data on dental microwear in nonhuman primates, we performed a series of feeding and microwear experiments with laboratory capuchin monkeys and lemurs in which dental impressions were taken immediately before and after the animals fed on a variety of foods. The goals were to provide answers to two questions:

1. Would new dental microwear be detectable in this short time interval of a single feeding event?
2. Would rates of dental microwear formation across events be correlated with the presence or absence of feeding on hard objects in those feeding sessions?

2. Materials & methods

2.1. Subjects

Feeding data and dental impressions were collected from three adult Sapajus apella (“UC-C,” “UC-M,” and “UC-S”) housed in the Animal Resources Center at the University of Chicago and two adult tufted capuchin monkeys, Sapajus apella (“NEO-C” and “NEO-M”) housed at the Comparative Medicine Unit at the Northeast Ohio Medical University (NEOMED). Feeding data and dental impressions were also collected from three adult Lemur catta (2 males [“NEO-K,” “NEO-T”], 1 female [“NEO-R”])
housed at the Comparative Medicine Unit at NEOMED. Not all animals were involved in every feeding experiment. The subjects had all of their teeth and displayed no obvious asymmetries, diseases, or deformities of their craniofacial and dental anatomy. All animals were housed in standard approved caging, given ad libitum water and fed daily a diet including monkey biscuits as well as various fruits and vegetables.

All procedures were approved by the relevant (UChicago or NEOMED) IACUC and conformed to the principles outlined in the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23, revised 1985) as well as with the requirements of the Animal Welfare Act.

At least three months prior to data recording, the three UChicago capuchins had been implanted with percutaneous Vitalium™ bone screws in the mandibles and zygomatic arches to serve as anchoring points for reflective markers used for recording of jaw kinematic data reported elsewhere [34–37]. Likewise, the NEOMED capuchins and lemurs were subject to analyses of craniofacial bone strain and EMG activity of chewing muscles [38,39]. As none of that work was of direct relevance to this study, readers interested in those methods are referred to the previously published work describing them.

2.2. Feeding experiments

Three feeding experiments were carried out in this study, and each involved the same basic protocol – i.e., the animal was anesthetized, baseline dental impressions were taken, the animal was allowed to wake up and feed on the offered items, after which the animal was anesthetized again and follow-up dental impressions were taken. Hence, baseline and follow-up impressions were taken within hours of each other on the same day in order to document rates of microscopic wear tied to the consumption of known food items fed between the pre- and post-impression time interval. The main difference between experiments involved the foods offered to the animals.

1. In one set of experiments, the animals (UC-C, UC-M, UC-S, NEO-C, NEO-M) were only offered unshelled brazil-nuts. UC-C and UC-M were used in two of these experiments; UC-S and NEO-C were used in one experiment each.

2. In another experiment, all five Sapajus monkeys were each offered a mixture of foods, including unshelled brazil-nuts and other shelled and unshelled nuts (i.e., almonds, walnuts, hazlenuts, pecans) plus additional food items (e.g., apple slices, dried apricots, raisins, banana chips, figs, cherry pits, and unpopped popcorn kernels).

3. In the third experiment, the lemurs were each offered a mixture of foods, focusing on soft or tough food items (e.g., dates, raisins, gummy bears, grapes, apple slices, figs, sucrose candy, and banana slices) but with no nuts.

2.3. Anesthesia

The animals were deprived of food for 24 h before each experiment.

At NEOMED animals were given an intramuscular injection of ketamine (10–25 mg kg\(^{-1}\); Ketaset1 Ft. Dodge Animal Health) and acetylpromazine (0.5–1.0 mg/kg; Acepromazine maleate—Boehringer Ingelheim) or ketamine (5–12 mg/kg) and medetomidine (0.05–0.07 mg/kg; Domitor1 Pfizer). Atropine (0.05 mg/kg) was administered by subcutaneous injection 10 min prior to anesthesia to reduce salivary secretions. At UChicago, the animals were sedated with ketamine (4 mg/kg) and dexmedetomidine (150–200 μg/kg) [40].

Animals were intubated orotracheally following sedation, and anesthesia was maintained using inhalant isoflurane delivered at 2–4% of inspired gas with a balance of pure oxygen for the duration of the impression session. Animal vital signs were monitored under anesthesia during the impression sessions and fluid support was provided by either subcutaneous or intravenous 0.9% saline as needed.

