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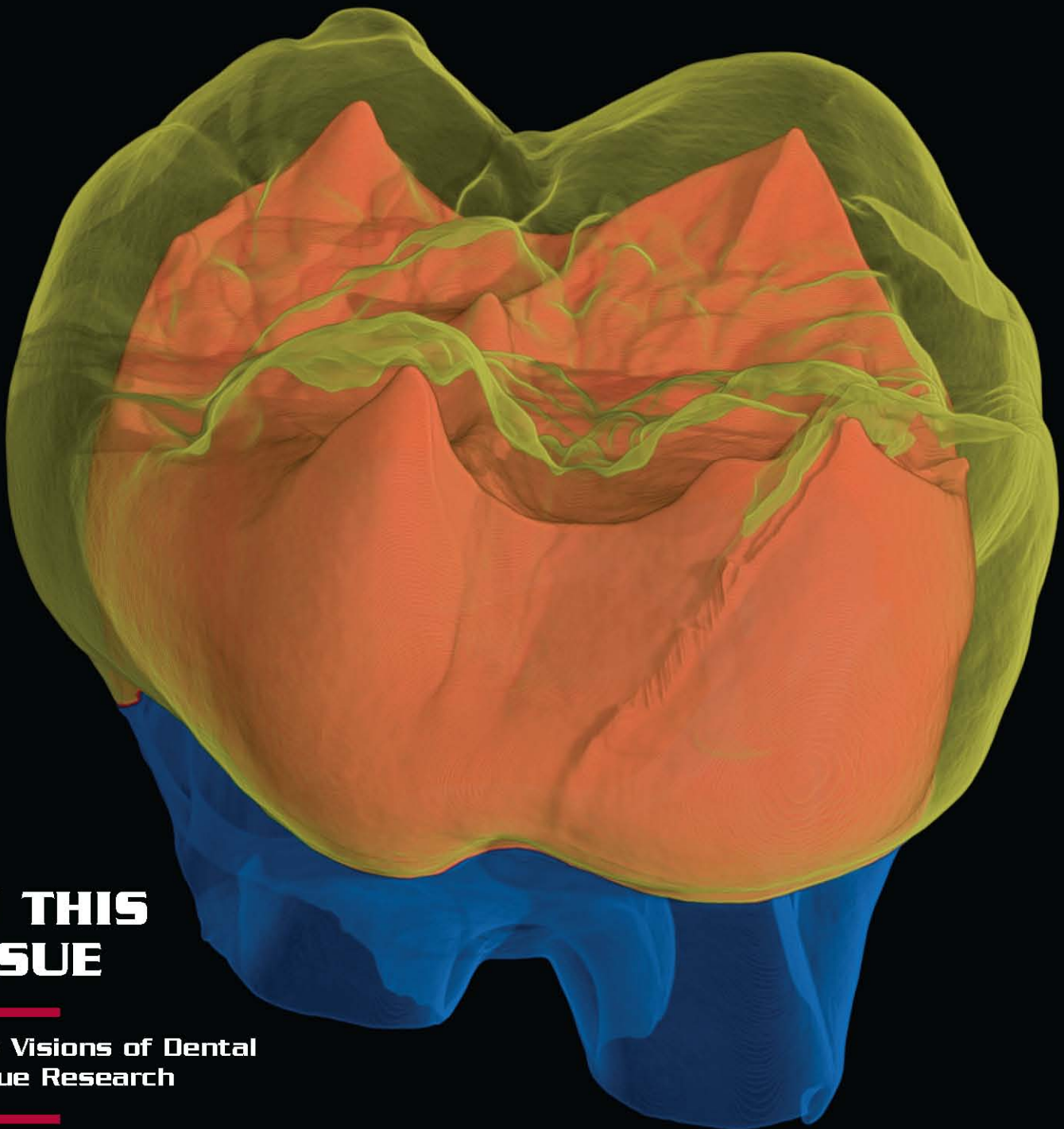

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ARTICLES

New Visions of Dental Tissue Research: Tooth Development, Chemistry, and Structure

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Teeth are one of the best preserved and most commonly recovered elements in primate fossil assemblages. Taxonomic, functional, and phylogenetic hypotheses often rely on dental characters, despite considerable evidence of homoplasy in tooth form and large variation in tooth size within and among primates.[1,2] Recent studies have led to new areas of research centered on incremental tooth development, chemical composition, and internal structure. Due to rapid technological developments in imaging and elemental sampling, these new approaches have the potential to increase our understanding of developmental biology, including not only changes in the pace of growth and reproduction, but also our assessments of diets, migration patterns, environments, and taxonomy. The integration of these temporal, chemical, and structural approaches heralds a bright future for the role of dental tissue research in evolutionary anthropology.

It is well established that dental tissues preserve a permanent record of their development through time, represented by incremental features in enamel and dentine microstructure.[3–8] Counts and measurements of these incremental features have been used to determine the rate and duration of tooth formation, stress experienced during development, and the age at death of juveniles. Incremental microstructure has traditionally been assessed through physical sectioning and preparation of histological sections, which reveal contrasting linear increments when viewed with light microscopy (Fig. 1). Developmental features are often classified as short- and long-period increments, based on their rhythmic repeat intervals (Box 1). Although histological investigations can yield highly accurate estimates of tooth formation, until recently these types of studies have been limited to small samples due to their time-consuming and semi-destructive nature.[6]

Because tooth growth begins before birth and continues throughout adolescence, assessment of incremental features may permit precise reconstruction of an individual's developmental history, including birth, subsequent stress during development, and death (Fig. 2). For example, a recent study of a young captive gorilla demonstrated a strong correlation between accentuated lines in tooth enamel and traumatic events, including eye injury, subsequent hospital visits, and transfers to different enclosures.[11] Other such studies have suggested that patterns of developmental stress inside teeth may correlate with social stress, weaning, ecological variation, and menarche.[12] Similar interpretations have been based on patterns of hypoplasias on external tooth surfaces,[13–15] although without knowledge of the precise timing of tooth formation. Unfortunately, relatively few data exist for individuals with corresponding behavioral, physiological, and ecological records, a fact that prohibits confident interpretations of accentuated lines or hypoplasias in fossil primates or archeological material. This line of research would benefit from study of additional individuals with known histories, as was done by Schwartz and colleagues.[11] One potentially complementary approach involves the assessment of changes in tooth chemistry, since dietary changes associated with birth or weaning may be integrated with the timing of tooth development.[16–19]

INCREMENTAL TOOTH DEVELOPMENT

Developmental Variation

Over the past 25 years, incremental features have been investigated in diverse primate taxa, particularly hominoid primates[6] (Table 1). During the past decade, researchers have taken a rigorous comparative approach through the use of large

