



Variation in enamel mechanical properties throughout the crown in catarrhine primates

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ABSTRACT

Enamel mechanical properties vary across molar crowns, but the relationship among mechanical properties, tooth function, and phylogeny are not well understood. Fifteen primate lower molars representing fourteen taxa (catarrhine, $n = 13$; platyrrhine, $n = 1$) were sectioned in the lingual–buccal plane through the mesial cusps. Gradients of enamel mechanical properties, specifically hardness and elastic modulus, were quantified using nanoindentation from inner (near the enamel–dentine junction), through middle, to outer enamel (near the outer enamel surface) at five positions (buccal lateral, buccal cuspal, occlusal middle, lingual cuspal, lingual lateral). Cuspal positions had higher mechanical property values than lateral positions. Middle enamel had higher mean hardness and elastic modulus values than inner and outer locations in all five crown positions. Functionally, the thicker-enameled buccal cusps of lower molars did not show evidence of increased resistance to failure; instead, lingual cusps—which show higher rates of fracture—had higher average mechanical property values, with no significant differences observed between sides. Preliminary phylogenetic results suggest there is relatively little phylogenetic signal in gradients of mechanical properties through the enamel or across the crown. There appears to be common mechanical property patterns across molar crowns in Catarrhini and potentially among primates more broadly. These results may allow more precise interpretations of dental biomechanics and processes resulting in mechanical failure of enamel in primates, such as wear and fracture. © 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Enamel forms the outer layer of tooth crowns in primates and has been characterized as the hardest biological material in the body (He and Swain, 2008; Jeng et al., 2011). Enamel consists of both inorganic and organic components, with organic components making up less than 5% of the enamel volume (Cuy et al., 2002). Enamel is hierarchical in structure and organization, containing adaptations to minimize mechanical failure at the nano (e.g., hydroxyapatite crystal orientation), micro (e.g., enamel arrangement and type, or ‘Schmelzmuster’), and macro (e.g., enamel thickness, crown morphology) levels (Maas and Dumont, 1999). These adaptations can vary throughout the thickness of enamel and across the surface of the tooth (e.g., Tafforeau et al., 2012).

Enamel is not evenly distributed on the molars of primates, typically being thicker on the lingual side of upper molars and on the buccal side of lower molars (Molnar and Ward, 1977; Macho and Berner, 1993; Schwartz, 2000). Cusps with thicker enamel are often termed ‘functional’, whereas cusps with thinner enamel are called ‘nonfunctional’ or ‘guiding’ cusps, due to differences in function; for example, functional cusps being able to withstand more sustained occlusal forces (Molnar and Gantt, 1977; Khera et al., 1990; Schwartz, 2000; Yang et al., 2022). While some researchers have suggested variation in cuspal enamel thickness is related to shearing and crushing forces (e.g., Schwartz, 2000), others have suggested the thicker enamel of functional cusps may protect against fracture (e.g., Grine, 2005). Functional cusps typically show more rapid wear (Macho and Berner, 1993; Schwartz, 2000; Kono, 2002), and nonfunctional cusps often present more chipping (Cavel et al., 1985; Eakle et al., 1986; Towle et al., 2020).

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Maxillary and mandibular teeth meet and interact with each other, and with food items, in a complex pattern in the different phases of mastication (Hiimeae and Kay, 1972; Kay and Hiimeae, 1974; Wall et al., 2006; see also a review by Ross and Iriarte-Diaz, 2014). If functional cusps are subjected to higher stresses during mastication (Lucas et al., 2008; Thiery et al., 2017), then this would require an explanation as to why they are much less likely to fracture than nonfunctional cusps (Towle and Loch, 2021). One potential explanation is that differences in cusp mechanical properties could reduce the risk of fracture under higher or more sustained stresses. Variation in mechanical properties of enamel has been identified in several primate taxa (Cuy et al., 2002; Park et al., 2008a; Lee et al., 2010; Darnell et al., 2010; Campbell et al., 2012; Constantino et al., 2012). In these previous studies, hardness and elastic modulus values were typically collected using nanoindentation, with hundreds to thousands of measurements taken across a single section of the crown. Hardness is a material's ability to withstand localized permanent deformation, whereas elastic modulus is a measure of its stiffness (Cheng and Cheng, 1998; Cuy et al., 2002; Oliver and Pharr, 2004). Increase in both hardness and elastic modulus values from inner (near the enamel-dentine junction, EDJ) to outer enamel (the exposed surface, OES) appears to be consistent in humans (e.g., Cuy et al., 2002; Park et al., 2008b). However, other studies have suggested this may not be the case for all primates (Campbell et al., 2012; Constantino et al., 2012). Factors that may influence variation in mechanical properties such as mineralization of dental tissues, prism orientation, enamel composition, and crystal arrangement/orientation, have been previously investigated in humans (e.g., Cuy et al., 2002; Angker et al., 2004; Shimizu and Macho, 2008; Xie et al., 2009; Jeng et al., 2011; Stifler et al., 2021).

It has been hypothesized that variation in mechanical properties across tooth crowns may be due to regional differences in stress distribution (Cuy et al., 2002). This is supported by research showing variation in enamel properties (e.g., microstructure, composition, hierarchical structure) affects the likelihood of tooth wear and fracture (Zheng et al., 2003; He and Swain, 2008; Roy and Basu, 2008; Jeng et al., 2011; Barani et al., 2012; Constantino et al., 2012). Mean mechanical property values often differ between species (e.g., Kaiser et al., 2018), although the magnitude of variation between primate taxa can be small (Constantino et al., 2012). Occlusal morphology and dental properties such as enamel thickness exhibit levels of genetic control, with less variation within than between species (Lee et al., 2010; Constantino et al., 2012; Winchester et al., 2014). Therefore, there may also be a phylogenetic signal in mechanical property gradients through enamel.

This study aims to extend previous research on enamel mechanical properties by investigating variation in hardness and elastic modulus across lower molar crowns in a taxonomically diverse sample of 13 catarrhine species, using one platyrrhine species as an outgroup for comparison. We hypothesize that mechanical properties vary in buccal, lingual, and occlusal regions of the crown and throughout the enamel thickness. Based on research on clinical human samples (e.g., Cuy et al., 2002; Park et al., 2008b) and assumptions relating to functional differences between lingual and buccal cusps (i.e., functional cusps showing additional adaptations to withstand fracture/failure; Grine, 2005; Yang et al., 2022), we predict that: 1) hardness and elastic modulus will be higher on the buccal side than on the lingual side of lower molars, 2) mechanical property values will also be higher in cuspal regions than in lateral positions, and 3) hardness and elastic modulus will increase from inner to outer enamel in all crown locations. We also explored the relationship between hardness and elastic modulus, and whether there is a phylogenetic signal in mechanical properties of molar crowns in primates.

2. Methods

2.1. Samples

This study included fifteen relatively unworn lower second ($n = 10$) and first ($n = 5$) molars from 13 catarrhine and one platyrrhine taxa: *Chlorocebus pygerythrus*, *Colobus* sp., *Macaca fascicularis*, *Macaca fuscata*, *Macaca mulatta*, *Macaca sinica*, *Miopithecus* sp., *Papio hamadryas*, *Papio ursinus*, *Gorilla* sp., *Pan troglodytes*, *Pongo* sp., *Cercocebus* sp., and *Cebus* sp. (Table 1). Selected molars were free of pathology and enamel defects and showed no macroscopic postmortem damage. All teeth were obtained from collections curated at museums or research institutes (see Table 1 for sample details) and were stored dry prior to testing. The individuals from which the samples were obtained from were young adults at death (except the *Pongo* sp.), with second molars in occlusion, but without substantial wear on the selected tooth (Table 1). The *Pongo* sp. tooth (right M₁) was not in occlusion and therefore, was likely from a younger individual. Each taxon was represented by a single molar except for *P. ursinus*, which included one male and one female molar. For statistical analyses, mean *P. ursinus* values were used. All catarrhines investigated were housed at the Auckland Museum (Auckland, New Zealand), University of Otago (Faculty of Dentistry, New Zealand), Otago Museum (Dunedin, New Zealand), and the Dunedin Museum of Natural Mystery (Dunedin, New Zealand), with two samples from Kyoto University (Primate Research Institute, Japan). One platyrrhine species (*Cebus* sp.), curated at the Otago Museum, was included as an outgroup comparison.

