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# Enamel nanocrystal misorientation increased with meat-eating and agriculture

<https://doi.org/10.1038/s41586-026-10583-8>

Received: 11 June 2025

Accepted: 22 April 2026

Published online: 3 June 2026

Open access

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Pupa U. P. A. Gilbert<sup>1,2,3✉</sup>, Daniel R. Green<sup>4</sup>, Patrick Mahoney<sup>5</sup>, Debbie Guatelli-Steinberg<sup>6</sup>, W. Scott McGraw<sup>6</sup>, Emma Lagan<sup>6</sup>, Fredrick Kyalo Manthi<sup>7</sup>, Samuel Muteti<sup>7</sup>, Emmanuel Ndiema<sup>7</sup>, Fernando Ramirez Rozzi<sup>8,9</sup>, Cayla A. Stifler<sup>1</sup>, Connor A. Schmidt<sup>1</sup>, Barat Q. Achinuq<sup>10</sup>, Andreas Scholl<sup>10</sup>, Benjamin Gilbert<sup>11,12</sup> & Mackie C. O'Hara<sup>13,14✉</sup>

Enamel covers teeth, is the hardest tissue in the vertebrate body and has a complex multiscale structure from nanometres to millimetres<sup>1</sup>. The structure comprises thin, long hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ) nanocrystals<sup>2</sup>, 50–70 nm wide, many micrometres long, parallel and bundled into approximately 5- $\mu\text{m}$ -wide rods. The rods undulate and cross into a microscale ‘decussation pattern’ that toughens enamel by deflecting cracks<sup>3,4</sup>. However, the crystallographic orientation of enamel nanocrystals is poorly understood. Here we show that the misorientation angle of adjacent nanocrystals varies markedly across 12 primate teeth spanning 9 species, 17.8 million years of evolution and diverse diets. Using a method called Polarization Enabled Large Input of Crystal Angles at the Nanoscale (PELICAN)<sup>5</sup>, we compare nanocrystals in the same (pre)molar locations and show that misorientation increases with food hardness in extant and fossil non-human apes and monkeys. We compare misorientation across three major dietary shifts in human evolution: the transition to meat-eating about 2.0–1.5 million years before present<sup>6,7</sup>, to agriculture (about 12,000 years before present)<sup>8,9</sup>, and the Industrial Revolution (about 250 years before present)<sup>10</sup>. We show that over the past 1.6 million years, in the human lineage misorientation increased with time, especially when meat and stone-ground grains were introduced into human diets, but not with the Industrial Revolution. Thus, besides macro-changes, teeth adapted to dietary change at the nanoscale and crystallographically. This observation suggests that misorientation may contribute to enamel’s resilience; thus, bioinspired materials may consider small misorientation angles for added resilience.

Enamel-like tooth structure is common to nearly all vertebrates and has been evolving since it first appeared in conodonts nearly 400 million years before present (BP)<sup>11</sup>. Enamel varies and evolves at multiple structural levels and developmental stages.

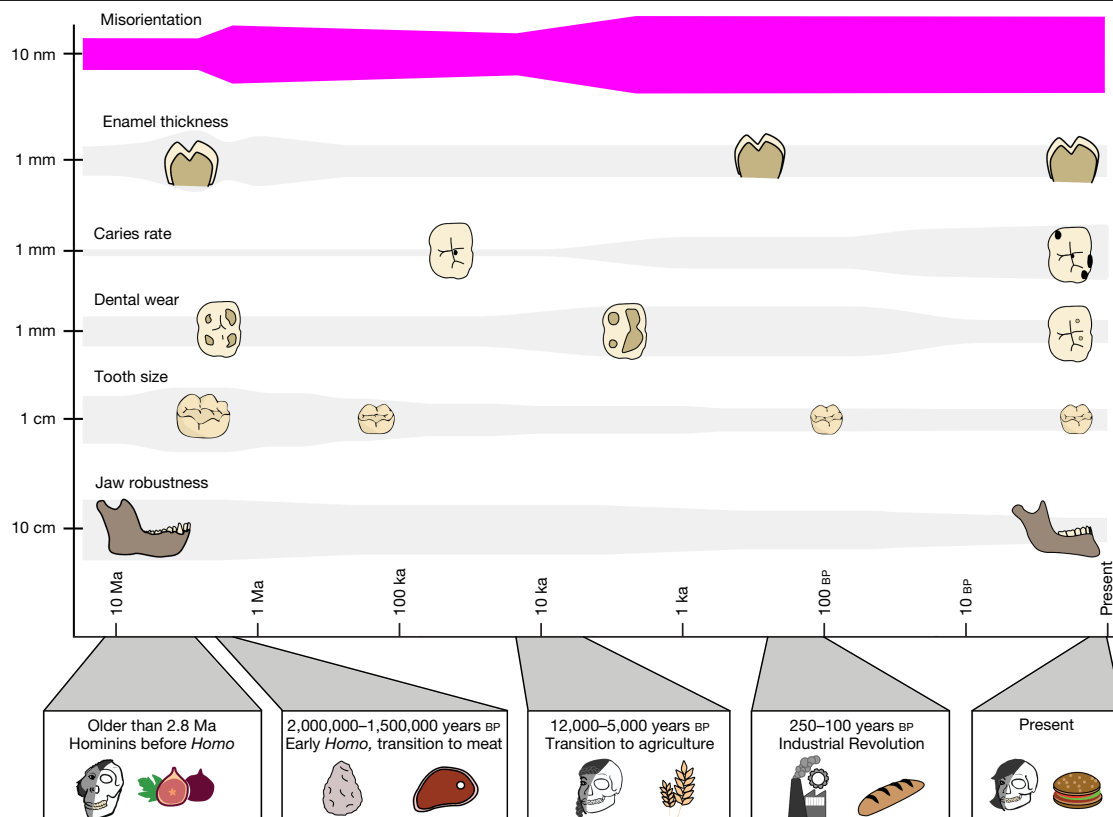
The structure and properties of enamel differ at different scales. At the macroscale, the thickness and shape of enamel can evolve quickly in response to the mechanical properties of food<sup>12,13</sup>. For example, teeth that have thick enamel<sup>14</sup> and blunt cusps are adapted to hard and abrasive foods, whereas teeth with thin enamel wear obliquely and have shearing edges suited for slicing soft foods<sup>13,15</sup>.

Hard-food consumption is associated with several dental adaptations in modern primates that resist crack propagation, including large teeth, low-crowned molars and premolars, and thick, unevenly distributed enamel<sup>13,16,17</sup>. Thick enamel at the millimetre scale helps prevent crack nucleation<sup>18</sup>.

At the micro- and nanoscales, enamel structure evolved to limit crack widening and propagation, thereby improving fracture resistance, also known as fracture toughness, or simply toughness<sup>3,19</sup>. At the microscale, decussating enamel prevents cracks from widening and propagating by deflecting them<sup>20</sup> at the interface between rod and interrod enamel<sup>21</sup>. At the nanoscale, researchers originally incorrectly assumed that adjacent nanocrystals within rods are co-oriented because they elongate parallel to one another morphologically, but they actually vary in orientation<sup>22</sup>. The angular distance of crystallographic *c*-axes in adjacent nanocrystals can vary by tens of degrees, and their orientation gradually changes within and across each rod by as much as 30–90° (ref. 22). Hereafter, we term this non-zero angular distance between the *c*-axes of adjacent nanocrystals simply ‘misorientation’. Molecular dynamics simulations have shown that misorientation deflects cracks and therefore toughens tooth enamel<sup>22</sup>.

<sup>1</sup>Department of Physics, University of Wisconsin, Madison, WI, USA. <sup>2</sup>Departments of Chemistry, Materials Science, Geoscience, University of Wisconsin, Madison, WI, USA. <sup>3</sup>Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. <sup>4</sup>Department of Human Evolutionary Biology, Harvard University, Cambridge, MA, USA. <sup>5</sup>School of Natural Sciences, University of Kent, Canterbury, UK. <sup>6</sup>Department of Anthropology, Ohio State University, Columbus, OH, USA. <sup>7</sup>Department of Earth Sciences, National Museum of Kenya, Nairobi, Kenya. <sup>8</sup>UMR 7206 Eco-anthropology, Musée de l'Homme, MNHN, CNRS, UParis Cité, Paris, France. <sup>9</sup>Oral Health, UMR\_S 1333, UParis Cité, Montrouge, France. <sup>10</sup>Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. <sup>11</sup>Energy Geosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. <sup>12</sup>Earth and Planetary Science Department, University of California Berkeley, Berkeley, CA, USA. <sup>13</sup>School of Anthropology and Conservation, University of Kent, Canterbury, UK. <sup>14</sup>Present address: Department of Biology, Ball State University, Muncie, IN, USA. ✉e-mail: pupa@physics.wisc.edu; mackie.ohara@bsu.edu





**Fig. 1 | Hominin dentitions have changed in relation to dietary changes over the past 10 million years.** Since hominins diverged from the last common ancestor shared with chimpanzees, significant dental changes occurred over the millimetre-to-centimetre scales. Compared with the earliest hominins, modern humans have less robust jaws, smaller posterior teeth and thinner

enamel, as well as less occlusal wear but greater caries prevalence (10 cm–1 mm, previous work; all references and details are presented in Supplementary Tables 3–8). At the nanoscale (10 nm), modern humans have more misoriented enamel than the earliest hominins, as reported here in magenta (this work).

The enamel formation mechanism, comprising organic matrix deposition, amyloid-like nanoribbons<sup>23</sup>, subsequent mineralization and resorption of the organic matrix, is conserved across all mammals, including primates<sup>24</sup>. Although primates differ in size (teeth, mouths, bodies, brains) and developmental rates<sup>25</sup>, they do not differ in enamel rod size, the presence of decussation patterns or nanocrystal sizes<sup>26</sup>. Thus, it is possible to make controlled comparisons of adjacent enamel nanocrystals across species without confounding size and developmental factors.

### Dietary shifts coincide with dental changes

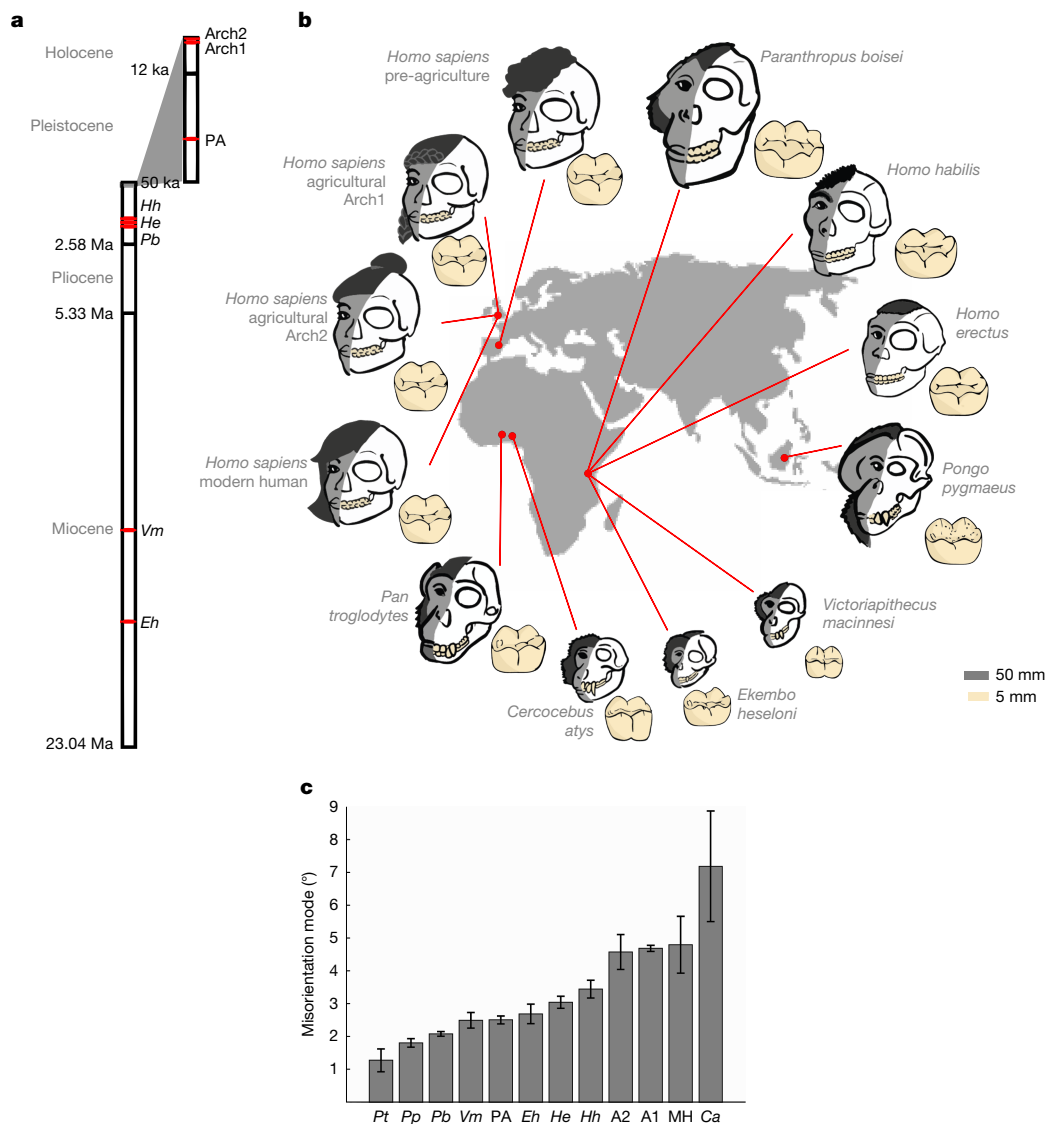
We investigated whether crystalline nanostructure and orientation changed with dietary hardness across primates, with particular emphasis on hominins, that is, members of the human lineage, including modern humans and our extinct fossil relatives. Supplementary Table 1 provides a summary of the terms frequently used for primates, including hominins, and to which group each of the nine species analysed in this study belongs. Supplementary Table 2 documents the 12 samples in this study.