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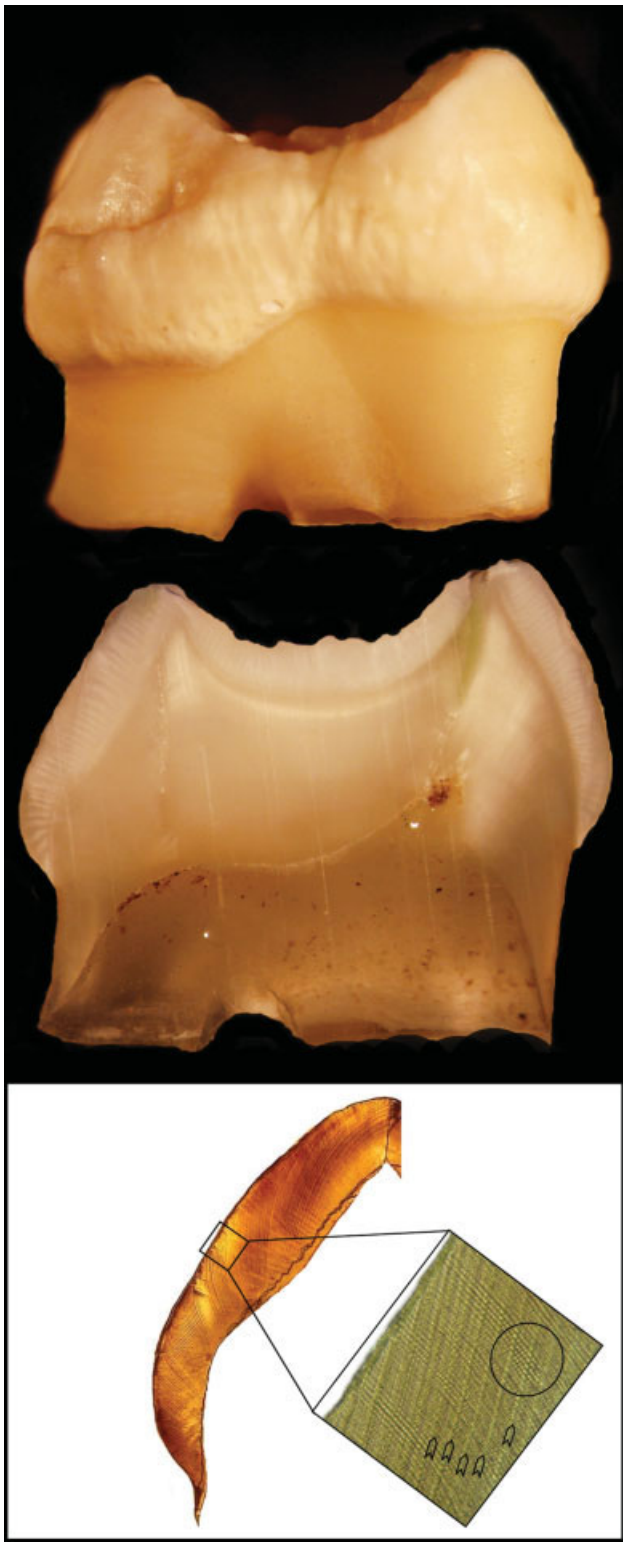

Figure 1. Developing chimpanzee molar illustrating the scale of dental microstructure studies, ranging from the whole crown (top), a plane of section across the mesial cusps (middle), and incremental features in the enamel observed with transmitted light microscopy (bottom). Not to the same scale. (Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.)

samples and analyses of variation within particular tooth types and among teeth of a single taxon.[20–23] These studies demonstrate that certain aspects of dental microstructure, such as the average rate of enamel secretion, are fairly conserved among hominoids, while the duration of secretion and periodicity of long-period lines varies considerably. For example, hominoid primate periodicities can range from 4 to 12 days, with living humans showing the widest-known range, 6 to 12 days.[6] Factors implicated in developmental variation include differences in brain size, body mass, metabolic rate, diet, and life history, defined here as the pace of growth and development, including ages at weaning and first and last reproduction, as well as total life span.[3,6,9,24,25] However, these patterns become more complex when considered within taxa such as humans or across diverse primate taxa,[25,26] necessitating further study of animals with known developmental and physiological variables, such as brain size, body mass, and metabolic rate.

Modern humans are one of the most well-documented primate taxa for which incremental data are available; this is largely due to the work of Reid and colleagues,[27–29] who have recently completed analyses of each tooth type in a relatively large sample of northern European and southern African individuals. These data reveal substantial developmental variation within humans, particularly for anterior teeth and premolars, with southern Africans consistently showing lower numbers of perikymata and shorter formation times. These results underscore the importance of considering intraspecific variation when comparing fossil hominins and modern humans.[21,30,31] Modern human populations also display an inverse correlation between Retzius line number and periodicity within a tooth and cusp type, leading to crown formation times with relatively small standard deviations.[23,32] These findings on modern human development have recently been applied to the interpretation of tooth growth and life history in Neanderthals, contributing to one of the more hotly

Box 1. Glossary of Aspects of Dental Microstructure and Tooth Development

Accentuated line—a pronounced internal line corresponding to the position of the developing enamel or dentine front; it relates to a stressor experienced during tooth development (as opposed to an intrinsic rhythm). The neonatal line found in teeth developing during birth is the most common example. See examples in Figure 2.