Impression sessions averaged approximately 30–45 min (depending on how many dental quadrants were sampled) after which isoflurane was discontinued. Animals receiving medetomidine were given atipamezole (Antisedan1 P) intramuscularly at an equivalent volume (5 mg/ml concentration) to the medetomidine to reverse its effects. After the initial implants and molding procedures, animals recovered to the point that they were alert, maintained a normal sitting posture, and readily took food in less than an hour after discontinuing anesthesia.

2.4. Dental impression protocol

Baseline impressions were taken using the techniques of Teaford and Oyen [41]. Thus, with the animal anesthetized, a plastic "mixing tip" (from the dental impression supplies) was cut to fit between the upper and lower canines on one side of the mouth. The teeth then were cleaned with a toothbrush and a dilute bleach solution before water-picking for 1–2 min. Then the teeth were dried for 2–3 min before impressions were taken using Coltene-Whaledent’s "President Jet Regular" polyvinylsiloxane. Once an animal recovered from anesthesia, it was fed the experimental food item(s) ad libitum before anesthesia was once again administered so that follow-up impressions could be taken on the same day.

2.5. Scanning electron microscopy and computation of rates of microscopic wear

As with previous analyses of rates of microscopic wear [13–16,18,42,43] epoxy casts of the first and second molars were examined at a magnification of 200X in a scanning electron microscope (either an AMRAY 1810 housed in the Functional Anatomy and Evolution Program at the Johns Hopkins University School of Medicine, or an FEI XL30 at Duke University’s Shared Materials Instrumentation Facility). Micrographs of the same areas on the same tooth were taken for baseline and follow-up casts of each animal. The baseline and follow-up images were then imported into Photoshop (together with an image of a reference grid) (Fig. 1). The follow-up image and the reference grid were placed in layers on top of the baseline image, so that the follow-up image could
be positioned optimally for visual comparison of its microwear features with those in the baseline image (using the reference grid merely as an aid in comparison) (Fig. 2). The number of features in each square of the follow-up micrograph was counted, as was the number of features that had appeared in the follow-up image as a result of the day’s feeding session (Fig. 3). These numbers were used to calculate the percentage of features in the follow-up micrograph caused during that day’s feeding session. If calculations were made for more than one facet on one tooth, or more than one tooth, those numbers were totaled, yielding a summary percentage of new features for that individual in that feeding experiment.

3. Results

3.1. Feeding experiment #1

While the data for NEO-M were not used (because the dental impressions were too poor to yield useful results), each
of the remaining Sapajus monkeys showed new microwear features after feeding on unshelled brazil-nuts, and the percentage of new features for each individual roughly paralleled the number of brazil-nuts consumed (Fig. 4a, Table 1). The one exception was NEO-C, whose overall rate of microscopic wear (calculated for molars from both left and right sides) masked a marked difference between rates calculated for the left and right sides (Fig. 4b, Table 1) which reflected his tendency to break nuts and chew them on the right side of his mouth.

3.2. Feeding Experiment #2

When the Sapajus monkeys were fed a mixture of food items (including softer items like dates, figs, and apples, plus other nuts including hazelnuts, almonds, and pecans) the percentage of new features generally decreased as compared with the results from Experiment 1 for the same animal (Fig. 5a, Table 1). The one exception was NEO-C, whose overall rate of microscopic wear (calculated for molars from both left and right sides) masked a marked difference between rates calculated for the left and right sides (Fig. 5b, Table 1) which reflected his tendency to break nuts and chew them on the right side of his mouth.

3.3. Feeding Experiment #3

The lemurs all showed little to no new microwear on their molars after a feeding bout (Fig. 6, Table 1).

4. Discussion

The described method of quantifying rates of wear is not without its limitations. For instance, taking dental impressions on live animals presents a formidable challenge; and every experiment is ultimately dependent on the quality of the resultant mold and cast. Under the current protocol, not every facet on every tooth was clean or dry when the impressions were taken. This may mean that, in rare cases, one impression may be excellent (e.g., NEO-M in second experiment) and the other may not be useable (NEO-M in first experiment), or organic films on a tooth may obscure details of a facet (Fig. 7a & b). It may also mean that analyses include data from both consumed very similar food items. In fact, as NEO-M's food items included some items not given to NEO-C (e.g., 2 popcorn kernels and 2 cherry pits), their differences in percentage of new features might be viewed as the opposite of what one might expect. However, one additional complication in this comparison was NEO-C's tendency to break into nuts and chew them on the right side of his mouth. Thus, the only new features seen on his molars in this experiment were on the right side (Fig. 5b, Table 1).