After diverging about 4–8 million years BP from the last common ancestor with chimpanzees<sup>27,28</sup>, hominins began incorporating meat into their diet about 2.0–1.5 million years ago (Ma); humans invented agriculture around 12,000 years BP, adopted it widely in the later Holocene, and ramped up production of processed food during the Industrial Revolution beginning around 250 years BP. During the past 2 million years of human evolution, teeth became smaller<sup>29</sup> with thinner enamel layers<sup>30</sup> and more pronounced decussation<sup>31,32</sup>. These dental changes occurred alongside brain expansion<sup>33</sup>,

increased body size<sup>34</sup>, reduced facial and oral structures<sup>35</sup>, language development<sup>36</sup>, and cultural expansion<sup>37</sup>. Figure 1 documents significant changes in the size of human teeth, enamel thickness, wear patterns and caries prevalence following each major shift in human diet and technological advance (see Supplementary Tables 3–8 for references).

### Nanocrystal misorientation in PELICAN

To determine whether the millimetre-to-centimetre-scale structural changes summarized in Fig. 1 were accompanied by nanoscale changes in *c*-axis crystal misorientation, we examined 12 polished histological cross-sections of primate molar or premolar teeth from fossil, archaeological and modern ape and monkey species (Supplementary Table 2). We analysed variations within a species and across species, and tested correlations with dietary hardness and time. The 12 samples included two Miocene primates (*Ekembo heseloni* and *Victoriapithecus macinnesi*, 17.8 Ma and 14 Ma), three Early Pleistocene hominins (*Paranthropus boisei*, *Homo habilis* and *Homo erectus*), four *Homo sapiens* spanning pre-agricultural to modern times (pre-agriculture about 40,000 years BP, two agricultural British archaeological samples from 1,550 years BP and 700 years BP, and one modern human), and three extant primates with markedly different diets (*Pan troglodytes*, *Pongo pygmaeus* and *Cercocebus atys*). The *H. sapiens* samples track temporal trends across 40,000 years, including dietary differences (pre-agriculture versus agriculture), and variation within groups (two pre-modern, agricultural samples and one modern sample). We used a method called Polarization Enabled Large Input of Crystal Angles at the Nanoscale (PELICAN), developed for this work, on sets



**Fig. 2 | Samples represent primates across time, taxa and diet.**

**a**, Archaeological and fossil samples are presented by epoch (black horizontal lines represent epoch borders; red horizontal lines represent sample ages):

*E. heseloni* (Eh) and *V. macinnesi* (Vm) date to the Miocene, *P. boisei* (Pb), *H. erectus* (He), *H. habilis* (Hh) and the pre-agricultural *H. sapiens* (PA) date to the Pleistocene, and two agricultural *H. sapiens* (Arch1 and Arch2) date to the Holocene (Supplementary Table 2). **b**, All locations of origin are indicated on the world map alongside a representative lower molar, skull and partial

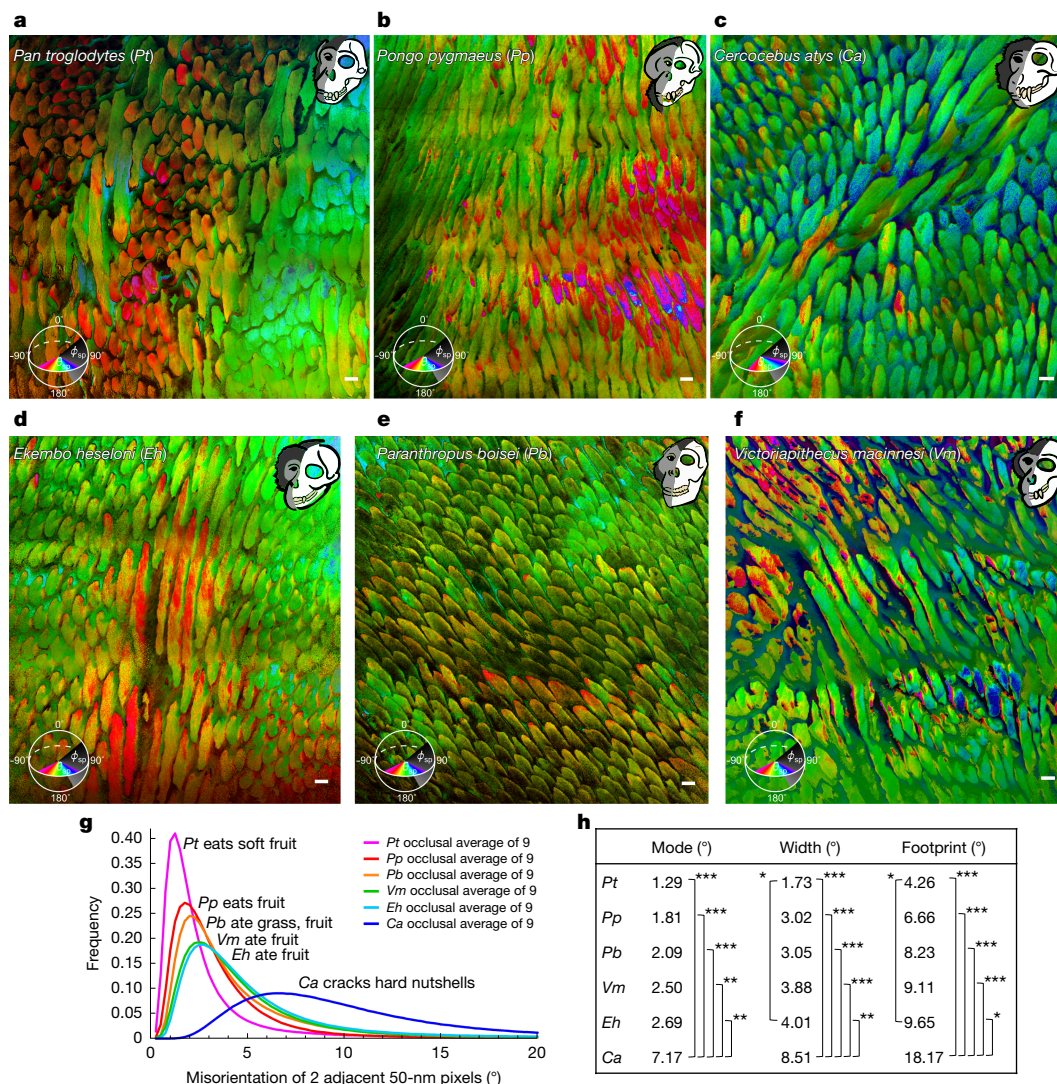
reconstruction. The grey scale bar for skulls is 50 mm; the beige scale bar for molars is 5 mm (Supplementary Table 2). **c**, The misorientation mode averaged over nine acquisitions in each sample; error bars are 1 s.d. (Supplementary Data 1). Extant species are abbreviated *P. troglodytes* (Pt), *P. pygmaeus* (Pp), modern human *H. sapiens* (MH) and *C. atys* (Ca). Arch1 and Arch2 are abbreviated A1 and A2. Credit: world map by Clker-Free-Vector-Images via Pixabay.

of photoemission electron microscopy (PEEM)<sup>38,39</sup> images. These ‘datasets’ were acquired similarly to polarization-dependent imaging contrast mapping<sup>40</sup>, but with revised image normalization and data processing that increases precision and provides high-throughput c-axis orientation information for every pixel. See Supplementary Methods for details. Extended Data Fig. 1 compares a PELICAN map with other imaging methods showing precisely the same region of enamel.

Using PELICAN, we compared the misorientation angle in three-dimensional space of two adjacent 50-nm pixels, matching the smallest possible size of enamel nanocrystal, which is also 50 nm. We compared each pixel with its adjacent eight pixels, identified the maximum misorientation angle and recorded that angle value. Choosing the maximum misorientation excludes co-oriented adjacent pixels and nanocrystals. We then produced a histogram of 1 million maximum misorientation angles per dataset, and 9 partly overlapping

datasets for each area analysed (Extended Data Fig. 2). We calculated the mode, width and footprint of each misorientation histogram to enable statistical comparison. Each PELICAN histogram therefore represents 9 million maximum misorientations, derived from 72 million pixel-pair comparisons per area. We repeated this analysis on the occlusal side of each tooth (Extended Data Fig. 2), and produced 3 × 3 partly overlapping PELICAN maps, in which colour quantitatively represents the orientation of the crystallographic c-axis in each pixel.

The full set of data and histograms is provided in the Supplementary Data 1. We used non-parametric tests to assess the significance of each comparison, owing to small sample sizes and a few non-normal distributions (Supplementary Table 9). Therefore, we used Kruskal–Wallis tests for pairwise comparisons between groups and Jonckheere–Terpstra tests to evaluate hypotheses of ordered, directional trends across dietary hardness categories and time; all *P* values were



**Fig. 3 | *Cercocebus atys* misorientation is significantly greater than that of all other fossil and modern apes and monkey. a–f**, PELICAN maps of two modern apes, *P. troglodytes* (Pt) (a) and *P. pygmaeus* (Pp) (b); and one modern monkey, *C. atys* (Ca) (c); two fossil apes, *E. heseloni* (Eh) (d) and *P. boisei* (Pb) (e); and one fossil monkey, *V. macinnesi* (Vm) (f). Sample abbreviations are in the Fig. 2 caption. Scale bars, 5  $\mu$ m.  $\theta_{sp}$  and  $\phi_{sp}$  are the spherical polar coordinates of the crystalline c-axis. They display in colour and grayscale, respectively, the

in-plane and out-of-plane orientation angles of the c-axis vector. **g**, Normalized histograms of misorientation for each of the samples in a–f. **h**, The histogram parameters averaged over nine datasets per area. Statistics: Kruskal–Wallis two-tailed Bonferroni significance is calculated based on 15 comparisons of 6 samples, each with nine datasets per area;  $*P < 3.3 \times 10^{-3}$  (0.05/15),  $**P < 6.7 \times 10^{-4}$  (0.01/15),  $***P < 6.7 \times 10^{-5}$  (0.001/15). All Kruskal–Wallis  $P$  values are in Supplementary Table 10.

Bonferroni-corrected where multiple comparisons were made. In Fig. 2, we present a summary of occlusal area results from all samples, along with representative skulls and reconstructions of faces for all species, drawn to scale with respect to one another, the size and shape of a molar tooth for each species, the foods each primate ate or eats, and the average and standard deviation across nine datasets of the most frequently occurring (mode) misorientations measured. Supplementary Tables 5–8 document what is known about the mechanical properties of each species' diet.

### Misorientation increases with diet hardness

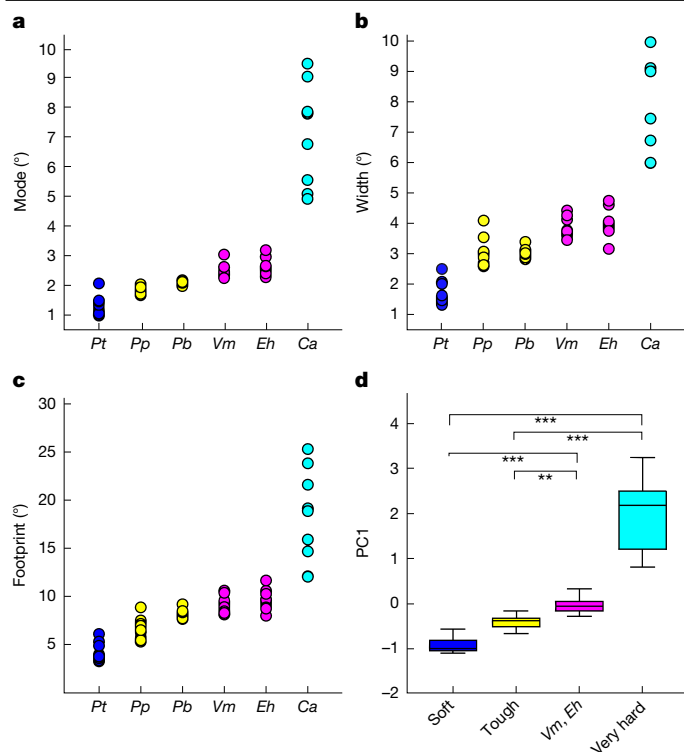
First, we sought to establish whether and how enamel nanocrystal misorientation varies with dietary hardness. To do this, we compared misorientation histograms from species with a range of dietary hardnesses: *P. troglodytes*, *P. pygmaeus*, *P. boisei*, *V. macinnesi*, *E. heseloni* and *C. atys*. Figure 3g reveals that (1) *C. atys*, a dedicated hard-object feeder, is significantly different from all the other species (Supplementary

Table 10); (2) *V. macinnesi* and *E. heseloni* show nearly identical histograms despite one being a monkey and another an ape; (3) *P. pygmaeus* and *P. boisei* plot together and are both known to consume a diet including tough objects; (4) *P. troglodytes* has significantly lower misorientation.

Both *V. macinnesi* and *E. heseloni* likely consumed primarily fruit and have statistically indistinguishable misorientation histograms with modes of  $2.5^\circ$  and  $2.7^\circ$ , respectively<sup>41,42</sup> (Kruskal–Wallis,  $P = 0.591$ ; Fig. 3). *P. boisei* falls between the modern (*P. troglodytes* and *P. pygmaeus*) and fossil (*E. heseloni*) apes and only differs significantly from *C. atys*. The misorientation mode, width and footprint of *C. atys* are significantly greater than those of all other samples (Kruskal–Wallis, all  $P < 8.3 \times 10^{-3}$ ; Supplementary Table 10).