2.2. Sample preparation

Sample preparation for mechanical properties analyses is summarized here following Loch et al. (2013a,b). Teeth were surface-cleaned with ethanol and mounted on a silicone mould, oriented to allow sectioning through the buccal–lingual plane of the mesial cusps. Specimens were embedded in epoxy resin (Epofix™ Cold-Setting Embedding Resin, Struers, Copenhagen). After setting at room temperature for 24 hours, teeth were longitudinally sectioned through the buccal–lingual plane of the mesial cusp tips using a MOD13 diamond blade (Struers, Copenhagen) under water irrigation in a high-speed saw (Accutom-50, Struers, Copenhagen). Two tooth segments were produced, with the mesial side being used for nanoindentation testing. The sections were surface-cleaned with ethanol and polished on a TegraPol-21 polisher (Struers, Copenhagen) with 1200-grit silicon carbide paper (Struers, Copenhagen), ultrasonic-cleaned for 3 minutes, and then further polished with 1- μ m diamond suspension (DP Suspension P, Struers, Copenhagen). A final ultrasonic cleaning was done in distilled water for 3 min, followed by drying using an air-jet for approximately 1 minute.

2.3. Nanoindentation and measurements

Prepared specimens were mounted on a steel base using thermoplastic cyanoacrylate glue. The mounting base contained a strong magnet to prevent lateral movement during nanoindentation. An optical microscope with a 20 \times magnification objective lens coupled with the nanoindenter allowed precise positioning for each indent array. Tests were performed using a Hysitron TI 950 Triboindenter (Bruker, Minnesota) equipped with a three-plate capacitive transducer capable of applying 10 mN of force with a 1-nN resolution, and 5- μ m displacement with 0.04-nm resolution. Each indent was performed using a three-sided pyramidal Berkovich tip (Bruker, Minnesota) calibrated on a fused

Table 1
Samples studied, including species, tooth type, sex, specimen number, and collection.

Species	Common name	Tooth	Collection	Specimen number	Sex
<i>Cercocebus</i> sp.	Mangabey	Lower right M1	Otago Museum	VT 2715	Not known
<i>Chlorocebus pygerythrus</i>	Vervet monkey	Lower left M2	Museum of Natural Mystery	n/a	Male
<i>Colobus</i> sp.	Colobus	Lower left M2	Auckland Museum	LM 957	Not known
<i>Macaca fascicularis</i>	Crab-eating macaque	Lower right M1	Museum of Natural Mystery	n/a	Female
<i>Macaca fuscata</i>	Japanese macaque	Lower left M2	Primate Research Institute	PRI 3383	Male
<i>Macaca mulatta</i>	Rhesus macaque	Lower left M2	University of Otago	CU 138	Female
<i>Macaca sinica</i>	Toque macaque	Lower right M2	Otago Museum	VT 2707	Not known
<i>Miopithecus</i> sp.	Talapoin	Lower right M2	Otago Museum	VT 2702	Not known
<i>Papio hamadryas</i>	Hamadryas baboon	Lower right M2	Primate Research Institute	PRI 7148	Male
<i>Papio ursinus</i>	Chacma baboon	Lower right M2	Museum of Natural Mystery	n/a	Male
<i>Papio ursinus</i>	Chacma baboon	Lower right M2	Museum of Natural Mystery	n/a	Female
<i>Gorilla</i> sp.	Gorilla	Lower right M1	Otago Museum	VT 2468	Male
<i>Pan troglodytes</i>	Chimpanzee	Lower right M1	Auckland Museum	LM 315	Not known
<i>Pongo</i> sp.	Orangutan	Lower right M1	Otago Museum	VT 3361	Not known
<i>Cebus</i> sp.	Capuchin	Lower left M2	Otago Museum	VT 2728	Not known

quartz standard. The Berkovich tip is made from diamond and has a tip radius of 100 nm, with a semiangle of 65.3°. The load function for each indent was a load-controlled, quasi-static, trapezoid load function with a 5-second loading time, 2-second holding time, and 5-second unloading time with a maximum load of 10 mN. This created indents with various contact areas depending on the hardness of the samples, ranging from an average diameter of 3575 nm for the sample with the highest hardness values (*M. mulatta*) to 5334 nm for the sample with the lowest hardness (*M. sinica*).

Mean values for inner (near the EDJ), middle, and outer (near the OES) enamel readings were calculated for five positions (buccal lateral, buccal cuspal, occlusal middle, lingual cuspal, and lingual lateral; see Fig. 1) within the buccal–lingual plane of the mesial cusps, generating a total of 15 mean measurements per sample. Positions were standardized as follows: buccal/lingual referring to the two sides of the mesial cusps (positions 1, 2, 4, 5; Fig. 1); lateral being within the lower half of the crown but before the crown begins to taper towards the cementum–enamel junction (positions 1 and 5; Fig. 1); cuspal position was close to the maximum height of the dentine horn (positions 2 and 4; Fig. 1), at a location in which both the OES and EDJ boundaries are approximately parallel. If there was evident cuspal wear, a point slightly cervical was chosen. The occlusal position was approximately halfway between the two cusps and typically directly below the central fissure (position 3;

Fig. 1). Areas with cracks and other postmortem artifacts were avoided.

To generate mean values for each location (inner, middle, and outer locations), indent arrays were standardized across samples, with 3 rows of 15 indents per position (i.e., 45 indents at each of the 5 positions in Figure 1, for a total of 225 indents/specimen). The last 2–4 columns indented into dentine and were discarded, leaving ~165–180 enamel indents per tooth. For each position, the first row of indents was undertaken slightly in (~10 μm) from the OES. Due to variation in enamel thickness between positions and among species, the distance between indents varied to obtain at least 165 indents within the enamel for each sample. A minimum distance of 15 μm between indents was set to avoid the influence of residual stresses from adjacent previous indents. Indents in dentine or near the EDJ were removed (Fig. 1). Indents were later classified as belonging to the inner, middle, or outer regions, by dividing indents into three equal components, providing ~11–13 indents per region (see Fig. 1, position 5). Thus, for each specimen, indents were taken at five positions (lingual lateral, lingual cuspal, middle occlusal, buccal cuspal, and buccal lateral), with mean values calculated for inner, middle, and outer locations at each position. This resulted in ~165–180 usable indents per specimen.