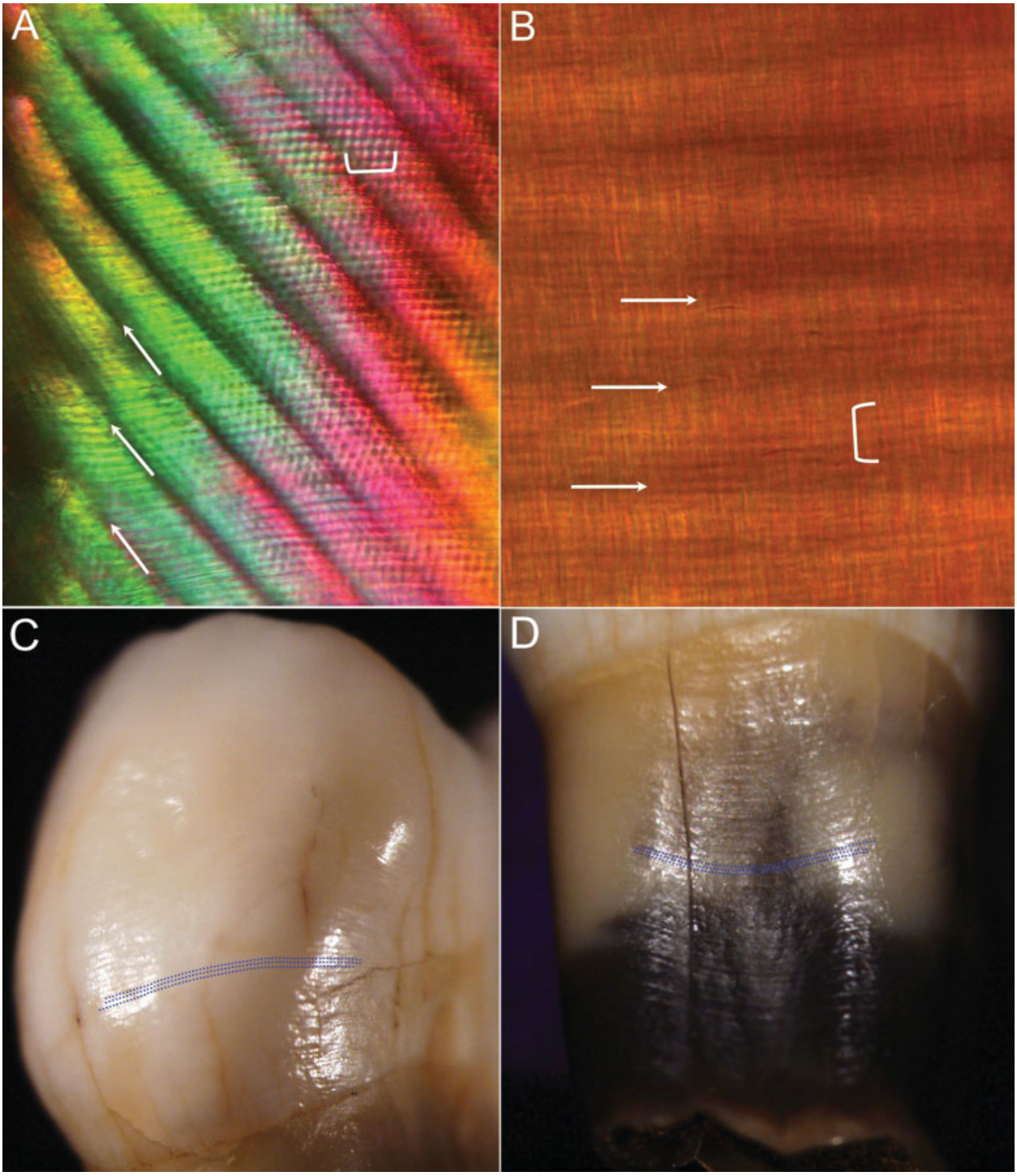

Figure B1. Incremental features in enamel (A,C) and dentine (B,D). Long-period lines are indicated by white arrows (Retzius lines in A, Andresen lines in B) and blue dotted lines (perikymata in C, periradicular bands in D). Groups of four short-period lines are indicated within each white bracket (cross-striations in A, von Ebner's lines in B). Images are not to the same scale.

Andresen lines—long-period (greater than circadian) incremental features in dentine representing the successive positions of the dentine-forming front, and corresponding to periradicular bands on the root surface. They are temporally equivalent to Retzius lines and perikymata in enamel. See examples in Figure B1B.

Cross-striations—short-period incremental features in enamel running at right angles to enamel prisms. Cross-striations represent a 24-hour cycle of ameloblast activity. Thus, the distance between adjacent cross-striations yields the daily rate of enamel secretion. These striations are temporally equivalent to von Ebner's lines in dentine. See examples in Figure B1A.

Crown formation time—the duration of tooth crown secretion, either for a single cusp or the entire crown of multicusped teeth, which is typically assessed through counts and measurements of cross-striations and Retzius lines.

Dental development—the continuous process of tooth initiation, matrix secretion, crown mineralization, dental eruption, and root completion. Primate dental development begins before birth with initiation of the deciduous dentition, followed by initiation of the permanent dentition.

Dental eruption—the process of tooth crown emergence; the tooth must move past the bone margin (alveolar eruption) and the gum (gingival eruption) in order to emerge into the oral cavity and eventually into functional occlusion.

Dentine development—dentine is formed when odontoblasts secrete a collagenous matrix (predentine), which rapidly undergoes mineralization to form primary dentine. Dentine formation begins at the dentine horn underlying the future cusp tip and progresses inward through secretion and downward through extension until it reaches the apex of the root.

Enamel development—enamel is formed when ameloblasts secrete enamel matrix proteins that mineralize into long, thin bundles of hydroxyapatite crystallites known as enamel prisms. As the secretory cells progress outward toward the future tooth surface, additional adjacent cells are activated through extension until the forming front reaches the cervix of the crown.

Hypoplasia—feature formed when hard tissue formation has been disrupted by a stressor experienced during development, which results in an anomalous depression (furrow or pit) on the surface of a tooth. These external features are often associated with accentuated lines internally.

Incremental features—microscopic markings that represent intrinsic temporal rhythms in dental hard tissue secretion. These can be annual (cementum annulations), long-period (for example, Retzius lines in enamel), or short-period (for example, cross-striations in enamel). See examples in Figure B1.

Laminations—daily incremental features in enamel running parallel to the developing enamel front and Retzius lines. Laminations are temporally equivalent to cross-striations but can be distinguished from them by their relatively oblique orientation.

Perikymata—external ridges and troughs encircling the tooth crown that are formed by Retzius lines as they reach the enamel surface. This region of the crown is often called the imbricational enamel. See examples in Figure B1C.

Periodicity—the number of days between long-period lines (Retzius lines or perikymata in enamel, Andresen lines or periradicular bands in dentine). Periodicity typically is assessed by counting daily cross-striations between pairs of Retzius lines. The value for long-period line periodicity is consistent within all teeth in an individual's dentition, but may vary among individuals in a taxon.

Periradicular bands—external ridges and troughs encircling the tooth root that are temporally equivalent to the Andresen lines in the dentine (in addition to the long-period lines in enamel). See examples in Figure B1D.

Retzius lines—long-period incremental features in enamel that represent the position of the developing enamel front at successive points in time. They are manifest on the tooth surface as perikymata and are analogous to the Andresen lines in dentine. The temporal repeat interval of Retzius lines is known as their periodicity. See examples in Figure B1A.

von Ebner's lines—short-period incremental features in dentine that reflect a 24-hour cycle of dentine secretion and are temporally equivalent to cross-striations and laminations in enamel. See examples in Figure B1B.

For additional information and illustrations, see Dean,[3] FitzGerald,[4] Smith,[5,6] Bromage,[7] Tafforeau and colleagues,[8] Dean and Scandrett,[9] and Smith and colleagues.[10]

debated topics in dental tissue research.