*P. troglodytes* and *C. atys* show the lowest and highest misorientation angles, respectively, at  $1.3^\circ \pm 0.4^\circ$  and  $7.2^\circ \pm 1.7^\circ$ , which represents a 6-fold difference (Kruskal–Wallis,  $P = 7.5 \times 10^{-11}$ ; Figs. 2c, 3 and Supplementary Table 10). These two species have the most distinct diets: *P. troglodytes* occasionally eats meat or termites, but mostly consumes





**Fig. 4 | Enamel nanocrystals are more misoriented and heterogeneous when diets include harder foods.** **a–c**, Misorientation histogram parameters, mode (**a**), width (**b**) and footprint (**c**), for the six non-hominin primate samples from Fig. 3, ordered by increasing dietary hardness (Fig. 3): *P. troglodytes* (Pt, soft, dark blue), *P. pygmaeus* (Pp, tough, yellow), *P. boisei* (Pb, tough, yellow), *V. macinnesi* (Vm, unknown hardness, magenta), *E. heseloni* (Eh, unknown hardness, magenta), *C. atys* (Ca, very hard, cyan). **d**, Boxplot of PC1 factor scores plotted versus dietary hardness categories: soft (dark blue, Pt), intermediate (yellow, Pp, Pb), Vm, Eh (magenta, unknown fossil taxa with unknown diets), and very hard (cyan, Ca). Statistics: box plots show median, interquartile range and range. Kruskal–Wallis comparisons identify significant differences between dietary groups, denoted by black bars and asterisks. Kruskal–Wallis two-tailed Bonferroni significance was calculated based on six comparisons of four dietary hardness categories; \* $P < 8.3 \times 10^{-3}$  (0.05/6), \*\* $P < 1.7 \times 10^{-3}$  (0.01/6), \*\*\* $P < 1.7 \times 10^{-4}$  (0.001/6). All Kruskal–Wallis  $P$  values can be found in Supplementary Table 12.

ripe fruit<sup>43</sup>, whereas *C. atys* feeds preferentially on the oily seeds of *Sacoglottis gabonensis* trees, which it cracks with its enlarged molars and premolars. The nutshell-like-casing of the seeds is very hard and stiff, requiring powerful isometric bites to crack<sup>44,45</sup>. The high misorientation mode and heterogeneity in *C. atys*, combined with its specialized hard-object diet, suggest that greater misorientation is an adaptation to more mechanically challenging diets. This nanoscale pattern mirrors known macroscale adaptations: *C. atys* evolved thicker enamel and larger molars for hard-food processing, whereas *P. troglodytes* evolved molars with thinner enamel to maintain sharp cutting edges<sup>15</sup>. These data suggested a trend of misorientation with food hardness that needed further validation.

From each set of 9 million misorientations per sample in Fig. 3, we compared mode, width and footprint of the histogram and found that they all increased with hardness of food. All three histogram parameters increased significantly with dietary hardness across both the full non-hominin comparison (6 samples, 54 histograms) and an extant-only subset (3 samples, 27 histograms; Jonckheere–Terpstra, all  $P < 5 \times 10^{-4}$ ; Fig. 4a–c and Supplementary Table 11). This positive directional trend indicates that misorientation (mode) increases and becomes more heterogeneous (width,

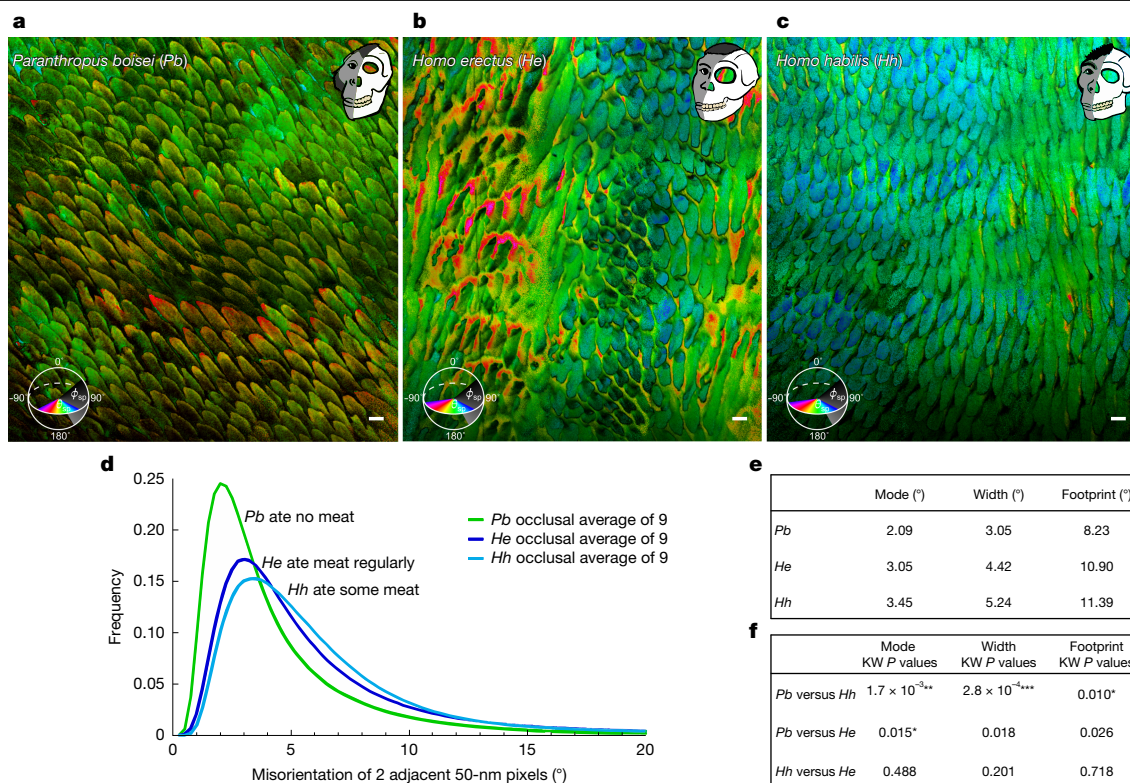
footprint) as dietary hardness increases from softest to hardest (Fig. 4a–c). Significant pairwise differences were observed between the softest diets and those of *V. macinnesi* and *E. heseloni* categories, between soft and very hard diets, and between intermediate and very hard diet categories (Kruskal–Wallis; Supplementary Table 10).

As mode, width and footprint are independent histogram parameters, but they all similarly increase with hardness (Fig. 4a–c), we used principal component analysis (PCA) to obtain a single principal component (PC1) capturing the magnitude (mode) and heterogeneity (width and footprint) in misorientation with one component or dimension. Mean PC1 factor scores ranged from  $-0.95$  (median  $-1.02$ ) for soft diets to  $+1.94$  (median  $2.18$ ) for very hard diets, effectively separating food hardness categories (Fig. 4d, and Supplementary Tables 11 and 12). PC1 factor scores increased significantly as dietary hardness increased in extant samples (Jonckheere–Terpstra,  $P = 1.8 \times 10^{-7}$ ; Supplementary Tables 11 and 12), consistent with the pairwise comparisons of harder and softer diets in Fig. 3h and Supplementary Table 10.

### Genus *Homo* misorientation and diet

Having established the misorientation versus hard-food trend, we analysed 7 hominins, fossil and modern, that span approximately 1.6 million years and 3 major dietary shifts. Misorientation mode differed significantly between *P. boisei* and both *H. erectus* and *H. habilis* (Kruskal–Wallis,  $P = 1.5 \times 10^{-2}$  and  $P = 1.7 \times 10^{-3}$ , respectively; Fig. 5). Despite living at the same time (1.6–1.8 Ma) and in the same place (Koobi Fora, Kenya), *P. boisei* had lower misorientation mode than either early *Homo* samples, whereas *H. erectus* and *H. habilis* misorientations were statistically similar to one another in mode, width and footprint (Kruskal–Wallis,  $P > 0.05$ ; Fig. 5). Thus, we can identify a correlation between meat-eating and misorientation, with modes increasing from  $2.1^\circ \pm 0.1^\circ$  (Pb) to  $3.1^\circ \pm 0.2^\circ$  (*H. erectus*) (Figs. 2 and 5). *H. habilis* ate meat but not as regularly as *H. erectus*, yet this hominin had slightly more misoriented nanocrystals, with a misorientation mode of  $3.5^\circ \pm 0.3^\circ$  (Kruskal–Wallis,  $P = 0.488$ ). We found no discernible difference between the fossil ape and monkey (*E. heseloni* and *V. macinnesi*), but differences in misorientation were observable in the later hominins (*P. boisei*, *H. habilis* and *H. erectus*; Kruskal–Wallis; Fig. 5f). Taking into consideration the significant relationship between misorientation and hard foods identified in primates (Figs. 3 and 4), the increase in misorientation with the introduction of meat (Fig. 5) can be interpreted as meat-eating being more challenging for enamel, because meat is tougher than fruit.

Figure 6 documents misorientation changes in *H. sapiens* from a pre-agricultural individual from Spain (PA, around 40,000 years BP), two British archaeological samples from agricultural populations (Arch1, around 1,550 years BP, and Arch2, around 700 years BP), and a recent clinically extracted molar from a modern British individual (around 50 years BP). Unexpectedly, the pre-agricultural misorientation mode, width and footprint are significantly lower than those of any more recent agricultural or industrial *H. sapiens*. Thus, the transition to grain-based agriculture in Europe around 12,000 years BP correlated with a significant increase in enamel nanocrystal misorientation. Misorientation mode was significantly higher in Arch1 than the pre-agricultural individual ( $4.7^\circ \pm 0.1^\circ$  versus  $2.5^\circ \pm 0.1^\circ$ ; Kruskal–Wallis,  $P = 2.9 \times 10^{-3}$ ; Supplementary Table 13). Comparison of the pre-agricultural individual with Arch2 yielded very similar results, with misorientation mode at  $4.6^\circ \pm 0.5^\circ$  (Kruskal–Wallis,  $P = 3.9 \times 10^{-3}$ ; Supplementary Table 13). The misorientation of crystals in the 2 archaeological teeth is almost double (1.9- or 1.8-fold) that of the pre-agriculture tooth. Following the transition to agriculture, Arch1, Arch2 and modern human *H. sapiens* (MH) showed consistent misorientation modes around  $4.7^\circ$ , with no significant pairwise differences among them



**Fig. 5 | The consumption of meat in hominin diets correlates with an increase in misorientation angle. a–c**, PELICAN maps of the occlusal enamel region from *P. boisei* (Pb; no meat; **a**), *H. erectus* (He; regular meat consumption; **b**) and *H. habilis* (Hh; occasional meat consumption; **c**). Scale bars, 5  $\mu$ m. **d**, Normalized histograms of misorientation for each area.

**e**, Histogram parameter averages for each sample. **f**, Kruskal–Wallis (KW) test *P* values comparing the histogram parameters from nine datasets per area across species. Kruskal–Wallis two-tailed Bonferroni significance is calculated based on three comparisons of three samples; \* $P < 1.7 \times 10^{-3}$  (0.05/3), \*\* $P < 3.3 \times 10^{-3}$  (0.01/3), \*\*\* $P < 3.3 \times 10^{-4}$  (0.001/3).

(Kruskal–Wallis, all  $P > 0.05$ ; Fig. 6 and Supplementary Table 13). The more recent Industrial Revolution did not result in a significant change in misorientation, although increases in caries and dental crowding indicate other dental consequences of an industrialized diet over the past 250 years<sup>10,46</sup>. This demonstrates that this 4.6–4.8° misorientation mode has remained stable for the past 1,550 years, at least in agricultural Britain.

Over the past 1.76 million years of *Homo* evolution, though, we can identify a trend towards increasing misorientation angles (Supplementary Table 14 and Extended Data Fig. 3). This trend stands out clearly when *Homo* misorientation is compared over time (Fig. 6g). PC1 factor scores increased significantly across four major time categories: early *Homo*, pre-agricultural *H. sapiens*, archaeological *H. sapiens* and modern humans (Jonckheere–Terpstra,  $P = 5.0 \times 10^{-5}$ ; Supplementary Tables 11 and 14). Although the composite PC1 misorientation factor score decreased between around 1.7 million years BP (Hh and He) and 40,000 years BP (PA) (Fig. 6c, Supplementary Fig. 16 and Supplementary Table 16), the trend is still very significant (Supplementary Table 14 and Extended Data Fig. 3). Pairwise comparisons of the individual parameters reveal similar results, with misorientation mode, width and footprint all differing significantly when pre-agriculture is compared with agriculture and today (Kruskal–Wallis,  $P < 1.7 \times 10^{-4}$ ; Supplementary Table 13). Between early *Homo* and agricultural and today, only mode differed significantly (Kruskal–Wallis,  $P < 1.6 \times 10^{-3}$ ; Supplementary Table 13), and between early *Homo* and pre-agriculture, only footprint differed (Kruskal–Wallis,  $P = 6.9 \times 10^{-4}$ ; Supplementary Table 13). The tight correlation among misorientation mode, width and footprint (Supplementary Table 15) suggests that as misorientation increases (mode), so does the misorientation heterogeneity (width and footprint). Many other parameters were tested and found to not