In nanoindentation, the nanoscale elastic deformation experienced by the diamond indenter during testing cannot be ignored. Nanoindentation data analysis from the raw data requires a

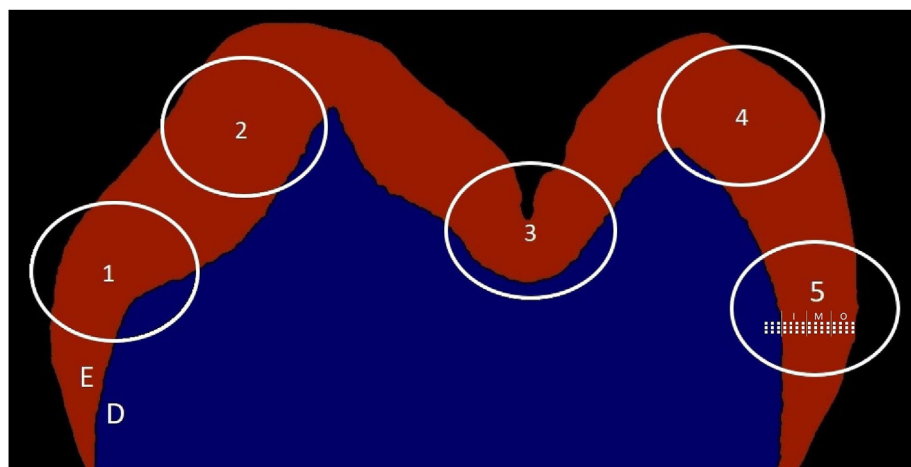


Figure 1. Sampling locations of the five positions, illustrated by a slice from buccal (left) to lingual (right), midway through the mesial cusps. Positions: 1) buccal lateral; 2) buccal cuspal; 3) middle occlusal; 4) lingual cuspal; and 5) lingual lateral. An example of the array in terms of location across a position is illustrated in position 5 (indents not to scale). How indents are split into inner, middle, and outer is also shown. Indents in the dentine were removed prior to analysis. Abbreviations: Enamel (E) is in red, dentine (D) is in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

parameter called reduced modulus, which is affected by the elastic moduli and the Poisson's ratios of the indenter and sample. Reduced modulus (E_r), and hardness (H) values were calculated using the TriboScan 9 software (Bruker, Minnesota) based on the Oliver–Pharr method (Oliver and Pharr, 1992). Calculation of elastic modulus (E) from reduced modulus values assumed a Poisson's ratio of $\nu = 0.30$. Generally, $E_r \cong E$ (within ~1.0%) for samples with significantly lower elastic modulus than the indenter (elastic modulus of diamond ≈ 1140 GPa) and similar Poisson's ratios, and so the reduced modulus was considered representative of elastic modulus in this study. All nanoindentation tests commenced after the instrument achieved thermal equilibrium. Tests were performed under standard laboratory conditions (~21 °C, ~56% relative humidity).

2.4. Comparisons and analysis

Specific positions for three taxa were removed due to data unreliability (i.e., likelihood of extensive wear in that area, cracks that were not visible prior to sectioning, or large preparation artifacts): *P. troglodytes* (buccal lateral), *M. sinica* (buccal lateral and buccal cuspal), and *Gorilla* spp. (occlusal middle). This means that data for 66 positions are presented, including 198 mean values for inner, middle, and outer comparisons (i.e., 66×3 ; Supplementary Online Material [SOM] Table S1). Qualitative comparisons were made among positions and among species using the mean values for each sample combined, the mean values for each of the five positions (i.e., five values for sample) and individual mean values for each inner, middle, and outer location (i.e., 15 values for each sample). **Statistical analysis** Each analysis utilized one mean value per species. To explore the potential relationship between hardness and elastic modulus, we conducted five separate Pearson's product-moment correlation analyses using the average values from each of the five positions. A two-tailed paired t-test was used to compare hardness and elastic modulus between cuspal and lateral positions, using the average lingual cuspal and lingual lateral values since all 14 species could be included. A two-tailed paired t-test was also used to compare hardness and elastic modulus values in buccal vs. lingual positions. Comparisons were done using one average buccal and one lingual value for each species, with the average of the two positions for each side calculated, and *M. sinica* and *P. troglodytes* being removed ($n = 12$ pairs of values). Lastly, a two-tailed paired t-test was used to compare hardness and elastic modulus values between inner, middle, and outer positions (i.e., outer vs. middle; outer vs. inner; middle vs. inner). Comparisons were performed for one average inner, middle, and outer value per species, using the mean values across all five regions. Significance level was set at $p < 0.05$; however, due to the relatively small sample sizes, trends toward significance were also considered ($p < 0.10$). The raw data that support the findings of this study are available in the SOM Table S1.

Phylogenetic signal When testing for phylogenetic signal, a large sample size ($n \geq 100$ species) is recommended to reduce the likelihood of type I and type II errors (Münkemüller et al., 2012). Given the relatively small sample in this study ($n = 14$ taxa), the phylogenetic analysis presented here suffered from low statistical power. Four metrics for phylogenetic signal were considered for use: Pagel's λ , Abouheif's Cmean, Moran's I, and Blomberg's K. Under strong Brownian motion, Pagel's λ is more efficient at identifying phylogenetic signal and, compared to Abouheif's Cmean, Moran's I, and Blomberg's K, has the smallest type I error with small samples (e.g., $n = 20$ taxa; Münkemüller et al., 2012). Under weak Brownian motion, e.g., when mutation rate is high and the evolutionary history of the character state is overwritten (Harmon et al., 2019), Pagel's λ is more susceptible to type I error but performs similarly to

other metrics (Münkemüller et al., 2012). Blomberg's K performs similarly to Pagel's λ under weak Brownian motion with small sample sizes and outperforms other metrics of phylogenetic signal in some more complex, non-Brownian models of trait evolution (Münkemüller et al., 2012).

In our sample, we do not know which evolutionary forces (e.g., Brownian motion, natural selection) have shaped primate enamel mechanical properties or the strength of these forces. We therefore used both Pagel's λ and Blomberg's K and not Abouheif's Cmean or Moran's I to test for phylogenetic signal in this analysis as they tend to perform well under a larger variety of evolutionary scenarios (Münkemüller et al., 2012). The two variables can yield distinct insights into the data: Pagel's λ is restricted between 0 and 1, indicating the phylogenetic tree can explain relatively little ($\lambda = 0$) to all ($\lambda = 1$) of the variation in the data, although values greater than one are possible (Harmon, 2018). Blomberg's K is interpreted similarly to Pagel's λ , but can be greater than 1, indicating there is less variation within clades than would be expected under pure Brownian motion (Blomberg et al., 2003).

To test for phylogenetic signal in gradients in enamel properties, three ratios were calculated for both hardness and elastic modulus. These ratios investigated gradients through the enamel (outer to inner enamel positions), across the width of the tooth (buccal and lingual positions), and radially from the cusps moving outwards (cuspal to lateral positions). Ratios were therefore calculated as follows:

1. Properties of outer to inner enamel: enamel properties were measured at five locations across the enamel cap (Fig. 1) producing five sets of outer and inner enamel measurements per tooth, and therefore five sets of ratios of outer to inner enamel properties.
2. Properties of buccal to lingual cusps of the tooth: enamel properties were measured at six locations on the buccal and lingual cusps of the tooth, respectively (i.e., cuspal inner, cuspal middle, cuspal outer, lateral inner, lateral middle, and lateral outer). Ratios using properties from homologous locations (e.g., buccal cuspal inner:lateral cuspal inner) were taken, creating six ratios per tooth.
3. Properties of the cuspal to the lateral side of the cusps: enamel properties were measured at two locations on each cusp: a cuspal location (2 and 4 in Fig. 1) and a lateral location (1 and 5 in Fig. 1). Ratios of inner, middle, and outer properties at locations 2 and 4 to inner, middle, and outer properties at locations 1 and 5, respectively, were calculated, producing six sets of ratios.