Hominoid and Hominin Life History

Dental microstructure analysis has frequently been employed in studies of the evolution of hominoid and hominin life histories.[6,33] A key advantage of using incremental features is their ability to yield chronological age estimates of specific developmental stages, such as first molar eruption, which are correlated with other developmental variables.[10,25,33,34] These studies suggest that, relative to other catarrhine primates, a prolonged hominoid life-history pattern may have arisen in the early Miocene, as shown by long crown formation times in *Proconsul nyanzae* and *Afropithecus turkanensis*, slow rates of root extension in *P. nyanzae*, and a chimpanzee-like age at first molar eruption in *A. turkanensis*.[33–36] However, determination of an ancestral great ape pattern has been complicated by recent documentation of substantial variation in extant great ape crown formation times and molar eruption ages,[11,22,37] as well as life-history parameters.[38] Additional data on developmental parameters in other early and middle Miocene taxa, as well as in addi-

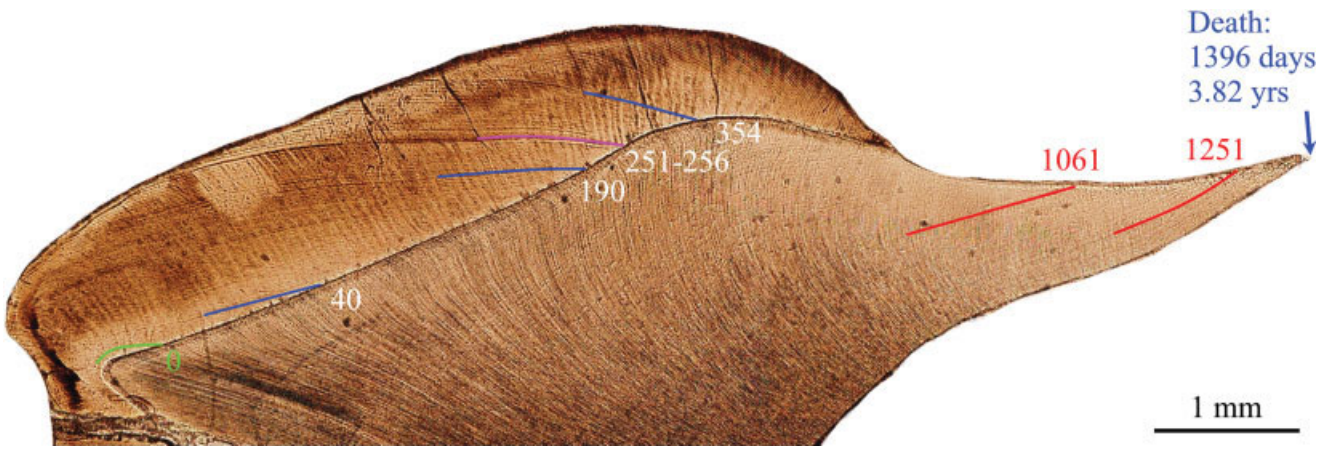

Figure 2. Illustration of histological reconstruction of an individual chimpanzee's developmental history from birth (green line on left) to death (blue arrow on right). The colored lines indicate unspecified postnatal developmental stress, with the corresponding age in days given. The age at death determined from field notes was 1,372 days, or 3.76 years, suggesting that histologically derived times of stress events are likely to be less than 2% different from the actual timing.

tional extant wild individuals, would help in further clarifying the evolution of great ape life histories.

Dental microstructure studies of fossil hominins have almost completely replaced determinations of dental eruption sequences or comparisons of relative degrees of development. Dental eruption sequences are known to be polymorphic within taxa,[39] while comparisons of the degree of dental development requires an assumption of developmental time or chronological age, leading to circular reasoning.[40] Bromage and Dean[41] first applied dental microstructure analysis to estimate the age at first molar emergence in several juvenile Plio-Pleistocene hominins, which appeared to be several years younger than modern human children at a similar stage of dental development. This and several subsequent studies have led to a general consensus that the prolonged childhood period and slow life history that are characteristic of living humans originated fairly recently, most likely within later members of the genus *Homo*.[6,33]

Unfortunately, little is known about incremental tooth growth in Middle Pleistocene hominins, save for counts of perikymata on anterior teeth of *Homo antecessor* and *Homo heidelbergensis*.[42] As reviewed by Smith and colleagues[10] and Guatelli-Steinberg,[101] data on Neanderthal tooth growth are conflicting. For example, counts of perikymata have been interpreted as suggesting rapid development relative to recent humans[42] or as indicating substantial overlap between Neanderthals and modern humans.[29,31] However, since perikymata numbers do not directly yield formation times, information from internal features is critical to determine precise crown formation and eruption ages. Recent histological studies of Neanderthal permanent molar formation illustrate similarities in certain aspects of internal development, such as daily rates of enamel secretion,[43] but differences in cuspal enamel thickness and rates of crown extension, leading to shorter molar formation times.[10] Building on these differences, a study of the developmental status of the nearly complete dentition of a juvenile Belgian Neanderthal suggested that Neanderthals did not share the prolonged life history of living and fossil *Homo sapiens*.[10] Exactly where and when this transition took place within the genus *Homo* remains unresolved.

Virtual Histology

A promising new area of incremental feature research is the application of virtual histology via propagation-phase contrast X-ray synchrotron microtomography,[44,45] which has greatly benefited from recent advances in technology. A synchrotron is a machine that produces beams of light from accelerated electrons deviated by magnetic fields. Depending on the energy, the light spectrum may range from radio frequencies to high-energy X-rays (hard X-rays). This type of light source facilitates more efficient and diverse imaging possibilities than do conventional laboratory X-ray sources.[45] This is a consequence of specific physical properties such as high flux, monochromaticity, parallel geometry, and spatial coherence (defined in Box 2). The first three of these special properties produce high-quality absorption scans with gray levels that can be used to quantify mineral densities.[8]

A unique and critical aspect of synchrotron imaging for dental tissue research is based on phase-contrast techniques, which reveal subtle variations in tissue structure that are invisible with other absorption contrast imaging techniques such as radiography or conventional microtomography. Over the past few years, improvements in the microtomography beamline ID19 at the European Synchrotron Radiation Facility in Grenoble, France, have permitted rapid imaging of microstructure in fully mineralized dental tissues at submicron resolution.[8,44–47] This nondestructive technique yields images of incremental

Box 2. Glossary of X-ray and Synchrotron Imaging Terms

Absorption—the amount of incoming X-rays stopped by a material for a given thickness (expressed in cm^{-1}). The different tissues of a tooth have different absorptions, largely depending on their density and chemical composition.