![Graphs showing the percentage of microwear features created in each individual feeding bout within experiment 1 (feeding on unshelled brazil-nuts). Numbers represent overall averages of numbers calculated for right and left M1s and M2s (including upper and lower molars when available). A. Percentage for NEO-C is based on numbers from right and left molars. B. Percentages for NEO-C showing the separate values for right and left molars.](image)

Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Experiment date</th>
<th>Total # Features</th>
<th># of New features</th>
<th>% New features</th>
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<tr>
<td>UC-M</td>
<td>4-6-09</td>
<td>746</td>
<td>12</td>
<td>1.6</td>
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<tr>
<td>UC-M</td>
<td>5-6-09</td>
<td>929</td>
<td>9</td>
<td>1.0</td>
</tr>
<tr>
<td>UC-S</td>
<td>5-5-09</td>
<td>333</td>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>UC-C</td>
<td>4-6-09</td>
<td>407</td>
<td>26</td>
<td>5.2</td>
</tr>
<tr>
<td>UC-C</td>
<td>5-6-09</td>
<td>1005</td>
<td>40</td>
<td>3.98</td>
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<tr>
<td>NEO-C</td>
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<td>815</td>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>(NEO-C – right side)</td>
<td>61</td>
<td>3</td>
<td>4.9</td>
<td></td>
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<tr>
<td>UC-S</td>
<td>5-28-09</td>
<td>456</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
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<td>555</td>
<td>6</td>
<td>1.1</td>
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<td>0.4</td>
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<tr>
<td>NEO-C</td>
<td>5-21-09</td>
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<td>17</td>
<td>3.7</td>
</tr>
<tr>
<td>(NEO-C – right side)</td>
<td>266</td>
<td>17</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>NEO-R</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NEO-K</td>
<td>6-8-10</td>
<td>193</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*NEO-C routinely broke the brazil-nuts and chewed them on his right side. Unfortunately, there was only one pair of molar facets from the right side for which features could be counted. If figures for the left (i.e., the side where NEO-C was doing minimal chewing) side are included into an overall average, his % of new features drops dramatically.*
shearing and crushing facets averaged together, which limits the possible resolution of some interpretations. Also, as all casts have aluminum pin-type mounts glued to their bases (while still in their impressions), there is no way to guarantee that baseline and follow-up images will be in precisely the same orientation (Fig. 2), and this, plus any foreshortening due to tilting and rotation of images, can make comparisons between images difficult. Despite such limitations, this method does allow for the monitoring of microscopic changes on the tooth surface on a day-to-day (or even event-to-event) basis, and it has been shown to accurately track more traditional macroscopic measures of rates of tooth wear [44].

The results of this study clearly show that dental microwear can be produced as a result of feeding on hard objects. Moreover, these features are often detectable after just one feeding bout. The features produced include pits and scratches, but the pits, in particular, are generally small and formed near the margins of existing features (Fig. 3).
Comparisons between feeding experiments, however, demonstrate that the relationship between the amount and type of items consumed and the rate of formation of dental microwear is not a simple one. For instance, even in the first experiment, where there was a clear correlation between the number of brazil-nuts consumed and the rate of microscopic wear, the feeding behavior and chewing patterns of one individual (NEO-C) impacted the results in two ways. First, this individual consumed more brazil-nuts than did any of the other monkeys, yet his overall rate of microscopic wear was less than that of most of the other monkeys that consumed less. Second, this individual had the habit of breaking into the brazil-nuts and chewing on them on the right side of his mouth. Perhaps not surprisingly, calculations of rates of microscopic wear for his left and right sides differed dramatically (Table 1, Fig. 4b), and the values for the right side were more like what one would expect (based on comparisons with the other monkeys in this experiment). These results demonstrate the importance of inter-individual differences in feeding and oral behaviors (at least in the short-run), and that, in turn, emphasizes the need for more feeding experiments – even when only one food item is used in those experiments.