An updated phylogeny of our sample was downloaded from timetree.org (Kumar et al., 2017) and used for analyses (SOM Fig. S1). The 'multi2di' function in the R package 'ape' was used to resolve the polytomy in the tree (Paradis and Schliep, 2019). As species are treated as the unit of measurement in phylogenetic comparative methods, it is not possible to input more than one measurement per taxon. To account for interspecific variability in enamel mechanical properties, one of the ratios was chosen at random (e.g., outer:inner enamel properties at location 2) and used for analysis. Doing so treats each ratio as if it were the species' average. Pagel's λ and Blomberg's K were calculated using this data using the 'phylosig' function in Phytools (Revell, 2012). For Blomberg's K, the number of simulations in the randomization test (nsim) was set to 999. This process of randomly selecting a ratio and testing for phylogenetic signal was repeated 9999 times to obtain a distribution of possible Pagel's λ and Blomberg's K values. The percentage of times that Pagel's λ and Blomberg's K were significant ($\alpha = 0.05$) was calculated. Sampling with replacement vs. using species averages allows for intraindividual variation in ratio

measurement to be considered. As the gradient in elastic modulus through the enamel's thickness is expected to be greatest through the tooth's cusp (Cuy et al., 2002), two sets of analyses were performed to investigate phylogenetic signal in the gradient through the enamel, one using all measurements ($n = 5$) and one using the measurements at the cusp tips ($n = 2$). All analyses were carried out using the Phytools package in R v. 4.1.0 using RStudio (Revell, 2012; R Core Team, 2021; RStudio Team, 2021). The phylogenetic tree used is shown in SOM Figure S1.

3. Results

Average values of hardness and elastic modulus were similar among taxa, regardless of enamel position (Tables 2–5). Average tooth values for hardness ranged from under 4 GPa (*M. sinica* and *C. pygerythrus*) to just over 5 GPa (*Miopithecus* sp. and *M. mulatta*; Table 2). Most species had average elastic modulus values between 90 GPa and 115 GPa, with two specimens with values below this range (Table 2; *M. sinica*, *Colobus* sp.). Cuspal enamel (i.e., buccal and lingual cuspal) commonly had higher mechanical property values than lateral enamel (i.e., buccal and lingual lateral; Tables 4 and 5).

3.1. Statistical comparisons

There was a strong relationship between hardness and elastic modulus for each of the five regions, with two of the five regions reaching statistical significance and a third suggestive of significance (Fig. 2; buccal lateral: $n = 12$, $df = 10$, $r = 0.55$, $p = 0.062$; buccal cuspal: $n = 13$, $df = 11$, $r = 0.41$, $p = 0.163$; occlusal middle: $n = 13$, $df = 11$, $r = 0.40$, $p = 0.176$; lingual cuspal: $n = 14$, $df = 12$, $r = 0.54$, $p = 0.045$; lingual lateral: $n = 14$, $df = 12$, $r = 0.65$, $p = 0.012$). A two-tailed paired t-test comparing lateral and cuspal values revealed a significant difference between these locations for hardness ($n = 14$, $df = 13$, $t = 2.75$, $p = 0.016$; Fig. 3A) and trending toward significance for elastic modulus ($n = 14$, $df = 13$, $t = 1.87$, $p = 0.084$; Fig. 3B). A two-tailed paired t-test comparing lingual and buccal positions, using one buccal and lingual mean value for each species (i.e., an average of the six lingual and six buccal positions), revealed no significant differences between sides for hardness ($n = 12$, $df = 11$, $t = 1.76$, $p = 0.106$; Fig. 3C) or elastic modulus ($n = 12$, $df = 11$, $t = 1.20$, $p = 0.255$; Fig. 3D). Middle enamel had significantly higher hardness values than outer enamel (middle vs. outer: $n = 14$, $df = 13$, $t = -4.16$, $p = 0.001$; Fig. 3E) and trended toward significant higher values than inner enamel (inner vs.

middle: $n = 14$, $df = 13$, $t = 2.08$, $p = 0.058$; Fig. 3E). Inner and outer enamel showed no significant difference for hardness (inner vs. outer: $n = 14$, $df = 13$, $t = 0.25$, $p = 0.804$; Fig. 3E). Elastic modulus was significantly higher in middle than in inner enamel (inner vs. middle: $n = 14$, $df = 13$, $t = 3.64$, $p = 0.003$; Fig. 3F) and trended toward significantly higher values than outer enamel (middle vs. outer: $n = 14$, $df = 13$, $t = -1.79$, $p = 0.096$; Fig. 3F). Outer enamel values are suggestive of being significantly higher than those of inner enamel (inner vs. outer: $n = 14$, $df = 13$, $t = 1.94$, $p = 0.074$; Fig. 3F).

3.2. Phylogenetic analyses

Results from the phylogenetic analyses indicated that phylogenetic signal was significant between 0–24.7% and 0.1–18.3% of the time for Blomberg's K and Pagel's λ , respectively (Table 6; SOM). Blomberg's K showed little to no evidence of phylogenetic signal for mechanical property gradients through the enamel (outer to inner all positions: 0–1.4%). These results were generally concurrent with Pagel's λ , with the exception that Pagel's λ detected phylogenetic signal in elastic modulus gradients in the outer cusps 18.3% of the time. Pagel's λ showed little to no evidence of phylogenetic signal for buccal to lingual mechanical property gradients (0–3.7%). Blomberg's K showed similar results for elastic modulus (1.6%) but found evidence of phylogenetic signal in hardness 13.5% of the time. Phylogenetic signal was found in the central to lateral mechanical property gradients 8.3–24.7% of the time.

4. Discussion

4.1. Hypotheses testing and comparisons with previous studies

Hardness and elastic modulus values were higher in cuspal than in lateral positions, significantly so for hardness ($p < 0.05$), and trending toward significance for elastic modulus ($p < 0.10$). Although lingual positions displayed higher average values for both mechanical properties than buccal positions, these differences were not significant. Middle enamel was significantly harder than outer enamel and trended toward higher values than inner enamel. Elastic modulus also had the highest mean values in middle enamel, was significantly higher than in inner enamel, and trended toward being significantly higher than outer enamel. There was also low phylogenetic signal detected for both mechanical property values analyzed. Therefore, the only prediction that clearly supports the hypotheses tested in this study is that cuspal enamel has higher mechanical property values than lateral enamel. In contrast, there was no support for higher mechanical property values in buccal than in lingual positions in the lower molars analyzed, and the prediction that mechanical property values would increase from inner to outer enamel was also not supported.

Although we observed variation in mechanical properties across the crown, this variation fell within a relatively narrow range (e.g., typically <0.5 GPa for hardness; <10 GPa for elastic modulus) and much lower than reported in humans by Cuy et al. (2002). For example, Cuy et al. (2002) reported that enamel hardness decreased from 4.6 GPa to 3.4 GPa from outer to inner enamel, and for elastic modulus, some crown positions decreased from 115 GPa near the OES to ~ 50 GPa approaching the EDJ. In Campbell et al.'s (2012) study on lemurs and humans, which used methods similar to those used here, substantial differences in mechanical properties between inner and outer enamel were observed in both hardness and elastic modulus in humans but less so in other primates. Constantino et al. (2012:174) also observed this difference between humans and extant nonhuman primates, concluding that “in several species, the point of maximum modulus or hardness

Table 2

Average values for hardness (GPa) and elastic modulus (GPa) for each species studied.