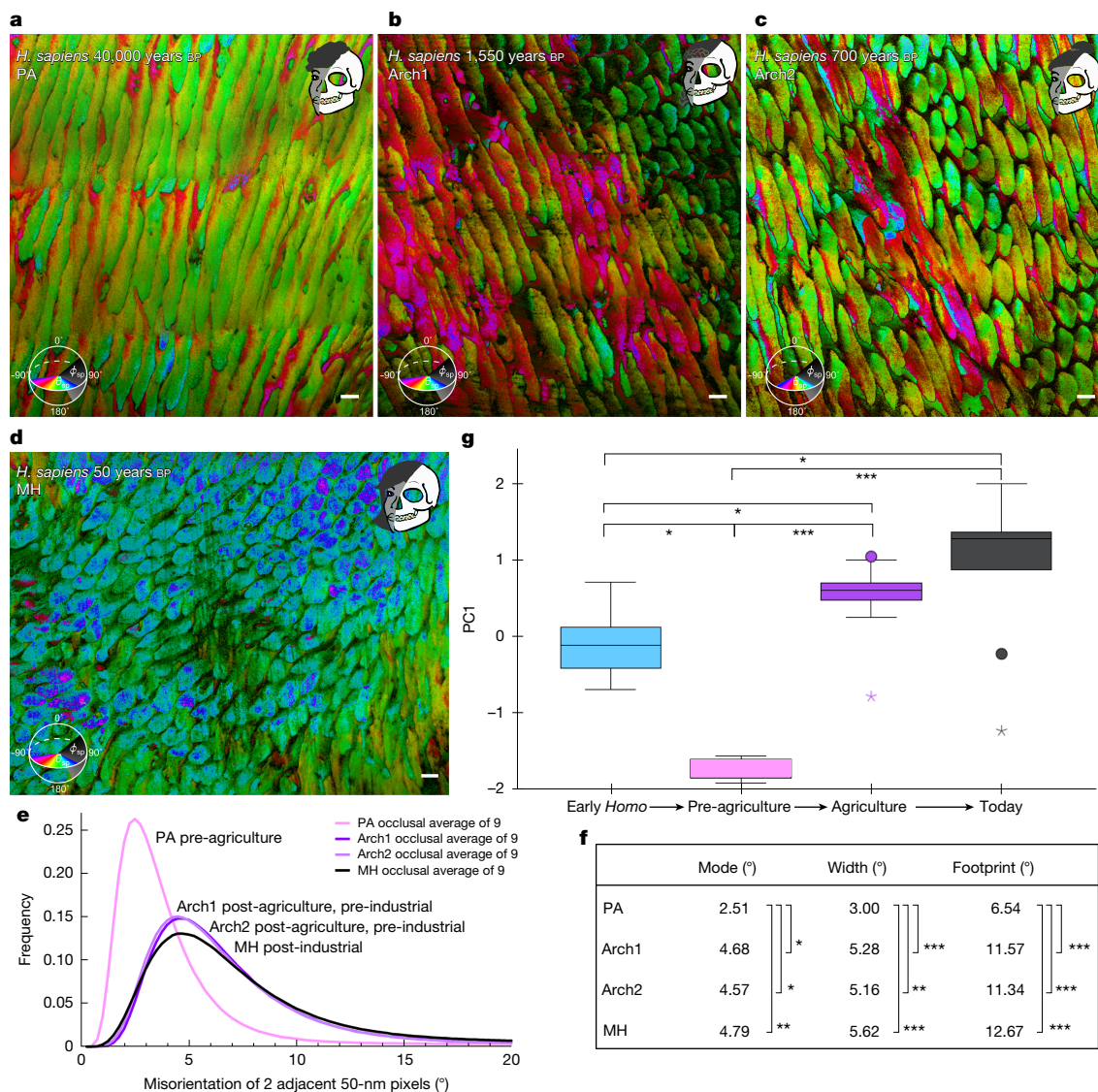
correlate with mode, width and footprint misorientations, nor with one another (Supplementary Table 15). The PC1 comparison, therefore, allows quantitative evaluation of misorientation change over time within the genus *Homo* (Extended Data Fig. 3 and Supplementary Tables 14–16).

### Misorientation within and across teeth

The results found in recent humans with an agricultural diet ( $\leq 1,550$  years BP) indicate that misorientation is consistent across individuals within a group. We obtained the Arch1 and Arch2 results from the functional cusp of a lower and upper first molar, respectively, suggesting that teeth on the upper and lower jaw have consistent misorientations. Within a tooth, the rod and interrod enamel crystals are oriented differently from one another; therefore, they differ in colour in PELICAN maps. However, when the misorientation of adjacent nanocrystals within rods and interrod enamel in the same area is compared, they are not statistically distinct (Kruskal–Wallis, all  $P > 0.05$ ; Extended Data Fig. 4). We established this using the two samples with the most extreme diets: the most misoriented *C. atys* and the least misoriented *P. troglodytes*. Separating rods from interrod enamel across species, therefore, does not change any of the above results. Supplementary Data 2 provides all the data relevant to this comparison.

For three samples (PA, MH and Eh), we acquired and compared two PELICAN areas, on the occlusal and lateral sides of the dentin horn (Extended Data Figs. 2, 5 and 6). We found no significant differences in misorientation histogram parameters for the pre-agricultural and most recent human (Kruskal–Wallis, all  $P > 0.05$ ; Extended Data Fig. 5) and *E. heseloni* (Kruskal–Wallis, all  $P > 0.05$ ; Extended Data Fig. 6) between





**Fig. 6 | Transition to agriculture correlates with increased misorientation, whereas the Industrial Revolution produced no significant change in misorientation.** **a–d**, PELICAN maps of *H. sapiens*: PA (pre-agriculture; **a**), Arch1 (**b**) and Arch2 (**c**) (post-agriculture, pre-Industrial Revolution), and MH (post-Industrial Revolution; **d**). Sample labels and abbreviations are at the top of all PELICAN maps. Scale bars, 5  $\mu$ m. **e**, Normalized histogram for each area in **a–d**. **f**, Kruskal–Wallis tests reveal significant differences between the histogram parameters of different samples, again from nine datasets per sample. Kruskal–Wallis two-tailed Bonferroni significance is calculated based on 6 comparisons of 4 samples; \* $P < 8.3 \times 10^{-3}$  (0.05/6), \*\* $P < 1.6 \times 10^{-3}$  (0.01/6), \*\*\* $P < 1.7 \times 10^{-4}$  (0.001/6). All sample comparison (PA, Arch1, Arch2, MH)

occlusal and lateral sides. Therefore, the misorientations in decussating enamel on occlusal and lateral sides of a dentine horn of a functional cusp of one tooth are probably representative of decussating enamel under all functional cusps of all molar and premolar teeth. The four *H. sapiens* teeth demonstrate that misorientations are also probably representative of all teeth in the same species. Therefore, the comparison of misorientation in occlusal areas done across all 12 samples in this study is well supported.

For the oldest sample, *E. heseloni*, we also analysed a region of radial enamel near the cusp tip and found that misorientation mode, width and footprint were greater in radial than in decussated enamel (Kruskal–Wallis, all  $P < 0.017$ ; Extended Data Fig. 6). This contrasts with previous observations showing that enamel at the outer enamel

Kruskal–Wallis  $P$  values can be found in Supplementary Table 13. **g**, The PC1 factor scores for samples grouped into four time periods: early *Homo* (light blue, *H. habilis* and *H. erectus*), pre-agriculture (pink, PA), agriculture (purple, Arch1 and Arch2) and today (dark grey, MH). The box plot shows median, interquartile range and range. Outliers (falling within 3 times the height of the interquartile range) are noted by circles and extreme values (falling further than 3 times the height of the interquartile range) are stars. Kruskal–Wallis comparisons identify significant differences between time periods (denoted by black bars and asterisks; Bonferroni-corrected thresholds for 6 comparisons; \* $P < 8.3 \times 10^{-3}$  (0.05/6), \*\* $P < 1.6 \times 10^{-3}$  (0.01/6), \*\*\* $P < 1.7 \times 10^{-4}$  (0.001/6); full  $P$  values in Supplementary Table 16).

surface (aprismatic and radial) exhibited lower toughness than the decussated enamel near the dentin–enamel junction<sup>47</sup>. However, none of the comparisons in this work are based on radial enamel, only on decussating enamel.

## Closing thoughts

The primary observation described here is that the misorientation of adjacent nanocrystals changed in conjunction with dietary shifts and, within the human lineage, misorientation increased when hominins introduced both meat and agricultural products into their diet. This observation suggests that nanocrystal misorientation may contribute to a tooth's resilience against hard or tough foods, complementing



previously documented adaptations such as tooth size, shape, enamel thickness and microstructure that confer resistance to wear and/or crack propagation. Thus, dietary specialization drives evolutionary changes across multiple hierarchical levels of enamel structure, down to the nanoscale.

The nut-cracking *C. atys* has an extremely mechanically demanding diet that requires the most resilient teeth<sup>44,45</sup>. We found that this monkey also has the greatest misorientation and exhibits the widest range of misorientation of all teeth measured (Figs. 3 and 4). We tentatively conclude, therefore, that we can add nanoscale misorientation to the suite of macro- and microscale adaptations that make enamel either harder, stiffer or tougher, or a combination of these material properties in relation to more mechanically challenging diets.

Although foods became easier to masticate over human evolutionary history with improvements in food processing and cooking<sup>48,49</sup>, we find that misorientation increased across both the transitions to meat-eating and agriculture. To test whether the misorientation shifts observed in *Homo* reflect factors other than diet, we calculated correlations between misorientation and sample age, tooth size, enamel thickness, maximum bite force and surface microwear values, but identified no significant correlations (Spearman's rho, all  $P > 0.05$ ; Supplementary Table 15). Ruling out those other factors, we returned to diet. One possible cause for the observations here might be that greater misorientation compensated for the marked and continuing reduction in molar size that characterizes our human lineage (Fig. 1). And, although agricultural food products may be more processed, the by-products of that processing (for example, millstone grit) may have contributed to high abrasion and significant wear in recent modern humans<sup>17</sup>.

We note that, even if greater misorientation is better from a materials perspective, all angles measured in all 12 samples were small misorientation angles, with modes between 1° and 7°, widths between 2° and 9°, and even the footprints of all histograms were small angles, between 4° and 18°. These misorientation angles fall within what material scientists call low-angle boundaries, which are typically defined as <15°. Such low-angle grain boundaries are low-energy surfaces, with surface energy of approximately 1 J m<sup>-2</sup>, increasing monotonically with misorientation angle<sup>50</sup>. This excess energy hinders dislocation motion, and thus stabilizes nanostructures<sup>50</sup>. Low-angle boundaries could have a similar role in ionic crystals such as hydroxyapatite or calcium carbonates, for example, under grinding conditions common for posterior teeth. Intriguingly, in molybdenum disulfide<sup>51</sup> and graphene<sup>52</sup>, low-angle grain boundaries have materials properties strongly dependent on misorientation angle. The results presented here, particularly the contrast between the fruit-eating chimp and the nut-cracking monkey, suggest a strong dependence of mechanical properties on misorientation angle.

## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10583-8>.

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## Methods

### Sample

The nine species analysed in this study are listed in Supplementary Table 1. The 12 tooth samples, locations of origin, date, collection ID and precise tooth analysed are listed in Supplementary Table 2. Whenever possible, we selected lower first molars (LM1) and the mesio-buccal cusp, which experiences crushing and grinding forces and is the first to wear<sup>53,54</sup> (Extended Data Fig. 2). When an LM1 was not available (for example, fossil samples), we used the functional cusp of the available molar or premolar, which is equivalent to an LM1's mesio-buccal cusp in its masticatory function (Extended Data Fig. 2). In all cases, analysis was restricted to molar or premolar teeth and focused on the occlusal region proximal to the dentine horn to control for functional use and avoid worn enamel.

### Histological sample preparation

Previous studies generated histological sections of PA, Arch1, Arch2, MH, *Pt*, *Pp* and *Ca*, but all used a standard set of histological methods<sup>55,56</sup>. Details of materials and consumables used for each sample can be found in their respective published articles (Supplementary Table 2). We identified no pathologies or taphonomic features in any sample. When wear was present, it was minimal and did not reach the dentine horn. All samples are permanent posterior teeth from adult individuals, with the exception of *Ca*, which was a subadult. Teeth were embedded in epoxy resin before being sectioned. Each tooth was sectioned through a plane intersecting the two mesial cusps (in the case of premolars, the two primary cusps; Extended Data Fig. 2) using a precision saw. The section was mounted on a microscope slide and used for other studies. For this study, we used the 'off-cut', which is the remaining portion of the tooth that was not mounted to the microscope slide. This off-cut was re-embedded, ground and polished as described below ('Sample preparation for PEEM').

The histological sectioning and analysis of the *Eh* sample was published in ref. 57; *Vm* was published in ref. 58; *Hh*, *He* and *Pb* were published in ref. 59. C. M. Dean led the histological preparation for these five samples, following standard protocols<sup>55,56</sup>. For these samples, no pathologies or taphonomic features were identified; when wear was present, it was minimal and did not reach the dentine horns.

### Sample preparation for PEEM

Samples arrived sectioned; we re-embedded, then ground and polished them. For re-embedding, we soaked blocks or slides in anhydrous ethanol and placed them face-down in two-part plastic moulds (Ted Pella) and covered them with freshly mixed EpoFix (EMS; 25 g epoxy, 3 g hardener, mixed with a Thinky planetary mixer, mixing for 1 min and defoaming for 1 min. We cycled the moulds three times through vacuum with a diaphragm pump kit (Ted Pella) and vented with nitrogen, to remove ethanol, ensure full epoxy penetration and burst surface bubbles. EpoFix cured overnight in air.