Beam geometry—the shape of the x-ray beam, which affects the image resolution and the way that cross-sectional slices are reconstructed from radiographs. Most conventional microtomographs are based on cone beam geometry, as the beam diverges from a microfocus X-ray source. The resolution limit is determined by the source size. Changing the position of the sample in the cone modifies the magnification factor of the radiographs. Synchrotron beams have such small divergence that they are considered to be parallel. The resolution is limited only by the detector. In parallel geometry, the algorithms used for slice reconstruction are exact, in contrast to the approximated algorithms used for cone beam reconstruction.

Beam hardening—differential absorption of the beam by the sample leading to artifacts in reconstructed slices, such as brightening of the tissue borders and false bridging of dense structures. Beam hardening occurs when the beam leaving the sample has relatively more hard X-rays than does the beam entering the sample. When micro-tomography is performed using a white X-ray beam (polychromatic continuous X-ray spectrum), the low energies of the beam are absorbed by the samples more than the high energies (the hard X-rays); this increases with sample thickness and density.

Beamline—experimental station where experiments are conducted, which uses the very narrow conical beam of synchrotron light generated at each position where accelerated electrons are deviated by bending magnets or insertion devices (periodic magnetic devices that cause electrons to undulate). A large synchrotron can have more than 40 beamlines devoted to diverse techniques.

Coherence—a physical property of synchrotron radiation related to X-ray wave geometry, which facilitates phase contrast imaging. Third-generation synchrotron radiation facilities exhibit small ($<$100 μm) X-ray source sizes and long source-to-sample distances (in the 50–150 m range). In such conditions, the spherical X-ray waves originating from the nearly punctual source can be considered, at the level of the sample, as quasi-plane waves, with an extended lateral coherence. Structures imaged with this coherent light produce interferences when the beam propagates, leading to the propagation phase contrast effect.

Flux—essentially, the number of photons for a given surface during a given time. Synchrotrons have far higher flux than do conventional sources (up to 10^{14} times). In addition to rapid scans and very high data quality, high flux allows the use of monochromators.

Monochromaticity—selection of only a narrow part of the incoming white X-ray (polychromatic) beam spectrum produced by the source by using a monochromator. A monochromatic beam produces images without beam hardening artifacts and allows quantitative imaging.

Propagation phase contrast—imaging technique based on the detection of interference patterns generated by interfaces in the sample, seen when imaged with a partially coherent X-ray beam. Increasing the sample-to-detector distance increases the phase contrast effect. This technique often reveals structures that are invisible with absorption contrast.

Resolution and voxel size—resolution is the size of the smallest detail that can be detected with a given setup (see "beam geometry"). Voxel size corresponds to the size of each elemental component of the 3D volume. It is related to the number of pixels of the detector and the magnification factor (in the case of conical or fan beam geometry). For an equivalent voxel size, the resolution would be better with a synchrotron than with a conventional microtomograph due to the beam geometry.

Tomography—computerized reconstruction of virtual slices through an object from a set of radiographs taken during rotation of the sample.

For additional information, see Tafforeau and colleagues.[45]

features in tooth enamel and dentine, as well as the neonatal line, which can be seen in highly mineralized fossils that are millions of years old.[44] Highly precise assessments of tooth formation are now possible though the nondestructive virtual determination of long-period line periodicity. In combination with virtual data on cuspal enamel thickness and the developmental status of unerupted teeth, this approach can be used to characterize dental development and age at death in material that is unavailable for traditional physical sectioning.[47] It may also be used to provide novel internal information from previously studied fossils. Beyond revealing developmental information in fossilized dentitions nondestructively, it is hoped that virtual histology will eventually facilitate precise understanding of the three-dimensional growth of a tooth at the cellular level.

DEVELOPMENTAL TIME AND TOOTH CHEMISTRY

Studies of tooth chemistry, particularly the proportions of specific elements such as strontium and calcium or ratios of elemental isotopes such as $^{13}C/^{12}C$, $^{18}O/^{16}O$, and

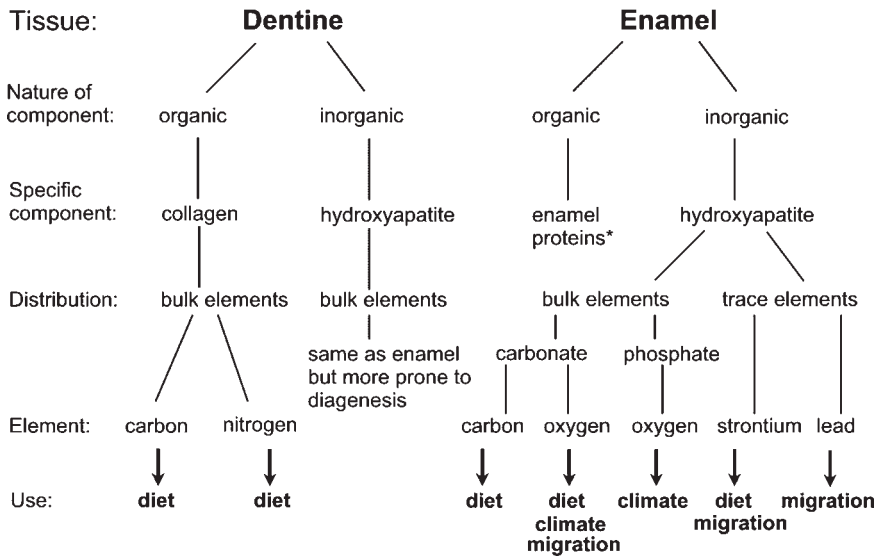

Figure 3. Stable isotopes studied in dental tissues (reviewed in Mays[49]). *Enamel proteins may one day yield isotopic data from individual amino acids.[48]

$^{87}Sr/^{86}Sr$, have expanded over the past decade to integrate information on the pattern and duration of dental development. Initial analyses required bulk samples taken from a large proportion of the tooth crown or root to infer an organism's diet geographic location, or climate of the region in which the individual lived during tooth formation[48,49] (Fig. 3). In contrast to bone, dental tissues mineralize over a relatively short time without subsequent remodeling,[50] providing a narrower window of recorded time. Isotopic studies of dental tissues may provide information about young individuals; once the tooth roots are complete, relatively little tissue is added later in life (in the form of small amounts of secondary/tertiary dentine and cementum).