Species	Hardness \pm STD (GPa)	Elastic modulus \pm STD (GPa)
<i>Cercocebus</i> sp.	4.90 \pm 0.62	96.72 \pm 7.51
<i>Chlorocebus pygerythrus</i>	3.98 \pm 0.97	95.64 \pm 15.10
<i>Colobus</i> sp.	4.60 \pm 0.59	62.99 \pm 8.31
<i>Macaca fascicularis</i>	4.54 \pm 0.65	90.56 \pm 11.38
<i>Macaca fuscata</i>	4.77 \pm 0.62	95.11 \pm 15.27
<i>Macaca mulatta</i>	5.06 \pm 0.58	114.26 \pm 9.53
<i>Macaca sinica</i>	3.93 \pm 0.97	67.97 \pm 12.84
<i>Miopithecus</i> sp.	5.01 \pm 0.65	111.51 \pm 9.73
<i>Papio hamadryas</i>	4.87 \pm 0.68	107.04 \pm 9.42
<i>Papio ursinus</i>	4.91 \pm 0.54	105.36 \pm 5.80
<i>Papio ursinus</i>	4.76 \pm 0.68	100.29 \pm 11.16
<i>Gorilla</i> sp.	4.34 \pm 1.01	104.43 \pm 11.58
<i>Pan troglodytes</i>	4.54 \pm 0.72	108.90 \pm 12.99
<i>Pongo</i> sp.	4.08 \pm 0.94	96.60 \pm 18.38
<i>Cebus</i> sp.	4.74 \pm 0.87	105.46 \pm 13.10

STD = standard deviation.

Table 3
Average elastic modulus (GPa) and hardness (GPa) for each specimen, separately for inner, middle, and outer enamel positions.

	Elastic modulus ± STD (GPa)			Hardness ± STD (GPa)		
	Inner	Middle	Outer	Inner	Middle	Outer
<i>Cercocebus</i> sp.	94.57 ± 8.57	98.51 ± 6.79	97.49 ± 6.76	4.83 ± 0.77	5.02 ± 0.54	4.83 ± 0.46
<i>Chlorocebus pygerythrus</i>	93.48 ± 11.39	97.21 ± 13.89	95.56 ± 18.01	3.93 ± 0.99	4.06 ± 1.00	3.91 ± 0.93
<i>Colobus</i> sp.	58.95 ± 7.66	65.88 ± 6.74	66.39 ± 8.30	4.54 ± 0.50	4.78 ± 0.46	4.54 ± 0.67
<i>Macaca fascicularis</i>	80.64 ± 9.00	95.32 ± 8.07	96.17 ± 8.81	4.02 ± 0.56	4.88 ± 0.53	4.75 ± 0.43
<i>Macaca fuscata</i>	90.85 ± 15.48	97.47 ± 12.97	94.79 ± 17.93	4.74 ± 0.76	4.83 ± 0.49	4.66 ± 0.62
<i>Macaca mulatta</i>	113.57 ± 8.78	114.19 ± 10.65	114.79 ± 8.70	5.24 ± 0.54	4.98 ± 0.65	4.97 ± 0.50
<i>Macaca sinica</i>	70.53 ± 12.74	74.51 ± 10.28	71.99 ± 11.25	4.00 ± 0.96	4.34 ± 0.78	4.22 ± 0.84
<i>Miopithecus</i> sp.	112.61 ± 9.31	112.86 ± 6.97	109.03 ± 11.24	5.15 ± 0.57	5.04 ± 0.64	4.84 ± 0.68
<i>Papio hamadryas</i>	105.17 ± 12.26	108.95 ± 8.40	106.43 ± 5.49	4.97 ± 0.88	4.96 ± 0.58	4.69 ± 0.48
<i>Papio ursinus</i> (female)	106.51 ± 5.88	105.90 ± 5.50	103.24 ± 5.58	5.12 ± 0.43	4.95 ± 0.52	4.59 ± 0.56
<i>Papio ursinus</i> (male)	100.52 ± 11.49	101.77 ± 10.65	98.13 ± 10.93	4.94 ± 0.66	4.79 ± 0.64	4.49 ± 0.66
<i>Gorilla</i> sp.	102.72 ± 11.67	108.39 ± 10.86	102.28 ± 11.66	4.15 ± 1.08	4.58 ± 1.03	4.29 ± 0.83
<i>Pan troglodytes</i>	104.02 ± 14.09	112.15 ± 13.03	110.39 ± 10.76	4.55 ± 0.78	4.58 ± 0.78	4.48 ± 0.64
<i>Pongo</i> sp.	84.69 ± 20.67	100.59 ± 15.25	104.89 ± 12.31	3.49 ± 1.02	4.31 ± 0.90	4.46 ± 0.63
<i>Cebus</i> sp.	107.23 ± 15.93	105.81 ± 13.74	103.25 ± 13.07	4.85 ± 1.00	4.81 ± 0.91	4.57 ± 0.87

STD = standard deviation.

Table 4
Mean elastic modulus values (STD) for each position studied. All measurements are in GPa.

Species	Buccal lateral	Buccal cuspal	Occlusal middle	Lingual cuspal	Lingual lateral
<i>Cercocebus</i> sp.	95.23 ± 8.70	101.13 ± 5.14	97.15 ± 7.26	92.89 ± 7.59	97.88 ± 6.47
<i>Chlorocebus pygerythrus</i>	85.70 ± 5.86	90.95 ± 5.42	86.65 ± 7.05	112.32 ± 19.94	98.28 ± 8.27
<i>Colobus</i> sp.	53.98 ± 4.50	61.54 ± 5.34	70.62 ± 4.12	69.39 ± 8.36	65.39 ± 7.74
<i>Macaca fascicularis</i>	84.49 ± 11.03	86.18 ± 9.50	99.41 ± 8.57	91.65 ± 12.36	90.95 ± 9.18
<i>Macaca fuscata</i>	69.20 ± 7.77	106.00 ± 7.63	98.90 ± 9.88	99.10 ± 11.91	96.66 ± 8.17
<i>Macaca mulatta</i>	110.13 ± 8.66	120.70 ± 7.74	113.24 ± 10.05	117.66 ± 6.09	108.75 ± 10.23
<i>Macaca sinica</i>	–	–	68.55 ± 6.44	77.50 ± 7.73	72.93 ± 12.57
<i>Miopithecus</i> sp.	112.61 ± 8.08	112.93 ± 8.52	108.76 ± 8.05	112.03 ± 12.33	111.45 ± 10.7
<i>Papio hamadryas</i>	110.09 ± 5.48	110.47 ± 7.72	97.25 ± 13.05	107.91 ± 4.96	108.60 ± 7.20
<i>Papio ursinus</i> (female)	107.08 ± 3.62	104.96 ± 5.23	106.06 ± 7.60	106.27 ± 5.03	102.44 ± 5.58
<i>Papio ursinus</i> (male)	112.54 ± 4.89	106.74 ± 4.78	90.97 ± 11.30	94.80 ± 5.97	96.08 ± 8.38
<i>Gorilla</i> sp.	106.74 ± 15.67	102.99 ± 12.97	–	101.39 ± 6.71	106.22 ± 7.21
<i>Pan troglodytes</i>	–	105.14 ± 6.93	114.70 ± 13.52	111.05 ± 16.13	105.25 ± 13.37
<i>Pongo</i> sp.	79.99 ± 17.59	99.06 ± 15.38	108.18 ± 12.89	98.34 ± 18.31	89.08 ± 18.88
<i>Cebus</i> sp.	93.20 ± 14.68	117.22 ± 11.37	108.34 ± 5.49	108.89 ± 9.12	97.77 ± 7.84

STD = standard deviation.

Table 5
Mean hardness values (STD) for each position studied. All measurements are in GPa.