All grinding and polishing used water as a coolant, but supersaturated with either 1 g l<sup>-1</sup> CaCl<sub>2</sub> or 0.2 g l<sup>-1</sup> Na<sub>3</sub>PO<sub>4</sub> (pH 9) in MilliQ water to prevent hydroxyapatite dissolution, dispensed by a peristaltic pump (Drive MFLX07522-30, Head MLFX07519-06, Tubing MFLX96410-14, all by MasterFlex, Avantor). We ground tooth surfaces with 600, 1,200 and 4,000 grit SiC paper (Buehler) using 5 N, 30 s each, 40/140 rpm co-rotating on a Buehler AutoMet 250 PM. We then polished the tooth surfaces on the same Buehler AutoMet 250 PM with 300-nm alumina (MicroPolish 0.3 µm, Buehler) using 10 N, 2 min, 40/140 rpm counter-rotating, then with 50-nm alumina (MasterPrep, Buehler) with 10 N, 1 min, 30/30 rpm counter-rotating. Both 300-nm and 50-nm suspensions were dispensed automatically via burst modules (Buehler). This protocol yields mirror-flat surfaces with ≤1 nm residual roughness, as previously verified by atomic force microscopy. When possible, we thinned the samples to 2 mm thickness, which is ideal for PEEM experiments. For

the fossil samples (*Vm*, *Eh*, *Hh*, *He* and *Pb*) this was not possible; thus, we used a modified sample holder for the PEEM experiment. We trimmed the epoxy blocks to remove excess EpoFix resin and then cleaned them repeatedly with anhydrous ethanol and a new TexWipe Dry Cotton Cleanroom Wiper using gentle force until clean under differential interference contrast (DIC) microscopy (×20 objective). This step is key to obtaining perfect surfaces for the surface-sensitive PEEM method.

We then transferred all samples to ultrahigh vacuum (approximately 10<sup>-8</sup> mbar) to remove any residual water. Several samples took many hours to outgas: we attempted the PA sample during 3 beamtimes over 2 years and it failed until we built a dedicated ultrahigh-vacuum chamber, called the Dali chamber (Supplementary Fig. 1). The ultrahigh-vacuum chamber included a 660 l s<sup>-1</sup> turbo pump ceramic-bearing turbomolecular Agilent Twistorr 704 (Agilent Technologies). The pump is mounted at the bottom of the Dali chamber, equipped with a fast-entry flange on a side, and a drawer that comes out to receive the sample, and goes back into the chamber directly above the turbo, where the sample sits while pumping down.

We coated all samples twice using a Cressington 208HR (Cressington, Ted Pella) sputter coater. First, we coated the samples with 40 nm platinum and a 4 mm × 4 mm silicon wafer mask (MTI) to cover the functional cusp of the tooth and expose everything else to platinum, with no motion of the sample stage. Then, we removed the silicon mask and coated the whole sample with 1 nm platinum while spinning and tilting it. This coating method makes it possible to analyse with photoemission spectroscopy and microscopy any insulating sample including minerals<sup>60</sup>, biominerals<sup>61,62</sup> and most importantly enamel<sup>22,63–66</sup>. At the calcium L edge, the maximum probing depth of the PEEM experiment is 3 nm (ref. 67); hence, a 1-nm coating enables much of the signal to originate from the sample under investigation. The 40 nm surrounding coating ensures good electrical contact between sample and sample holder, so that the sample can float at -18 kV without arcs and sparks, and the photoelectrons it emits can be accelerated towards the electrostatic optics column.

We defined the region of interest identically in all samples. Whenever possible, we focused PELICAN analysis on specific occlusal regions of lower first molars (LM1) near the dentine horn to control for functional use, and selected the mesio-buccal cusp of each tooth because this region experiences both crushing and grinding forces<sup>53,54</sup> (Extended Data Fig. 2). When an LM1 was not available (for example, fossil samples), we used the 'functional cusp' of the available molar or premolar, which is closest in its masticatory function to an LM1's mesio-buccal cusp (Extended Data Fig. 2). The region selected for analysis in all tooth cross-sections was well below the surface of the functional cusp tip; the region of interest was approximately 200–300 µm occlusal to the apex of the dentine horn where both parazonal and diazonal zones of Hunter-Schreger bands in the decussation pattern were visible (Extended Data Fig. 2). We also analysed regions 200–300 µm lateral to the apex of the dentine horn for some samples. We precisely recorded the precise location of each area analysed in PEEM into a high-resolution optical image of each sample using Adobe Photoshop. Extended Data Figs. 5c and 6c, and Supplementary Fig. 2c provide examples of this precise localization in Photoshop, including the tooth cusp, the dentine horn, and the location of occlusal and lateral areas analysed with PEEM. The optical images were obtained with either DIC microscopy<sup>68</sup> or polarized light microscopy (PLM), and show the whole sample surface. Extended Data Figs. 5c and 6c, and Supplementary Fig. 2c show examples of the DIC images. The PLM images used crossed polarizers on the illumination and reflected light channels at 90° ± 30° from one another. All DIC and PLM images were acquired using a Zeiss AxioScope 7 (CTK Instruments) and stitched together automatically using the Zeiss software.

### Scanning electron microscopy

We acquired scanning electron microscopy (SEM) images using a Zeiss EVO LS10 microscope, operated at 10 kV accelerating voltage



and 9.5 mm working distance, in either secondary electrons or back-scattered electrons mode. The SEM images in Extended Data Fig. 1 present both the secondary electrons or the backscattered electrons of the same region of a *Ca* sample. We imaged the sample as it had been coated for the PEEM experiment; thus, the SEM, PEEM and PELICAN images are all directly comparable from precisely the same sample and accurately the same region of sample surface, even though the greater penetration and scattering depths in SEM and the larger depth of field provide the impression of more topography in SEM than in surface-sensitive PEEM.

## PEEM beamtime experiments

We acquired all data using the PEEM-3 microscope at the Advanced Light Source at Lawrence Berkeley National Laboratory. PEEM-3 is the end-station of beamline 11.0.1.1 at the Advanced Light Source and is a photoemission electron microscope<sup>38,39</sup>, with the sample floating at high voltage (−18 kV), fully electrostatic lenses<sup>69</sup> magnifying the image formed by photoemitted electrons under soft-X-ray illumination, and a best resolution of 20 nm (ref. 40).

PEEM-3 has an elliptically polarizing undulator source, which enables rotating the linear polarization of the soft-X-rays illuminating the sample surface. For this work, we acquired stacks of 38 full-field PEEM images while rotating the polarization from horizontal to vertical in 5° intervals. At each of 19 polarizations, we acquired 2 images at 2 photon energies on either side of the sharpest and most intense calcium  $L_2$  peak, termed peak 1, typically at 352.6 eV. We acquired the 2 images either 0.2 eV above and below peak 1, or +0.1 and −0.3 eV, whichever gave the greatest contrast in ratio images at each particular beamtime<sup>22,65</sup>. Both settings are effective, but the optimal choice varies every time the undulator, the monochromator or the beamline optics are tweaked and must be determined each time. The beam was shuttered off between exposures to prevent radiation damage<sup>70,71</sup>.

## Data-taking and normalization for PELICAN

It is important for PELICAN quantitative analysis<sup>5</sup> to use image ratios, so that the relative intensity is normalized and the amplitude of the  $\cos^2$  curve in each pixel is representative, as much as possible, of the crystal orientation in that pixel, not of the unevenly distributed illumination, polarization or sample-dependent image brightness. This is never perfect, but it improves substantially with normalized images. The image ratio can be acquired on-peak and off-peak if only one peak is dichroic, or on two anticorrelated dichroic peaks. Example energies for the oxygen K edge in carbonates are on-peak of the  $\pi^*$  peak at 534 eV and off-peak in the pre-edge at 530 eV. For the calcium L edge in calcium phosphates, instead, the  $L_2$  peak is the superposition of multiple anticorrelated peaks; thus, the peak appears to shift in energy with polarization by 0.4 eV (ref. 65). In Supplementary Fig. 3, we highlight two energies, 0.4 eV apart, where they usually give the greatest contrast, 0.1 eV above and 0.3 eV below the  $L_2$  peak at 352.6 eV. In either case, after normalization by ratio of these two images, each pixel has a  $\cos^2$  dependence on the elliptically polarizing undulator polarization angle. Immediately after acquisition, we aligned all 38.P3B PEEM images in PeemVision (<https://sites.google.com/lbl.gov/bl11-peem3-als/tools>), then ratioed them so that at each elliptically polarizing undulator polarization angle, the higher energy image is divided by the lower energy one, then saved as 19.P3B images.

We recorded the precise position where each dataset was acquired during data acquisition in the beamtime notebook as  $x, y$  coordinates. We recorded and saved both the PEEM coordinates and the visually identified features in PEEM and DIC or PLM images in the Photoshop file.

For each area, we acquired a  $3 \times 3$  map, with  $50 \pm 5$   $\mu\text{m}$  field of view, 1,030 pixels horizontally and 1,054 pixels vertically. Each pixel was therefore  $50 \pm 5$  nm, which matches the cross-section of enamel nanocrystal. The 9 stacks in a  $3 \times 3$  area were partly overlapping, as the sample position was shifted by 40  $\mu\text{m}$  between images.

## PELICAN analysis

We describe the PELICAN analysis in detail in Supplementary Methods and we have published the software and the manual to use it on Zenodo<sup>5</sup>, where a link to GitHub is provided. PELICAN is a method developed for this study to quantitatively measure the orientation angle of the crystallographic  $c$ -axis of crystals that exhibit X-ray linear dichroism. This method significantly improves on polarization-dependent imaging contrast<sup>22,40,62,65,72</sup>; it now calculates and displays the inferred  $c$ -axis orientation in spherical polar coordinates  $\theta_{\text{sp}}$  and  $\phi_{\text{sp}}$  in each pixel, calculates the misorientation angle of the two  $c$ -axes in two adjacent pixels, and calculates the histograms of all values for each spherical polar coordinate as well as the histogram of misorientations of adjacent pixels. PELICAN analysis runs in Igor Pro 8 (WaveMetrics) or a later version, and uses the Gilbert Group Macros (GG Macros), freely available via Zenodo<sup>5</sup>. For all areas, we calculated the histograms using identical PELICAN parameters ( $a_{\text{max}} = 1$ ,  $b_{\text{min}} = 1$ ,  $b_{\text{max}} = 120$  and  $\phi_{\text{zymin}} = 45$ ). For display purposes, however, we modified the parameters for several images, as described in Supplementary Methods, and the colour legends differ for each area in Figs. 2, 4 and 5, Extended Data Figs. 1 and 4–6, but in all figures they display precisely the range in which  $\theta_{\text{sp}}$  and  $\phi_{\text{sp}}$  vary. In a nutshell,  $\phi_{\text{zymin}}$  was either 45°, 80° or 90°, the  $\phi_{\text{sp}}$  range was identical for all between 50° and 150° displayed as brightness, the  $\theta_{\text{sp}}$  range was either −45° to 45°, −25° to 70° or −75° to 45° and displayed as hue. In addition, most PELICAN maps were adjusted in brightness with the ‘ $b$  brightness’ tool in PELICAN, which displays the value of  $b$  as brightness. The effect of ‘ $b$  brightness’ is similar to ‘levels’ and ‘Auto’ in Photoshop. This was done for all samples except for *Ca* and *Vm*.

Once we obtained nine PELICAN maps per area, with colour showing the orientation of  $c$ -axes semi-quantitatively, we mounted and stitched the nine partly overlapping maps manually, using Adobe Photoshop. We positioned maps manually by placing the nine maps in nine separate Photoshop layers, then we blended the nine layers using the Photoshop tool ‘Auto Blend Layers’, with ‘Panorama’ option, and merged them into one layer called ‘merged’.

## Histograms of misorientations

PELICAN saves a.csv histogram file for each stack, which represents the 1 million misorientation maxima. PELICAN also fits the peak, that is, the mode of the histogram of 1 million misorientations with a log normal function (Supplementary Fig. 10, bottom left), and saves the fitted peak position and calls it ‘mode (°)’ at 100% of the fitted height, the full-width at half-maximum called ‘width (°)’ at 50% of the fitted height, and the ‘footprint (°)’ at 10% of the fitted height, as presented in Figs. 2, 4 and 5, and Extended Data Figs. 4–6. The histograms are all normalized to 1, such that the area under the curve is always 1; thus, the histograms can be compared with one another. In Figs. 3, 5 and 6, and Extended Data Figs. 4–6, we present the average histograms, obtained by adding the nine histograms from each area and then dividing by nine in Microsoft Excel. Complete data are provided in Supplementary Data 1, which contains a summary sheet (‘all samples, PELICAN results’) with all mode (°), width (°) and footprint (°) and their means and standard deviation for all areas. The histogram outputs of PELICAN are also included on a separate sheet for each sample. Each sample’s sheet contains nine histograms and the calculated average histogram. All histograms are normalized to 1, such that the area under the curve is 1, so all histograms or average histograms can directly be compared with one another.

## Statistical analysis of misorientation histograms

We performed all statistical analyses in IBM SPSS Statistics 30. As PELICAN histograms are not Gaussian, we characterized each dataset’s histogram by 3 parameters: mode (100% of the peak or maximum), width (full-width at 50% maximum) and footprint (full-width at 10% maximum). We compared these parameters across the  $3 \times 3 = 9$  datasets

in each area. Shapiro–Wilk tests found that these 3 parameters in 12 occlusal areas were normally distributed for most (31 out of 36), but not all cases (5 out of 36) (Supplementary Table 9); thus, we used non-parametric statistics. We calculated the means and standard deviations in Fig. 2 and Supplementary Data 1 across these  $n = 9$  values.

We used two-sided Kruskal–Wallis tests with Bonferroni correction ( $\alpha = 0.05$ ) to compare histogram parameters between samples (Supplementary Tables 10–13). We used two-sided Jonckheere–Terpstra tests to assess directional trends in misorientation with increasing dietary hardness in non-*Homo* samples (Fig. 4 and Supplementary Table 11) and temporal trends within *Homo* samples (Fig. 6g, Supplementary Table 14 and Extended Data Fig. 3).