Variation Within Individuals

Several recent studies have used information on tooth growth to look at migration patterns and dietary changes in human archeological contexts.[51] Sealy, Armstrong, and Schrire[52] examined skeletal and dental samples from five unknown individuals, including pairs of earlier- and later-forming teeth, and concluded that differences in carbon, nitrogen, and strontium isotope ratios revealed dietary shifts and migration events. Wright and Schwarcz[18] examined changes in carbon and oxygen isotopes across several teeth of the same individuals spanning the time from birth to adolescence. Their results suggested that infant diets in their archeological population were supplemented with solid food by two years of age, although breastfeeding appeared to continue for an extended period. Building on this bulk sampling approach, Fuller, Richards, and Mays[19] used the internal spatial pattern of tooth formation to track changes in nitrogen and carbon isotopes in serial sections of successively forming tooth crowns and roots, demonstrating differences between earlier- and later-formed regions within a tooth, which were interpreted as pre- and postweaning dietary signals.

Most recently, Humphrey and colleagues[16,102] used laser ablation inductively coupled plasma mass spectrometry to sample strontium/calcium ratios of pre- and postnatal enamel in histological thin sections of teeth from modern humans. This technique involves focusing a thin laser beam on a small area of a tooth surface or thin section that transforms (ablates) the solid into a plasma, which is then analyzed with a mass spectrometer to yield the elemental composition. The results of this study demonstrated that decreasing strontium/calcium ratios could be tracked through time from the inner to the outer enamel, revealing dietary shifts associated with birth and the beginning of the postnatal diet. Laser ablation is a promising new approach, as certain trace elements[16,17,53] and carbon within carbonate[54] can be detected in microscopic samples and related to incremental features. However, this technique is restricted to measurements of a limited number of elements and is typically performed on inorganic material.

The Problem of Enamel Mineralization

Isotopic studies of dental enamel have recently underscored the importance of considering the complex tooth mineralization process for accurate temporal interpretation of chemical signatures.[8,16,46,51,55–58] Mineralization proceeds in a series of stages as apatite crystallites grow in length and thickness, with the majority of mineral incorporation occurring some time after the end of secretion.[8,16,55] Isotopic studies of intratooth variation often sample enamel serially from one end of the crown to the other, assuming a rate of constant growth and mineralization, which allows isotopic values to be regressed against sampling position as a proxy for a specific point in developmental time (see, for example, Fig. 3, p. 208, in Balasse and colleagues[59]). However, the final mineralization process does not follow the pattern of matrix secretion, which is represented by incremental features.[8,60,61] This is particularly critical for studies that attempt to relate isotopic information to local incremental features.[8,16,17,54] It is still unclear when particular elements are incorporated into enamel, and thus if the recovered signal has been "dampened" (shifted in time or modified in degree) by subsequent mineralization during maturation.[16,51] Further complicating the situation, many studies of tooth mineralization

have been performed on nonprimate mammals,[8,55,57–59] and thus may not clarify how primate teeth mature, since tooth mineralization and maturation processes vary among mammals.[50,62]

Passey and Cerling[55] attempted to model the dampening effect that subsequent mineralization may have on the original "input signal" recorded as a tooth develops. By measuring phosphorous, an indicator of hydroxyapatite content, they found that tooth mineralization occurred in a spatially diffuse pattern that differed among teeth and continued after matrix secretion was finished. Based on this information, they proposed a model to predict the original input signal from knowledge of the "mature (measured) signal" and the process of amelogenesis for teeth growing at a constant rate throughout the life of the animal, with the aim of interpreting temporal information present in the pattern of chemical variation. However, this model appears to be of limited utility for hominoid teeth, as rates of enamel secretion and extension vary from the beginning to the end of crown formation (reviewed in Smith[6]). Additional study is of critical importance to document the timing and relative degree of incorporation of particular elements in primate tooth enamel.

Tafforeau and colleagues[8] applied X-ray synchrotron microtomography to the study of tooth mineralization, finding that rhinoceros enamel shows a 20-micron thick zone near the enamel-dentine junction that appears to mineralize rapidly after secretion. This suggests that isotopic analyses of intratooth variation may recover a near-primary signal from sampling along this zone (also see Balasse[56] and Zazzo, Balasse, and Patterson[58]). Even when the spatial pattern of mineralization is taken into account, it is unlikely that a single isotopic sample will represent a discontinuous or instantaneous point in time because some degree of time averaging will occur during mineralization.[8,56,57] However, a recent study of strontium/calcium ratios in human molar enamel suggests that the effect of time averaging may be minor for particular elements.[16] This study demonstrated a consistent change in ratios sampled before the neonatal (birth) line and after this line, suggesting that physiological differences in strontium discrimination were recorded at the time of matrix secretion and were not lost during subsequent mineralization. Humphrey and colleagues[17] also recently applied laser ablation to developmentally overlapping teeth in two baboon individuals and found some correspondence in ratio changes between simultaneously forming enamel (between teeth). This provides additional evidence that subsequent mineralization does not completely dampen the original (biogenetic) signal.

Fossil Hominins and Tooth Diagenesis

Isotopic analyses of fossil hominin dental tissues have primarily centered on elucidating southern African Plio-Pleistocene hominin diets[54,63,64] and potential environmental shifts,[65] and these studies are beginning to provide insight into hominin mobility.[53] Dental tissues are of particular importance for older time periods because little organic material (collagen) remains in fossil bone and diagenetic processes are highly likely to have modified the inorganic component.[66] Although most dietary studies have used bulk sampling, a recent approach by Sponheimer and colleagues[54] related sequential isotopic laser ablation samples from the tooth surface to counts of perikymata in four *Paranthropus* teeth. Their results showed a high degree of variation within and between teeth. This, based on estimates of the time represented by perikymata, was interpreted as seasonal and interannual variation, implying that *Paranthropus* was not a dietary specialist. Less research has been done on hominin migration. Richards and colleagues[53] recently presented isotopic evidence of Neanderthal mobility based on strontium isotopes in dental enamel. Differences in the strontium detected in the internal tooth enamel relative to the local geology were used to suggest that the individual had migrated from a region at least 20 kilometers away.

An advantage of using tooth enamel for isotopic studies is its greater resistance than bone to diagenetic change.[67,68] Diagenesis, or the process of chemical change after death (including fossilization) is highly variable and still not completely understood in hard tissues.[51,54,66] However, diagenesis can strongly affect the assessment of tooth chemistry in archeological or fully fossilized tissues. Because enamel is approximately 95% mineralized by the end of formation, in contrast to dentine or bone at approximately 70%–75%, the chemical composition of mature enamel is believed to provide a more faithful record of the original mineralization process. Furthermore, given the highly inorganic composition of enamel, it has been suggested to approximate a "closed system" that is more impermeable to alteration from the postmortem burial environment (but see evidence of enamel diagenesis below and in Wang and Cerling,[68] Kohn, Schoeninger, and Barker,[69] and Schoeninger and colleagues[70]). However, one limitation of inorganic component analyses is the lack of internal verification of results; in certain cases it is difficult to exclude environmental sources of particular elements that may bias results.