Likewise, in the second experiment (where Sapajus monkeys were fed a variety of food items), some results are what one might expect, with UC-S and UC-C showing declines in the rate of microscopic wear when compared with the first experiment, and UC-M showing a slight increase associated with his consumption of slightly more brazil-nuts than he had consumed in the first experiment (Fig. 5a). However, the results for NEO-M and NEO-C raise additional issues (regardless of NEO-C’s propensity to chew on his right side). If anything, as noted earlier, NEO-M might have been expected to show slightly more new microwear features than NEO-C, but he did not. One possible complication might relate to the fact that NEO-M was the oldest monkey in the experiment (at least in terms of molar morphology), with molars reduced to enamel rims. This could impact the results of this study in two ways. First, he may have been doing more chewing elsewhere along the tooth row, for instance, in the premolar region. As this particular study did not focus on the premolars, we cannot shed light on that possibility at this time. Second, even though traditional “Phase I” and “Phase II” facets are effectively obliterated by such extreme wear (and thus standard dental microwear analyses are generally not performed on such teeth), the dentin basins and enamel rims on his molars could have been used differently from each other. That could lead to one of two possibilities: either (1) the dentin exposures on his molars bore the brunt of the crushing impacts of the foods he was eating, or (2) with only enamel rims on the molars, one might expect to see an even faster rate of molar microwear on those rims if crucial cutting or crushing of foods was performed against them and/or if the enamel on those rims was indeed softer than that which was originally on the occlusal surface [45]. The results of this study suggest that the first interpretation is correct. In any case, the results of the second experiment raise another point of interest: what is causing the new microwear observed on the teeth of these animals? If NEO-C only processed a few almonds, hazelnuts, and monkey chow biscuits, in addition to apple slices, prunes, dried apricots, raisins, and figs, what caused the relatively rapid creation of dental microwear on his right molars? As NEO-C and NEO-M were the only animals fed monkey-chow in these experiments, this might have been the cause, as monkey chow has long been known to cause dental microwear [15]. But, if that is the case, then one might expect a similar result for NEO-M. Likewise, as UC-S, UC-C, and UC-M each consumed a mixture of “soft” foods and nuts, the most intuitively likely cause of the new microwear is the nuts, particularly pieces of their shells. However, as UC-M and U-C each consumed more nuts than did UC-S (Table 2), why are their rates of microwear not more different? These mixed results indicate that we still have much to learn about the interrelationship between oral food processing of specific foods and the creation of dental microwear.

By comparison, the third experiment yielded few surprises, as the lemurs (with no nuts among their food items) showed little to no new microwear. This differed from the Sapajus monkeys (with the occasional nuts among their food items) in Experiment 2, who showed at least some new microwear (Fig. 6).

In sum, Experiments 1 and 3 showed relatively straightforward results which seemed to implicate hard objects in the creation of new microwear features (Experiment 1), and the absence of hard objects in the absence of microwear creation (Experiment 2). Does this rule out the possibility of soft foods causing dental microwear? Probably not, for while enamel hardness, as measured by standard Mohs or Vickers scales, is indeed relatively high, the scale of those tests is likely too crude to rule out microscopic wear mechanisms and potential impacts of specific foods on the creation of dental microwear. If the protein bonds between hydroxyapatite crystals are susceptible to damage as a result of contact with materials traditionally viewed as being “softer” than enamel [22,23,32,33], then it may not matter if a food item is “harder” than enamel, or how those measurements are created. What may matter will be the microscopic and/or nano-scale approach angles of particles approaching the tooth surface and the shape of those particles. From this perspective, perhaps some dental microwear (e.g., in one of the lemurs in Experiment 3) should be expected from the consumption of soft food items. Moreover, we could easily be overlooking other complexities in the food items themselves, or in the
tooth-food-tooth interactions. Were any of the other foods acidic enough to facilitate the creation of microwear by slightly weakening the surface enamel before the consumption of subsequent items? Could stiffer foods provide a more effective platform than more malleable foods for the application of contact pressures by food/abrasive particles against the teeth [24]? Or, could the seeds within foods traditionally viewed as "soft" (e.g., grapes) actually cause microwear? Clearly more work is necessary.

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