We established dietary hardness categories for non-*Homo* samples based on the mechanical properties of diet documented in the literature (Fig. 2 and Supplementary Tables 3–8) as soft (*Pt*), intermediate (*Pp*, *Pb*), fossil frugivore (*Vm*, *EH*) and very hard (*Ca*). Jonckheere–Terpstra analyses identified trends across these categories in all non-*Homo* samples ( $n = 54$ ) and extant primates only ( $n = 27$ ) (Fig. 4 and Supplementary Table 11). Then, we performed PCA on mode, width and footprint parameters across all 12 samples. This yielded a new, single composite measure (PC1), which explains 97.5% of variation with comparable loadings across all 3 variables (range 0.980–0.995). We calculated the PC1 factor score for each of the 108 occlusal datasets (12 samples with 9 occlusal datasets per sample; Supplementary Data 1). We then ran Jonckheere–Terpstra and Kruskal–Wallis tests on PC1 factor scores across the four dietary hardness categories (Fig. 4 and Supplementary Table 12). We assigned the six *Homo* samples to four time periods and ran Jonckheere–Terpstra and Kruskal–Wallis tests on their PC1 factor scores to identify trends and pairwise differences (Fig. 6 and Supplementary Tables 13–16). Finally, we ran two-sided Spearman’s rho correlations between the three histogram parameters (mode, width and footprint) compared with published metrics from Supplementary Table 8 (Supplementary Table 15).

## Ethics permissions

UCL–Kent collection (MH): ethical approval for histology research on this sample of teeth was obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166; project ID: 203541). The Newcastle collection (PA, Arch2, *Pp*, *Pt*) consists of existing thin sections produced at Newcastle University by Dr. Don Reid. Skeletal Biology collections (Arch1) are archaeological, and an ethics license is not needed. Fossil research (*Hh*, *He*, *Pb*, *EH*, *Vm*) was authorized in Kenya with NACOSTI permits NACOSTI/P/22/20091 and NACOSTI/P/24/36662.

## Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information files.

## Code availability

All PEEM and PELICAN code used in this study is available on Zenodo at <https://doi.org/10.5281/zenodo.17314120> (ref. 5).

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**Acknowledgements** P.U.P.A.G. received 40% support from the Department of Energy, Basic Energy Science, Chemical Sciences, Geosciences, Biosciences, Geosciences (DOE–BES–CSGB–Geosciences) Grant DE-FG02-07ER15899 at University of Wisconsin, 40% support from award FWP-FP00011135 also from DOE–BES–CSGB–Geosciences at Lawrence Berkeley National Laboratory under contract number DE-AC02-05CH11231, and 20% support from the National Science Foundation (NSF), Biomaterials Grant DMR-2220274. M.C.O’H. received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement number 101026776. All PEEM experiments were done at the Advanced Light Source, a US DOE Office of Science User Facility under contract number DE-AC02-05CH11231. This research was additionally funded by the Turkana Basin Institute (D.R.G.), a Climate Center Grant from the Lamont-Doherty Earth Observatory of Columbia University (D.R.G.), and by the Harvard University American School of Prehistoric Research (D.R.G.). This research was authorized in Kenya with NACOSTI permits NACOSTI/P/22/20091 and NACOSTI/P/24/36662. We thank the National Museums of Kenya for permission to use their collection of hominoid tooth sections, and we thank C. Dean for sectioning them. P.U.P.A.G. previously published as Gelsomina De Stasio.

**Author contributions** P.U.P.A.G. conceived of the study, prepared samples, acquired most of the PEEM data, processed all data in PELICAN, co-developed the PELICAN method, prepared Figs. 2, 4a–e and 5a–d, Extended Data Figs. 1 and 4–6, Supplementary Figs. 1–3 and all data provided as Supplementary Data 1 and 2, and wrote the article. P.M. contributed samples Arch1, Arch2, MH, *Pt* (the one we used) and *Pp*. D.G.–S. contributed samples *Pt* (destroyed) and histological prep of Ca. W.S.M. contributed sample Ca. D.R.G., F.K.M., S.M. and E.N. contributed samples from Kenya: *EH*, *Vm*, *Pb*, *He* and *Hh*. F.R.R. contributed sample PA. E.L. did the artistic reconstructions of all heads in Figs. 2, 3, 5 and 6, and prepared Fig. 1. C. A. Stiffler and C. A. Schmidt prepared some of the samples and acquired some of the PEEM data. B.Q.A. and A.S. provided PEEM beamline operation and troubleshooting during beamtime. B.G. co-developed the PELICAN method and prepared Supplementary Figs. 4–6 and Supplementary Table O. M.C.O’H co-designed the study, selected all samples except those from Kenya, did all the statistical analysis, prepared Figs. 1, 2, 3h, 4, 5f, 6g, Extended Data Figs. 2 and 3, and wrote all the anthropology parts of the paper, especially Figs. 1 and 2 and Supplementary Tables 1–16. All authors read and edited the article before submission.

**Competing interests** The authors declare no competing interests

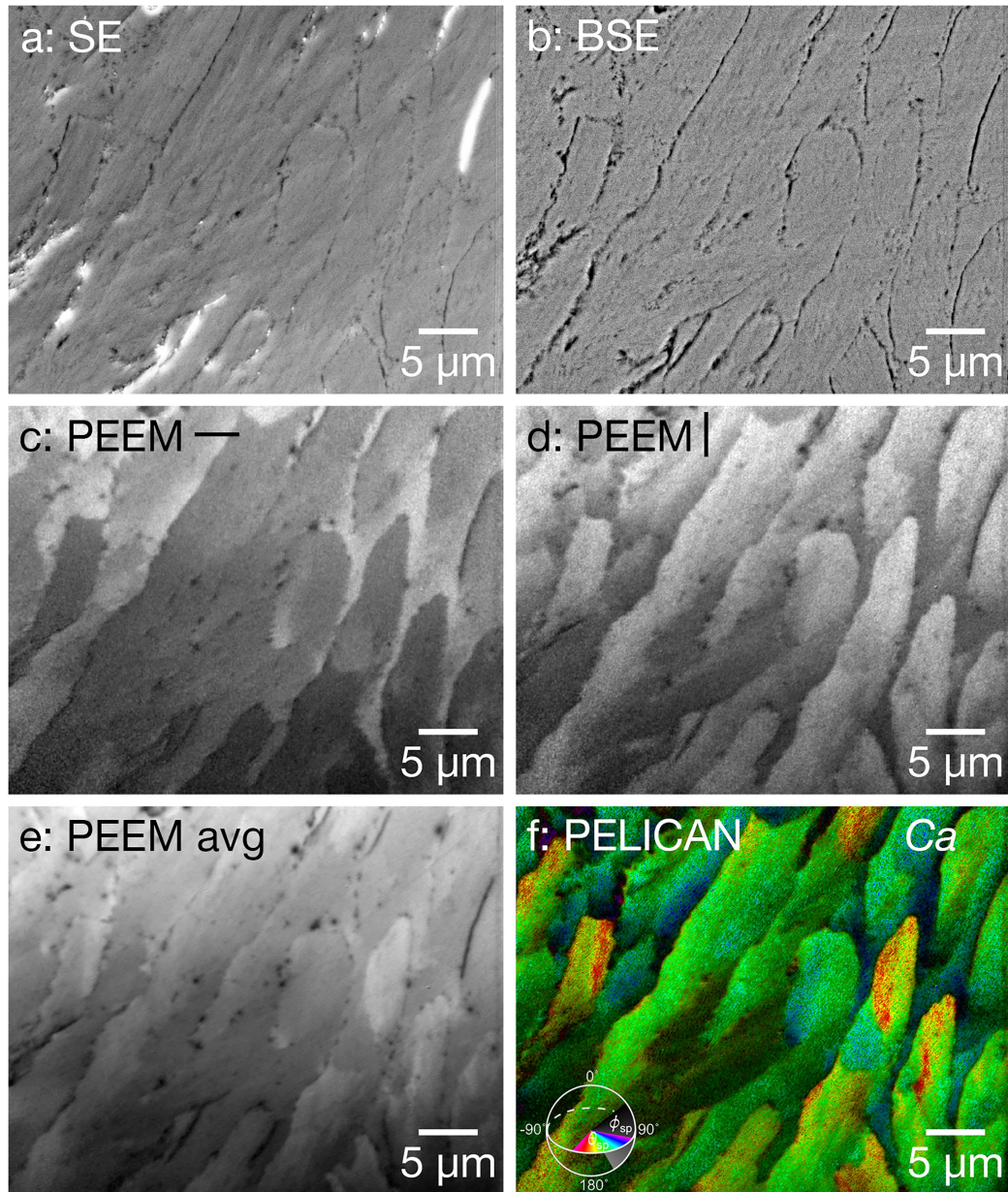
## Additional information

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41586-026-10583-8>.

**Correspondence and requests for materials** should be addressed to Pupa U. P. A. Gilbert or Mackie C. O’Hara.

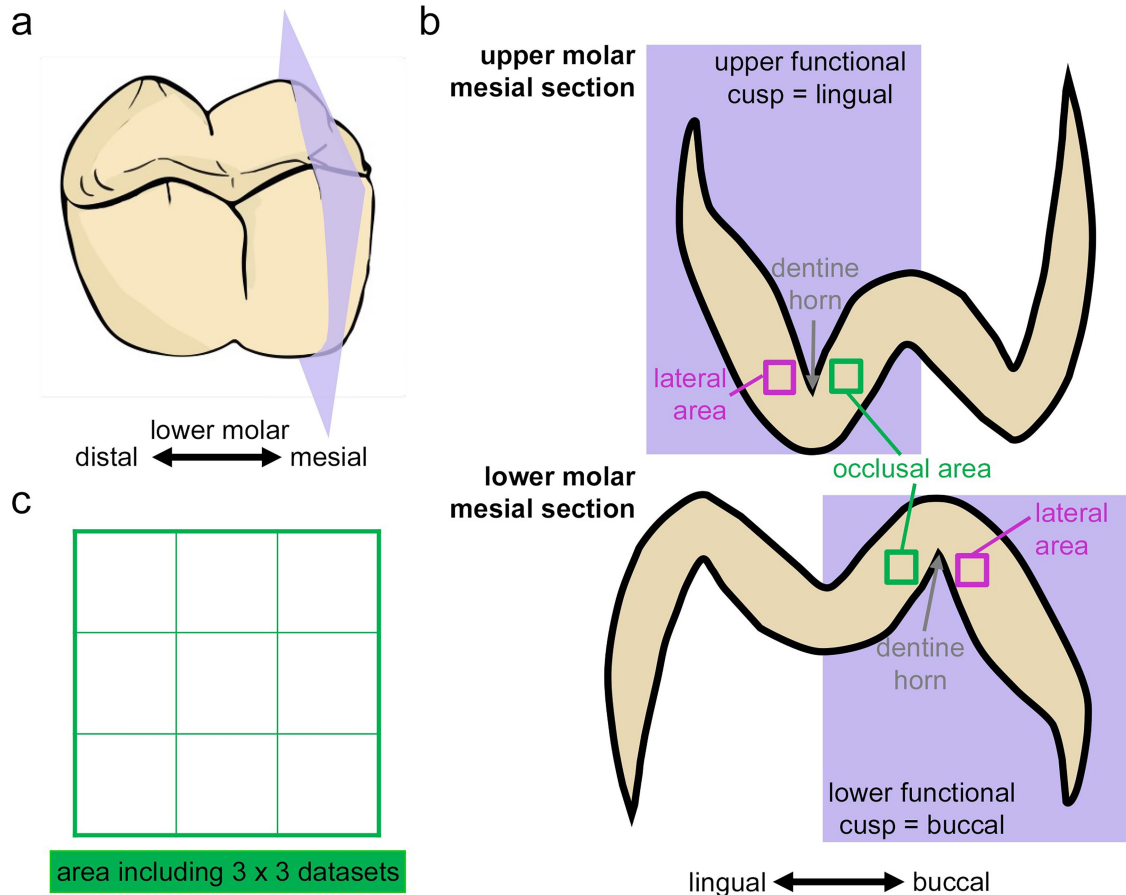
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**Extended Data Fig. 1 | The same region of a Ca tooth imaged with SEM, PEEM, and PELICAN. a: SE.** Scanning electron microscopy (SEM) image, acquired in secondary electrons (SE) mode, shows almost no contrast, except for some very bright organics surrounding rods. **b: BSE.** SEM image, acquired in back-scattered electrons (BSE) mode, shows contrast only where black organics surround each rod. **c: PEEM —** Ratio of two PhotoEmission Electron Microscopy (PEEM) Images acquired at 352.7 and 352.3 eV with horizontally polarized x-ray illumination at the Ca  $L_2$ -edge. **d: PEEM |** Same but with vertically polarized x-rays, illustrating the effect of x-ray linear dichroism in