In a study of modern and fossil southern African fauna, Sponheimer and colleagues[64] argued that similarity in strontium/calcium ratios between modern and fossil animals demonstrated that diagenesis was unlikely to have a major impact on these ratios in their sample of fossilized dental enamel. However, others have abandoned the use of trace-element ratios such as that of strontium to calcium, in part because it is difficult to detect when elements have leeched in or out of a tooth or bone.[71,72] Because even slight amounts of particular elements or isotopes will be detected in most cases, a ratio is always obtained, but it is often quite difficult to verify that it represents the original signal. Hydroxyapatite, the major mineralized component of enamel, dentine, and bone,

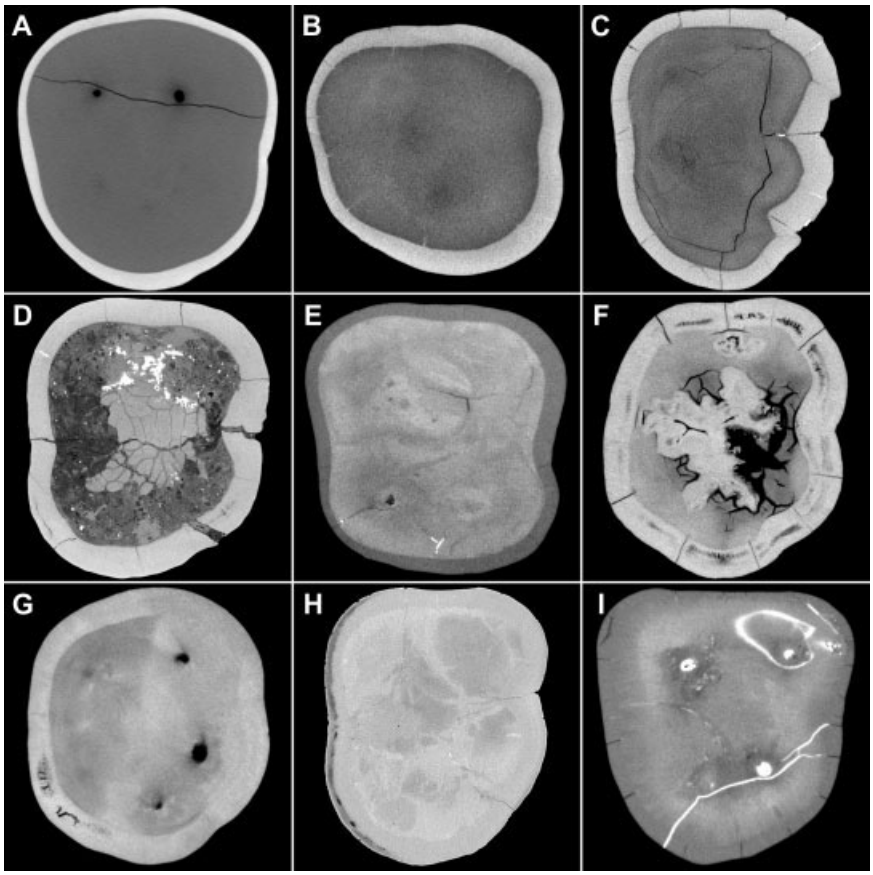

Figure 4. Examples of diagenetic patterns in fossil primate teeth imaged using monochromatic absorption synchrotron micro-CT (Box 2). A) Modern human tooth showing normal (homogenous) tissue contrast; enamel is white and dentine is gray. B) Pleistocene hominin tooth with good preservation; only slight heterogeneity is apparent within each tissue. C) Miocene hominoid tooth with similar preservation. D) Miocene hominoid tooth from the same site as C with good preservation of enamel but poor preservation of dentine. E) Eocene primate with inverse contrast between tissues. F) Pleistocene hominin tooth showing very strong alteration of enamel and dentine. G) Same individual as F (adjacent tooth) showing a complex gradient from moderate tissue contrast (top left) to no contrast (top right). H) Miocene hominoid with little to no tissue contrast and enamel demineralization (left). I) Eocene primate with no tissue contrast and infilling of metallic oxides (bright cracks). Patterns A through C would facilitate full 3D segmentation of enamel and dentine (semi-automatic discrimination of corresponding pixel values). Pattern D would permit only enamel segmentation. Patterns E and F would permit only measurements on virtual slices or fully manual slice-by-slice 3D segmentation. Pattern G would permit only partial assessments of enamel thickness. Patterns H and I prohibit accurate identification of internal dental tissue structure (position of the enamel-dentine junction).

can become unstable after death and may undergo chemical change (for example, ionic substitution, such as Ca^{2+} by Sr^{2+}) with or without fundamental structural change.[66,73] Furthermore, as Humphrey, Dean, and Jeffries[16] note, subsurface enamel is known to exchange mineral ions such as fluoride[74] with the oral environment. This is a potential complication for isotopic studies that attempt to use incremental features on the tooth surface to infer the timing of elemental incorporation.[54]

In summary, new isotopic approaches concerned with identifying dietary, environmental, and geographic transitions within the period of individual tooth formation or the development of the entire dentition may allow insight into the timing of weaning, the seasonality of particular environments, and behavioral adaptations. While evidence derived from small samples of living humans and baboons supports the utility of isotopic ratios such as strontium/calcium in enamel for identifying dietary transitions, additional study is needed to validate the utility of this approach for archeological or fossil material. Additional experimental work on the timing of elemental incorporation and the effects of mineralization would be particularly beneficial. For example, comparisons of simultaneously forming regions of the enamel within a tooth (that is, along the plane of a Retzius line) could be illustrative of the mineralization process in different regions of the tooth crown. Additional comparisons of temporal signals in slow-mineralizing enamel and fast-mineralizing dentine may allow better interpretation of previous analyses. Although dentine often is not ideal for studies of archeological and fossil material due to diagenesis and does undergo slight changes in mineralization through sclerosis, it could be used to cross-check the fidelity of elemental incorporation in the enamel of extant taxa.[75] Future studies of primate dental tissue chemistry may also benefit from the development of additional screening procedures, such as virtual sectioning, quantitative mineralization mapping, and refined chemical tests. These procedures could reveal changes in mineral density or composition since diagenetic patterns can vary dramatically within and between sites, as well as within a dentition or even within an individual tooth (Fig. 4).