Species	Buccal lateral	Buccal cuspal	Occlusal middle	Lingual cuspal	Lingual lateral
<i>Cercocebus</i> sp.	4.52 ± 0.62	4.63 ± 0.70	5.30 ± 0.50	4.84 ± 0.60	4.97 ± 0.44
<i>Chlorocebus pygerythrus</i>	3.50 ± 0.41	3.45 ± 0.29	2.91 ± 0.44	5.11 ± 0.67	4.75 ± 0.66
<i>Colobus</i> sp.	4.59 ± 0.48	4.59 ± 0.37	4.54 ± 0.54	4.92 ± 0.80	4.56 ± 0.80
<i>Macaca fascicularis</i>	4.34 ± 0.63	4.71 ± 0.37	4.57 ± 0.54	4.59 ± 0.90	4.53 ± 0.64
<i>Macaca fuscata</i>	4.37 ± 0.57	4.88 ± 0.54	4.84 ± 0.49	4.96 ± 0.66	4.65 ± 0.67
<i>Macaca mulatta</i>	5.12 ± 0.60	5.29 ± 0.34	4.70 ± 0.61	5.27 ± 0.43	4.97 ± 0.65
<i>Macaca sinica</i>	–	–	4.07 ± 0.69	4.45 ± 0.74	3.91 ± 1.07
<i>Miopithecus</i> sp.	5.18 ± 0.68	5.00 ± 0.65	4.74 ± 0.49	4.96 ± 0.77	5.26 ± 0.52
<i>Papio hamadryas</i>	5.34 ± 0.36	4.87 ± 0.61	4.09 ± 0.67	5.07 ± 0.47	4.98 ± 0.58
<i>Papio ursinus</i> (female)	5.14 ± 0.27	4.86 ± 0.58	4.88 ± 0.58	5.09 ± 0.54	4.59 ± 0.34
<i>Papio ursinus</i> (male)	5.16 ± 0.35	4.83 ± 0.58	4.18 ± 0.71	4.84 ± 0.47	4.84 ± 0.82
<i>Gorilla</i> sp.	4.54 ± 1.32	3.53 ± 0.90	–	4.66 ± 0.41	4.71 ± 0.60
<i>Pan troglodytes</i>	–	4.38 ± 0.57	4.71 ± 0.68	4.57 ± 0.70	4.56 ± 0.94
<i>Pongo</i> sp.	3.42 ± 0.82	3.90 ± 0.86	4.46 ± 0.81	4.63 ± 0.70	3.98 ± 1.06
<i>Cebus</i> sp.	4.07 ± 0.94	5.54 ± 0.67	4.69 ± 0.60	4.81 ± 0.66	4.46 ± 0.75

STD = standard deviation.

appears not at either the inner or outer surface but somewhere in between". The present study supports the findings of Constantino et al. (2012) and suggests this may be the most common pattern in Catarrhini. Many studies have highlighted the potential benefits of evolving graded enamel; this gradient may protect teeth against different types of failure (e.g., He and Swain, 2009; He et al., 2013). However, this does not explain the differences observed between humans and nonhuman primates.

Why human molars display substantial variation in mechanical properties values, increasing from inner to outer enamel, requires further investigation. Environmental factors during dental development or through life may change the mechanical properties of teeth. For example, Park et al. (2008b) observed an increase in hardness and elastic modulus values near the occlusal surface in older humans, although the inner enamel remained relatively constant across age groups. From this perspective, it is possible that

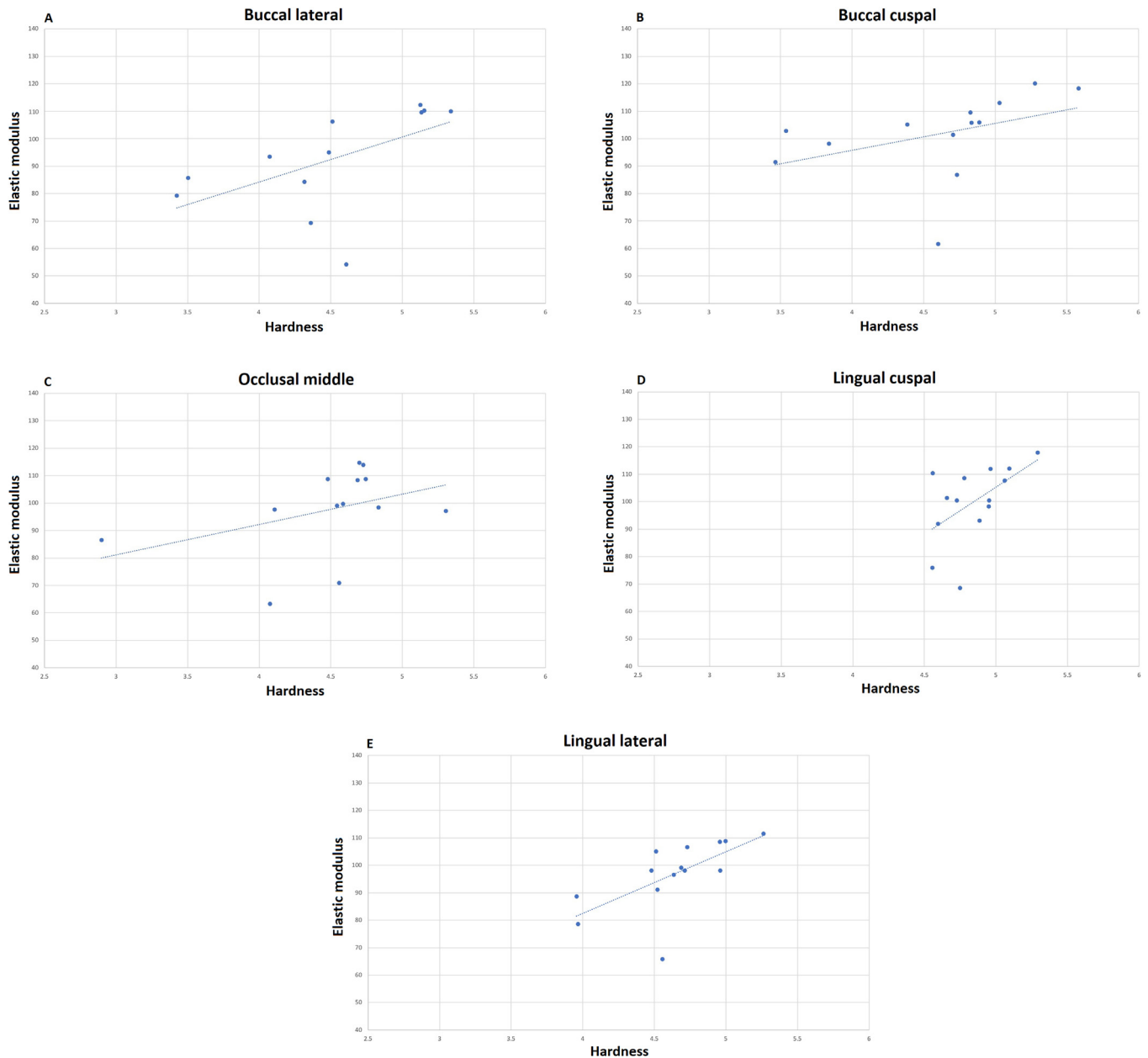


Figure 2. Scatter plots illustrating the bivariate relationship between hardness (x-axis) and elastic modulus (y-axis). All values are in GPa, with A) buccal lateral; B) buccal cuspal; C) occlusal middle; D) lingual cuspal; and E) lingual lateral. A trend line was added in each case. The relationship between hardness and elastic modulus was significant for lingual cuspal ($p = 0.045$) and lingual lateral positions ($p = 0.012$) and trended toward significance for buccal lateral enamel ($p = 0.062$).

findings of higher mechanical properties values in outer enamel may be related, at least in part, to the age of sampled individuals. Most of the teeth analyzed in the present study were from young adults, which may partially explain differences in enamel properties between humans and other primates (i.e., human samples from older individuals may show increased mechanical properties values for outer enamel; see Park et al., 2008b). However, additional factors could also account for differences in enamel properties between humans and nonhuman primates, including environmental factors during dental development and throughout life (e.g., incorporation of fluoride), as well as how different samples were stored and prepared (e.g., ‘fresh’ samples vs. ‘dry’ museum specimens).