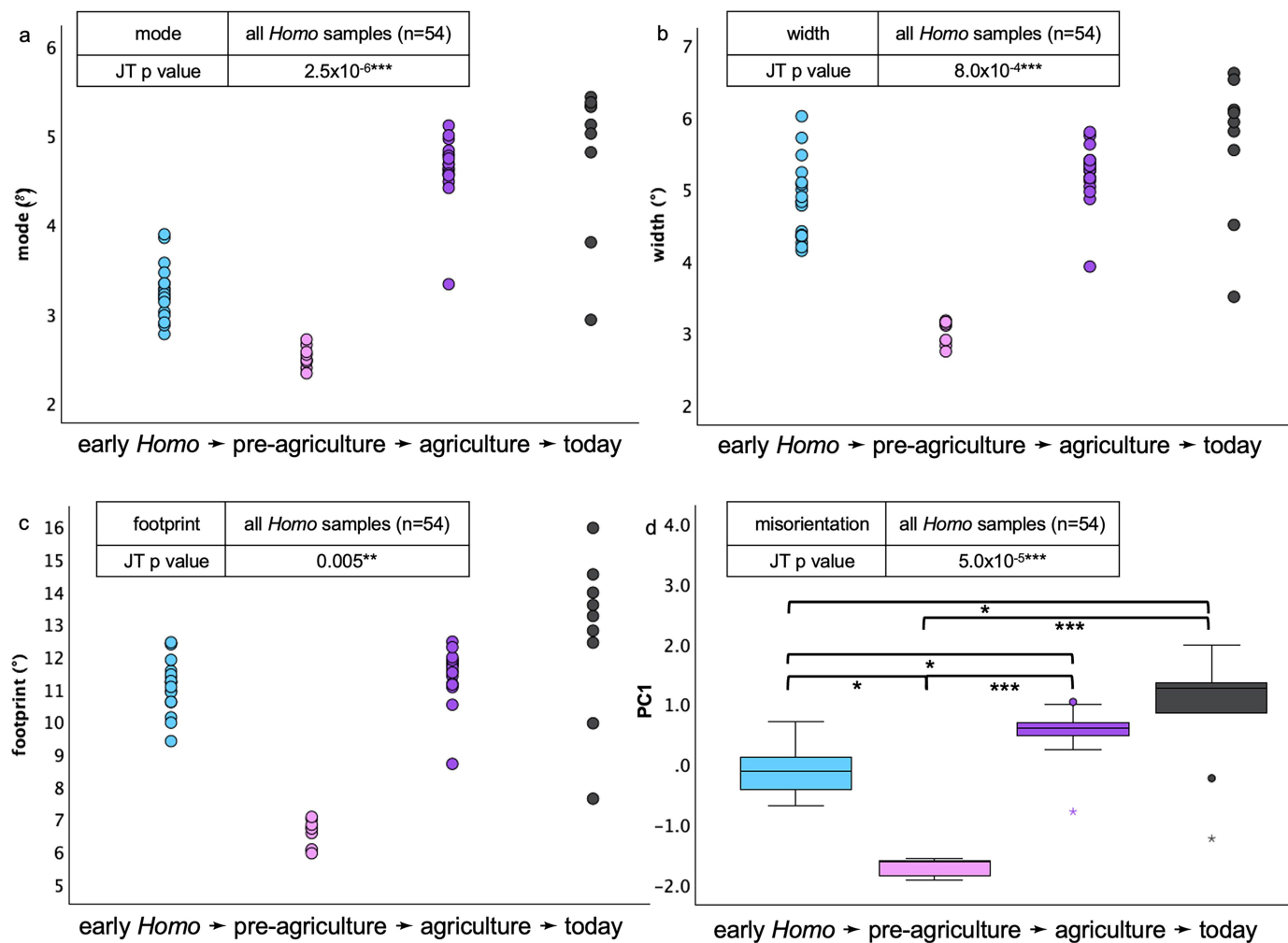
hydroxyapatite<sup>65</sup>. **e: PEEM avg.** Average of 19 PEEM image ratios acquired with polarization rotating from horizontal to vertical in 5° increments. **f: PELICAN.** A Polarization Enabled Large Input of Crystal Angles at the Nanoscale (PELICAN) map of precisely the same location on the Ca tooth. Colours quantitatively represent the orientation of the enamel nanocrystal c-axis in each pixel, including  $\theta_{sp}$  and  $\phi_{sp}$  angles describing the orientation of the crystallographic c-axis in each pixel. In spherical polar (sp) coordinates the in-plane  $\theta_{sp}$  angle is displayed as hue and the off-plane angle  $\phi_{sp}$  as brightness.



**Extended Data Fig. 2 | Sectioning location of all teeth in this study and location of areas analysed with PELICAN.** **a**, oblique sections of all molars were obtained by cutting through the mesial cusps, orthogonal to the cervical margin (purple plane). **b**, the area selected for analysis was on the occlusal side of the functional cusp (buccal for lower teeth, lingual for upper teeth). For the

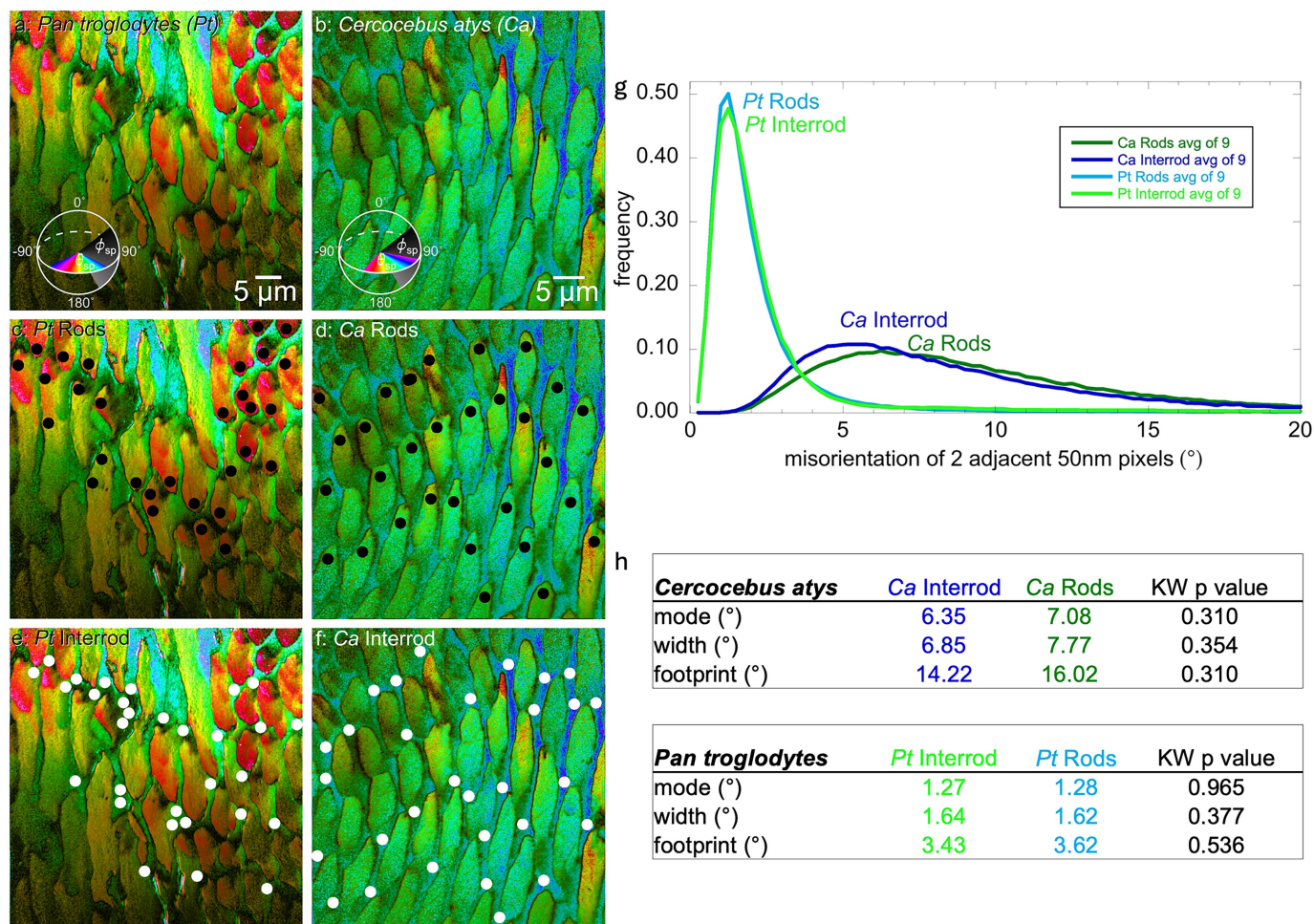
tooth in **a**, a lower molar, the mesio-buccal cusp is the functional cusp. The occlusal area is ~200  $\mu\text{m}$  away from the enamel dentine junction toward the occlusal basin; the lateral area is ~200  $\mu\text{m}$  away from the enamel dentine junction toward the outer enamel surface. **c**, each area includes 3 x 3 datasets, with 1 million pixels each, partly overlapping and stitched after data processing.





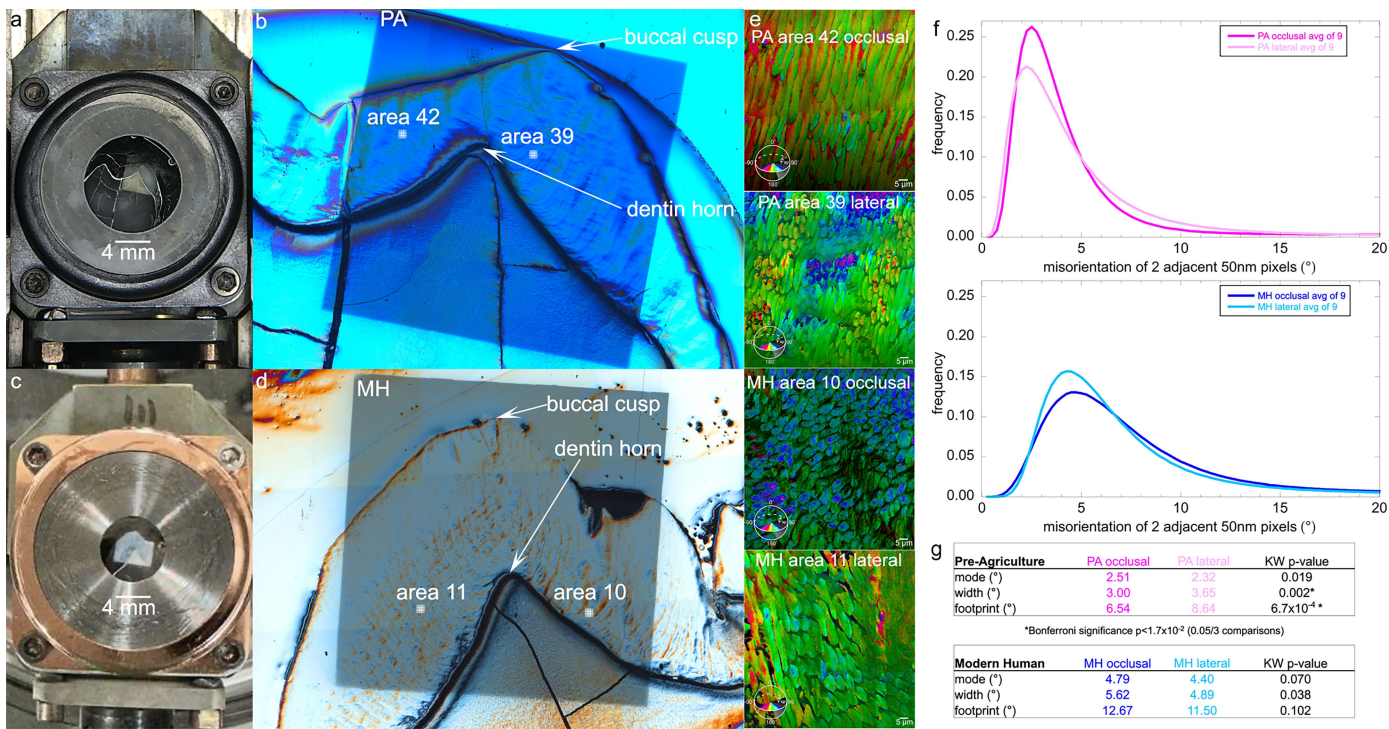
**Extended Data Fig. 3 | Enamel nanocrystals become more misoriented and heterogeneous over time in *Homo*.** **a-c:** Misorientation histogram parameters for six samples in Figs. 5 and 6, ordered from oldest to most recent: early *Homo* (*Hh*, *He*), pre-agriculture (PA), agriculture (Arch1, Arch2), and today (MH). Histogram parameters include misorientation mode (**a**), width (**b**), and footprint (**c**). Jonckheere-Terpstra (JT) test p values indicate significant trends for all samples (n = 54) (Supplementary Table 14). **d:** PC1 factor regression scores were obtained by combining all three histogram parameters and plotted vs.

time periods. Box plots show median, interquartile range, and range. Kruskal-Wallis (KW) comparisons identify five significant differences between time periods, denoted by black bars and asterisks. KW two-tailed Bonferroni significance was calculated based on six comparisons of four time period categories, so \* indicates  $p < 8.3 \times 10^{-3}$  (0.05/6), \*\*  $p < 1.7 \times 10^{-3}$  (0.01/6), and \*\*\*  $p < 1.7 \times 10^{-4}$  (0.001/6). All KW p values can be found in Supplementary Table 12.