Previous studies on extant primates and clinical human samples have shown that compared to buccal cusps, lingual cusps of lower

molars had higher prevalence of fractures but a low rate of wear (Cavel et al., 1985; Eakle et al., 1986; Towle et al., 2020; Towle and Loch, 2021). The higher prevalence of fractures on ‘nonfunctional’ cusps in both clinical human and other primate samples due to potentially ‘weaker’ enamel in ‘nonfunctional’ cusps was not supported by the results of the present study. Instead, with larger samples, it is lingual positions that seem more likely to display higher hardness and elastic modulus than that of buccal, given the higher mean values. If so, this could suggest an evolutionary response to localized increased susceptibility of fracture. However, given that both hardness and elastic modulus mean values are higher in lingual enamel, further testing is required to ascertain if these regions are truly more protected (i.e., if hardness and stiffness are higher in lingual enamel, could these regions be more susceptible to fracture than if the enamel was softer and more pliant?).

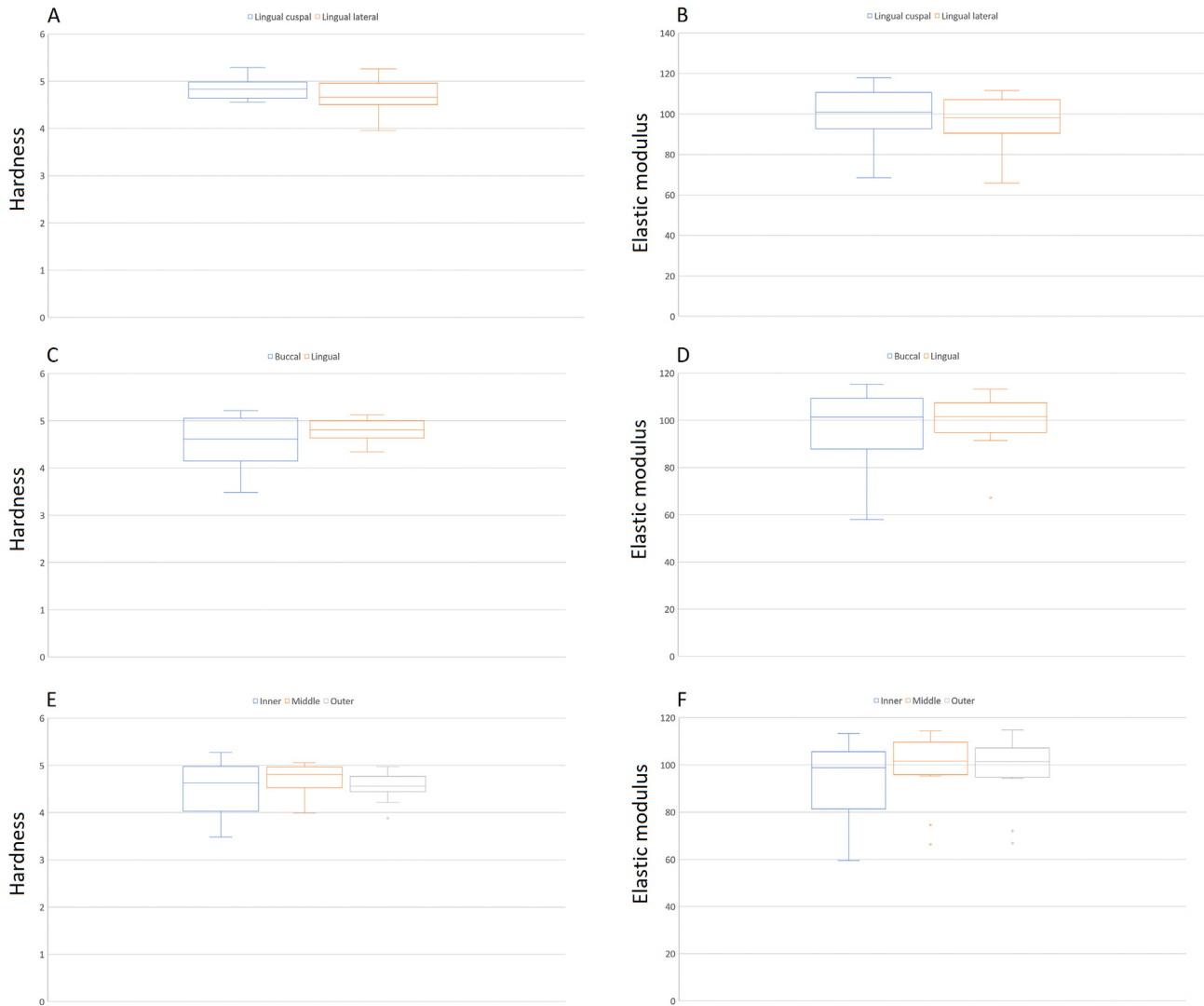


Figure 3. Box and whisker plots illustrating the univariate relationships comparing specific crown locations: Lingual cuspal and lingual lateral, for hardness (A) and elastic modulus (B); buccal and lingual, for hardness (C) and elastic modulus (D); inner, middle and outer, for hardness (E) and elastic modulus (F). All values are in GPa. Lateral and cuspal values were significantly different for hardness (A; $p = 0.016$) and trended toward significance for elastic modulus (B; $p = 0.084$). Lingual and buccal positions were not significantly different ($p > 0.05$ for hardness [C] or elastic modulus [D]). Middle enamel had significantly higher hardness values than outer enamel (E; $p = 0.001$) and trended toward significantly higher values than inner enamel (E; $p = 0.058$). Inner and outer enamel showed no significant difference for hardness (E; $p > 0.05$). Elastic modulus was significantly higher in middle than in inner enamel (F; $p = 0.003$) and trended toward significantly higher values than outer enamel (F; $p = 0.096$). Outer enamel trended toward being significantly higher than inner enamel (F; $p = 0.074$).

Table 6
Results of phylogenetic analysis. Phylogenetic signal was detected in only a small percentage of the data.

Ratio	Metric	% Blomberg's K significant	% Pagel's λ significant
Inner:outer all positions	Hardness	1.4	2.0
Inner:outer all positions	Modulus	1.3	2.6
Inner:outer cusps	Hardness	0.5	6.7
Inner:outer cusps	Modulus	0.0	18.3
Buccal:lingual	Hardness	13.5	3.7
Buccal:lingual	Modulus	1.6	0.1
Central:lateral	Hardness	24.7	12.9
Central:lateral	Modulus	15.3	8.3

There are many factors that could influence the variation in mechanical properties across primate molar crowns, including prism orientation, enamel composition, and the presence of large-scale enamel structures such as Hunter-Schreger bands, interprismatic/prismatic enamel proportions, crystal arrangement/

orientation, and chemical composition (Cuy et al., 2002; Angker et al., 2004; Shimizu and Macho, 2008; Xie et al., 2009; Bajaj and Arola, 2009; Jeng et al., 2011; Stiffler et al., 2021). Mineral content of enamel typically increases from inner to outer enamel, with a concomitant decrease of interprismatic material and organic content (Macena et al., 2014). Interestingly, as shown here and elsewhere (e.g., Constantino et al., 2012), primates do not show a clear increase in mechanical properties values from inner to outer enamel that might be expected to mirror the increase in mineral concentration. The underlying structural and compositional explanation for this needs to be further explored.

4.2. Phylogenetic comparisons

Phylogenetic analysis results suggest there was low (<25% of the time) to no (<5% of the time) phylogenetic signal in the gradients of hardness and elastic modulus through the enamel, across the width of the tooth, and radially from the center of the tooth moving

outwards. Based on qualitative observation of the data, there was consistency in mechanical property gradients across primate species, and we can speculate that there may be a genetic component to these gradients. It is likely that we were unable to detect a phylogenetic signal due to reasons such as: 1) low statistical power due to small sample size, 2) small differences in absolute measurements that led to large differences in ratios, 3) the variation in these ratios due to shared phylogenetic history has been overwritten, for example, by strong evolutionary forces or environmental factors, and/or 4) little change to properties in the species studied. While our comparative sample is relatively small, and therefore prone to both type I and type II error, our phylogenetic analyses can serve as null hypotheses for future studies. Based on our results, the teeth of species often considered to be hard-object feeders (e.g., mangabeys, orangutans, and possibly *Cebus*, though we do not know the species of our *Cebus* individual; [Wieczkowski, 2009](#); [Daegling et al., 2011](#); [Vogel et al., 2014](#); [Towle and Loch, 2021](#)) do not show high hardness or elastic modulus values or perceptible differences (based on qualitative observation of our data) compared to other species. As has been suggested previously (e.g., [Lee et al., 2010](#); [Constantino et al., 2012](#)), it seems other enamel properties such as occlusal morphology and enamel thickness may have evolved in response to changes in food-processing behaviors. Mechanical properties values of enamel seem relatively conserved compared to traits such as enamel thickness, which can vary substantially among closely related primates in response to dietary changes ([Pampush et al., 2013](#); [Thierry et al., 2017](#)).

4.3. Study limitations and future research

Most studies on the mechanical properties of teeth have relied on dried museum specimens (e.g., [Baker et al., 1959](#); [Sanson et al., 2007](#); [Braly et al., 2007](#); [Lee et al., 2010](#); [Constantino et al., 2012](#); [Erickson, 2014](#)). Other studies have used 'fresh' ('wet') specimens, to better represent the real mechanical properties of enamel during life. Differences in values have been reported between these two methods (e.g., [Kaiser et al., 2018](#)). Our samples, similar to many others reported in the literature, could be affected by factors such as changing hydration and dry storage. Dried samples are unlikely to retain the full organic and water content that would have been present in enamel during life. Ethanol, the chemical used to remove polishing debris and clean the outer surface of teeth, is a known dehydrating agent causing denaturation of protein components, including in dental tissue ([Klemm, 1998](#); [Nalla et al., 2006](#); [He and Swain, 2007](#); [Loch et al., 2013c](#)). However, ethanol effects on our samples would likely have been minimal, due to the chemical stability of hydroxyapatite when exposed to ethanol (i.e., the protein and water content in enamel are more commonly affected; [He and Swain, 2007](#)). On the other hand, dried storage would have influenced the dental tissues; thus, mechanical properties values reported here would likely have been slightly overestimated (see [He and Swain, 2007](#)). As the focus of this study is on changes across the teeth, overestimations in absolute values would probably not detract from the overall comparative findings that include the first reports of mechanical properties of dental tissues in a number of primate species based on methods and protocols that are the standard for this type of research. Thus, a strength of our study is that results can be compared to other nanoindentation studies that have been previously carried out on different species (e.g., [Lee et al., 2010](#); [Darnell et al., 2010](#); [Campbell et al., 2012](#); [Constantino et al., 2012](#)). Future studies would benefit from exploring the effects of storage methods (dry vs. wet) and cleaning protocols on estimations of mechanical properties values in dental samples.

Mechanical properties may also vary depending on the orientation in which the tooth is studied, due to the anisotropic nature of enamel ([Spears et al., 1993](#); [Habelitz et al., 2001](#)). Standardizing the positions in the present study likely reduced these effects. Additionally, as portions of the enamel thickness are likely composed of decussating prisms (e.g., Hunter-Schreger bands), the prism orientation in relation to the nanoindenter tip likely varied between each indent row and/or column, meaning slight orientation differences among samples are unlikely to significantly bias the results of the present study. Differences in elastic modulus and hardness have also been reported to be higher in enamel prism areas than in interprismatic regions ([Ang et al., 2012](#)), therefore, also potentially influencing results. In this study, we did not attempt to assess the orientation of the prisms or the coverage of prismatic/interprismatic enamel that was indented. Future studies that incorporate such factors may provide further information on the observed patterns. Furthermore, the overlap in mechanical property values among inner, middle, and outer positions (i.e., large standard deviations for a relatively small number of samples) suggests further comparisons with larger sample sizes may provide further insights into mechanical property gradients of primate enamel.

The absence of significant differences between 'functional' and 'nonfunctional' cusps, does not necessarily mean both sides are equally protected against fracture. Other structural features vary between these locations and are likely also crucial in explaining differences in fracture susceptibility. For example, functional cusps are typically lower, more rounded, and with thicker enamel that may also contain additional/denser areas of prism decussation (i.e., Hunter-Schreger band configuration and density; see [Yang et al., 2022](#), and references therein). Additional wear and mastication-simulation studies are required to assess links between these other properties and mechanical property gradients described here, especially in relation to factors such as enamel thickness and forces applied during the masticatory cycle ([Hiemae and Kay, 1972](#); [Kay and Hiemae, 1974](#); [Wall et al., 2006](#); see also a review by [Ross and Iriarte-Diaz, 2014](#)). Future work should also evaluate within-species variation in enamel mechanical properties and how these patterns link to other variables/factors, such as enamel structure and mineral concentration ([Yang et al., 2022](#); [Towle et al., 2023](#)). Larger sample sizes will allow the understanding of variation among individuals, across age groups and between sexes. The impact of environment and diet during dental development (e.g., among captive vs. wild primate samples) could also be assessed. Lastly, including data on the variation of mechanical properties across tooth crowns in finite-element analyses (e.g., [Sender and Strait, 2023](#)) will allow more accurate models to be generated than assuming that dental tissues are homogeneous.

5. Conclusions

This study adds evidence for variation in mechanical properties in catarrhine primate molars, further highlighting that enamel is a heterogeneous material. Cuspal enamel had significantly higher mechanical properties values likely related to more sustained/higher occlusal forces. Despite substantial differences in reported wear and fractures between buccal and lingual molar crown locations, our analysis did not reveal any significant differences in mechanical properties between these locations. This suggests that other dental tissue properties and/or masticatory factors, may be responsible for the observed wear/fracture patterns. Middle enamel positions showed higher mechanical property values than inner and outer enamel. However, variation in mechanical property values throughout enamel in primates was less than what has been observed in clinical human samples. Further research is needed to

explore the graded nature of these properties. There was little variation in mechanical properties among species, suggesting these characteristics may be evolutionarily conserved and are likely shared among Catarrhini (and potentially among primates) and suggesting other features (e.g., enamel thickness and occlusal morphology) may have been more important in recent adaptation. Understanding variation in properties across tooth crowns will allow more precise biomechanical interpretations, especially in relation to the masticatory cycle, jaw mechanics, tooth wear, and fracture patterns in primates.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Online Material

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