**Extended Data Fig. 4 | Rods and interrod enamel does not differ within an area.** Single PELICAN maps from *Pt* (a) and *Ca* (b), showing clean images with species label, colour bar, and scalebar. c-f, 30 black dots indicate rods, 30 white dots indicate interrod enamel. Each dot has a 30 pixel diameter. g, histograms measured exclusively within the rod or interrod dot diameters. h, table of results averaging histogram parameters for *Ca* (top) and *Pt* (bottom) by rod and

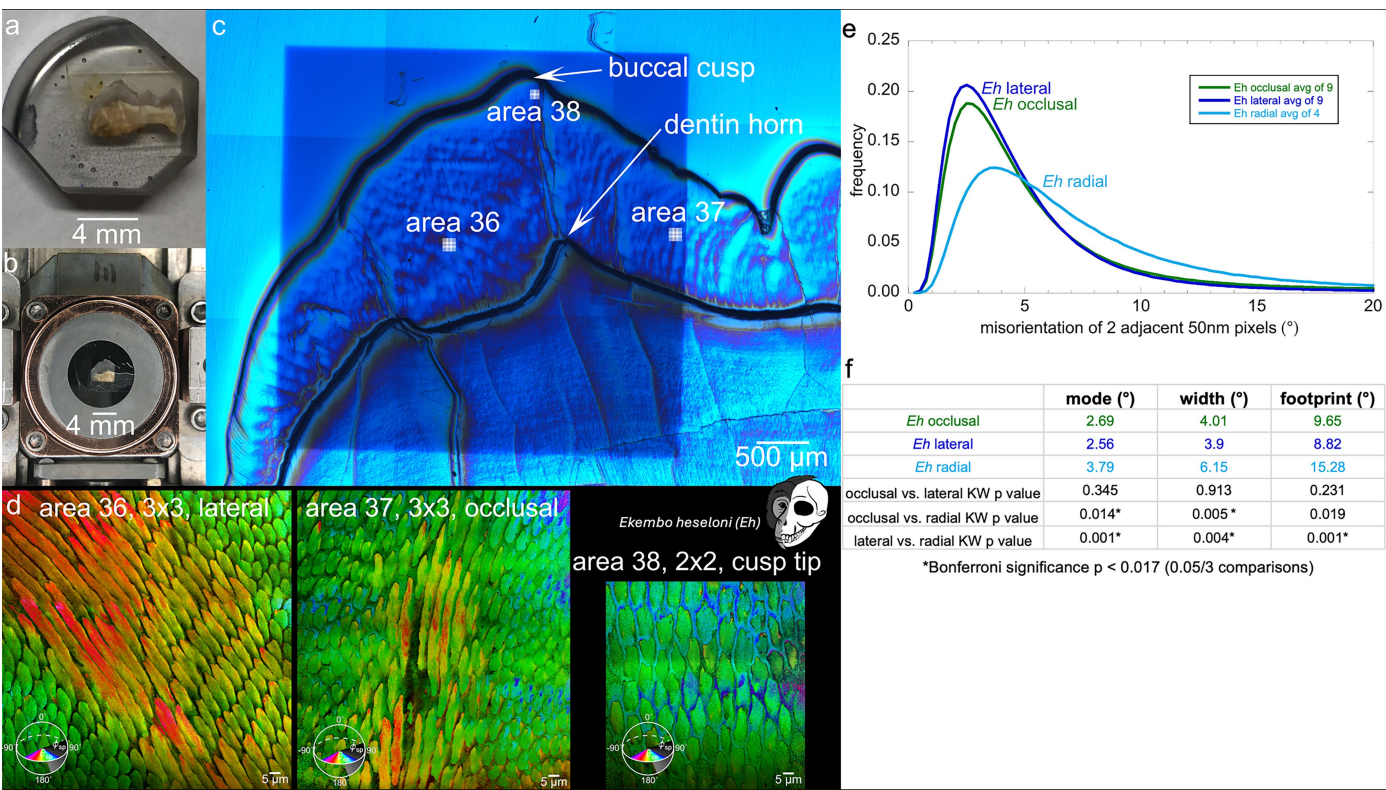
interrod enamel, and eight other, partly overlapping datasets adjacent to them, for *Pt* and *Ca* ( $\pi \times 15^2 \text{ pixels} \times 30 \text{ dots} \times 9 \text{ maps} = 1.9 \times 10^5 \text{ pixels}$ ). The table shows misorientation mode, width, and footprint, and their statistical analysis (Kruskal-Wallis). Within the same area of the same species, misorientations of adjacent pixels within rods are the same as in interrod enamel.



**Extended Data Fig. 5 | The PA and MH teeth, the precise locations of the four areas analysed, four PELICAN maps, and the histograms of misorientations.** PA=*Homo sapiens* pre-agriculture, MH= modern human sample of *Homo sapiens* post-industrial. PA (a) and MH (b) mounted in the PEEM sample holder. PA (b) and MH (d) samples in DIC images after coating the samples with 40 + 1 nm Pt. The precise locations of the three areas analysed in PEEM are shown by partly transparent and overlapping white 3 × 3 squares labelled “areas 42, 39, 10, and 11”. The central square with 1 nm Pt coating is

4 mm-wide e, four PELICAN maps acquired in the four areas on the occlusal and lateral sides of the dentin horn. f, normalized histograms comparing misorientations of adjacent pixels in occlusal (top, pink) vs. lateral (bottom, blue) decussation patterns for PA and MH. g, Kruskal-Wallis (KW) tests revealed no significant difference in PA or MH occlusal vs. lateral regions' misorientation modes. The PA lateral region's misorientation is significantly more variable (width, footprint) than that of the occlusal region, but no other differences were identified.





**Extended Data Fig. 6 | The *E. heseloni* tooth and the location of the three areas analysed with PELICAN.** **a**, the *E. heseloni* (*Eh*) tooth as received was embedded in clear epoxy. We show it after re-embedding, polishing, and trimming, but before coating so the whole tooth is visible. It is missing the bottom of the crown and the root, but two cusps are clearly visible thus it is simple to identify the lateral and the occlusal sides of each cusp. **b**, the *Eh* sample mounted in the PEEM sample holder. **c**, a DIC image of the *Eh* sample after coating with 40 + 1 nm Pt as described in Extended Data Fig. 5 caption and in Methods: Sample preparation for PEEM). The precise locations of the three areas analysed in PEEM are shown by partly transparent and overlapping white squares, either 3  $\times$  3 or 2  $\times$  2, labelled “areas 36, 37, 38”. **d**, three PELICAN maps

acquired in the three areas labelled in **c**, on the lateral and occlusal sides of the dentin horn, and at the cusp tip. Areas 36 and 37 have clear decussation patterns, whereas the cusp tip does not, it has “radial enamel”, that is, all rods are parallel to one another and perpendicular to the tooth surface (in elongation, not in c-axis orientation). **e**, misorientation histograms obtained for the three areas. **f**, table of statistical comparisons (Kruskal-Wallis) of histogram parameters in occlusal, lateral, and radial enamel. Occlusal and lateral decussated enamel does not have significantly different misorientation. Radial enamel misorientations are significantly different from both occlusal and lateral.



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*Give  $P$  values as exact values whenever suitable.*
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Data collection Gilbert, B. & Gilbert, P. U. P. A. GG Macros v1.0.0. (2025). <https://doi.org/10.5281/zenodo.17314120>

Data analysis IBM SPSS 30

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|                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reporting on sex and gender                                        | Archaeological human remains were used in this study, but sex was not included in the analysis as not all archaeological or fossil individuals were complete enough to sex.                                                                                                                                                                                                                                                                    |
| Reporting on race, ethnicity, or other socially relevant groupings | n/a                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Population characteristics                                         | n/a                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Recruitment                                                        | n/a                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Ethics oversight                                                   | UCL-Kent collection (MH): Ethical approval for histology research on this sample of teeth was obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166; project ID: 203541). The Newcastle collection (PA, Arch2) consists of existing thin sections produced at Newcastle University by Dr. Don Reid. Skeletal Biology collections (Arch1) are archaeological, and an ethics license is not needed.~ |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Study description        | This study uses PhotoEmission Electron Microscopy (PEEM) and PELICAN analysis to quantify enamel nanocrystal misorientation across 12 teeth from 9 primate and hominin species spanning ~17.8 million years of evolution. Specimens were grouped by dietary hardness (for non-Homo primates) and temporal period (for Homo). Non-parametric tests compare misorientation parameters across dietary hardness categories and Homo time periods.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Research sample          | 12 posterior teeth (molars and premolars) from 9 primate species were analyzed: two extant great apes (Pan troglodytes, Pongo pygmaeus), one extant monkey (Cercopithecus atys), one extinct monkey (Victoriapithecus macinnesi), four fossil apes (Ekembo heseloni, Homo habilis, Homo erectus, Paranthropus boisei), and four Homo sapiens specimens spanning pre-agricultural (~40,000 BP), archaeological (~700–1,550 BP), and modern (<50 BP) time periods. The sample was selected opportunistically to maximize phylogenetic breadth and ecological diversity given the extreme rarity of specimens suitable for destructive PEEM preparation. Specimens represent both extant (museum collections) and extinct (fossil) taxa. Sex and age data are not systematically available for most specimens; the Ca specimen is a known subadult. The sample is intended to represent broad patterns of enamel nanostructure evolution across dietary ecologies and hominin temporal trends, not population-level variation. |
| Sampling strategy        | No a priori power analysis was performed; sample sizes were constrained by the availability of rare, well-preserved fossil and museum specimens suitable for destructive PEEM preparation. The rationale for sufficiency is that PEEM/PELICAN analysis yields approximately 9 million individual nanocrystal misorientation measurements per tooth, providing exceptional within-sample statistical power. Across-specimen comparisons rely on non-parametric tests (Kruskal-Wallis, Jonckheere-Terpstra) applied to the 9 summary statistics (mode, width, footprint) per tooth area, which is consistent with the available specimen counts. The 12-tooth sample spans four dietary hardness categories and four Homo temporal periods, enabling both ecological and evolutionary trend analyses.                                                                                                                                                                                                                         |
| Data collection          | PEEM data were collected by the authors at the PEEM-3 end-station of beamline 11.0.1.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory, during multiple scheduled beamtimes. For each tooth, a 3x3 mosaic of PEEM image stacks was acquired in the occlusal region approximately 200–300 µm from the dentine horn apex, targeting areas where Hunter-Schreger decussating bands (parazones and diazones) were visible. Nanocrystal misorientation maps were subsequently generated using the PELICAN algorithm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Timing and spatial scale | PEEM data were collected across multiple beamtimes at ALS over a period of several years; the PA specimen required acquisition attempts at three separate beamtimes before successful data collection, which was ultimately achieved following construction of a dedicated UHV pre-pump chamber. Specimens originate from geographic locations spanning Kenya, Spain, and the United Kingdom, with fossil specimen ages ranging from ~17.8 Ma to ~700 BP.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Data exclusions          | no data were excluded                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

## Reproducibility

Within-specimen reproducibility was assessed through the 9-stack 3×3 acquisition design: mode, width, and footprint values were computed independently for each of the 9 stacks per area, and means and standard deviations are reported (ALL DATA.xlsx). Shapiro-Wilk tests assessed the normality of these within-specimen distributions. The standardized PEEM preparation protocol (fixed polishing sequence, coating procedure, UHV outgassing, imaging parameters) was applied identically across all specimens to maximize cross-specimen reproducibility. The PELICAN algorithm is deterministic given fixed input images. Histological preparation followed published standard protocols applied consistently across specimens.

## Randomization

Random allocation was not applicable. Specimens are pre-existing museum and fossil collections selected on the basis of taxonomic identity, preservation, and documented dietary ecology.

## Blinding

Blinding was not performed and was not feasible, as specimen identities (taxon, provenance, dietary ecology) are intrinsic and known prior to sample preparation. However, the primary data generation is algorithmic (PELICAN misorientation analysis), eliminating subjective observer bias in the main quantitative outputs.

Did the study involve field work? ☐ Yes ☒ No

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

- n/a Involved in the study
- ☒ ☐ Antibodies
  - ☒ ☐ Eukaryotic cell lines
  - ☐ ☒ Palaeontology and archaeology
  - ☒ ☐ Animals and other organisms
  - ☒ ☐ Clinical data
  - ☒ ☐ Dual use research of concern
  - ☒ ☐ Plants

### Methods

- n/a Involved in the study
- ☒ ☐ ChIP-seq
  - ☒ ☐ Flow cytometry
  - ☒ ☐ MRI-based neuroimaging

## Palaeontology and Archaeology

## Specimen provenance

All fossil and archaeological specimens are listed in Table S2 with accession numbers and institutional repositories. Fossil specimens (Eh, Vm, Hh, He, Pb) are held at the Kenyan National Museum (KNM), Nairobi, Kenya (accession numbers: KNM-RU 1695, KNM-MB 27844, KNM-ER 1805E, KNM-ER 3733, KNM-ER 801). Pre-agricultural Homo sapiens specimen (PA) is from Cueva del Humo, Málaga, Spain (Newcastle Collection, University of Kent; HT-11-97). Archaeological specimens Arch1 (Ozengell Grange, Kent, UK; Skeletal Biology Collection, University of Kent; Oz 195a) and Arch2 (St Gregory's Priory, Canterbury, Kent, UK; Newcastle Collection, University of Kent; NGB89 SK57) are from Anglo-Saxon contexts. Permits for access and destructive analysis of specimens were obtained through institutional material transfer and loan agreements with the respective holding institutions.

## Specimen deposition

All specimens analyzed in this study remain deposited in their respective institutional collections: Kenyan National Museum (fossil hominins Eh, Vm, Hh, He, Pb); Newcastle Collection, University of Kent (PA, Arch2, Pt, Pp); Skeletal Biology Collection, University of Kent (Arch1); UCLK Collection, University of Kent (MH); Tai Forest Collection, Department of Anthropology, Ohio State University (Ca). Off-cuts prepared for PEEM analysis have been returned to or are held under the agreements with the originating collections.

## Dating methods

No new dates were generated as part of this study. Chronological assignments for all specimens are based on previously published dates from the literature, cited in Table S2.

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

## Ethics oversight

UCL-Kent collection (MH): Ethical approval for histology research on this sample of teeth was obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166; project ID: 203541). The Newcastle collection (PA, Arch2, Pp, Pt) consists of existing thin sections produced at Newcastle University by Dr. Don Reid. Skeletal Biology collections (Arch1) are archaeological, and an ethics license is not needed. Fossil research (Hh, He, Pb, Eh, Vm) was authorized in Kenya with NACOSTI permits NACOSTI/P/22/20091 and NACOSTI/P/24/36662.

Note that full information on the approval of the study protocol must also be provided in the manuscript.



|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Seed stocks           | <i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>                                                                                                                                                                                                                                                                                          |
| Novel plant genotypes | <i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i> |
| Authentication        | <i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>                                                                                                                                                                                                                                       |