THREE-DIMENSIONAL ANALYSES OF TOOTH STRUCTURE

The recent development and increasing availability of nondestructive high-resolution micro-computed tomography (micro-CT), based either on conventional or synchrotron X-ray sources, has facilitated evolutionary and clinical studies of internal tooth structure and dental tissue volumes (reviewed in Tafforeau and Smith,[44] Hayakawa and colleagues,[76] Olejniczak, Grine, and Martin[77] and Olejniczak, Tafforeau, and Feeney[78]). Micro-CT imaging is an improve-

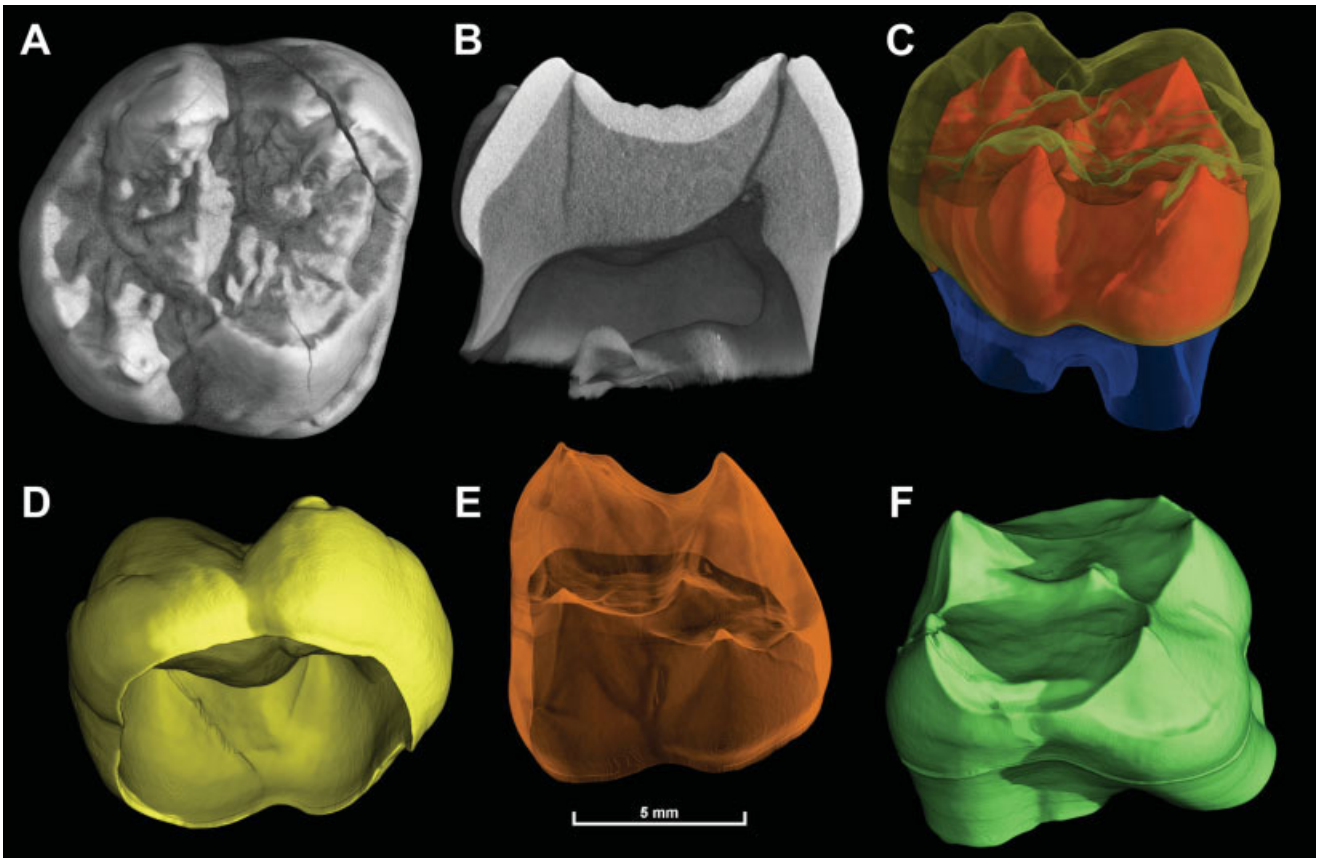

Figure 5. Developing chimpanzee tooth (shown in Figs. 1 and 2) imaged with an isotropic voxel size of 13.8 μm using a conventional micro-CT before sectioning. A) 3D rendering of the occlusal surface. B) Virtual vertical cut through the mesial cusps. C) 3D model of the tooth after segmentation showing the enamel cap (transparent yellow), EDJ (red), and dentine (transparent blue). D) Isolated enamel cap used for volume measurement (178.6 mm^3). E) EDJ used for surface area measurement (188 mm^2). F) Dentine used for volume measurement (293.3 mm^3). The volume of dentine and pulp in the enamel cap delimited by the basal plane (following Olejniczak and colleagues[86]) is 239 mm^3, yielding a 3D average enamel thickness of 0.95 mm and a 3D relative enamel thickness of 15.31 (following Tafforeau,[46] Olejniczak and colleagues,[78] and Kono.[84])

ment over medical CT imaging because fine anatomical details can be resolved (Fig. 5) and accurate metric assessment can be done on relatively small dental samples.[46,77,79] This is particularly important for studies of enamel thickness, given the limitations of conventional radiography or tomography,[79] as well as limitations in physically sectioning invaluable fossil material. Aspects of microscopic dental tissue structure can also be investigated nondestructively with high precision; this is one of the main features of synchrotron imaging. Despite these advantages, more commonly available conventional microtomographs may yield absorption data quite similar to synchrotron data for voxel sizes between 5 and 50 microns. For standard absorption imaging of well-preserved teeth (for example, cases A to C in Figure 4), there is little advantage in using a synchrotron. However, propagation phase-contrast X-ray synchrotron microtomography often reveals the enamel-dentine junction in strongly modified teeth even when there is little or no absorption contrast.[45,46] This is a particularly valuable tool for imaging diagenetically altered fossil teeth, especially tissue structure in older hominins and earlier primate fossils.

Tooth Crown and Root Structure

Most anthropological applications of micro-CT techniques have examined internal tooth structure from virtual two-dimensional planes of sections.[46,80–83] Reiko Kono's landmark study[84] firmly established the utility of micro-CT for assessment of three-dimensional (3D) molar enamel thickness and enamel distribution. Three-dimensional enamel thickness data are now available for a broad sample of extant primates,[46,78,84] the fossil ape *Gigantopithecus blacki*,[85] Neanderthals,[43,86] australopiths,[103] and Middle Stone Age *Homo sapiens*.[87] New 3D data have confirmed broad trends in primate enamel thickness derived from earlier two-dimensional studies[78] and have also demonstrated that thick molar enamel is not found in all fossil hominin taxa.[86] Future applications of micro-CT are needed to clarify phylogenetic or functional aspects of hominin enamel thickness, particularly given the diverse range of measurement techniques employed in previous studies. In particular, such data

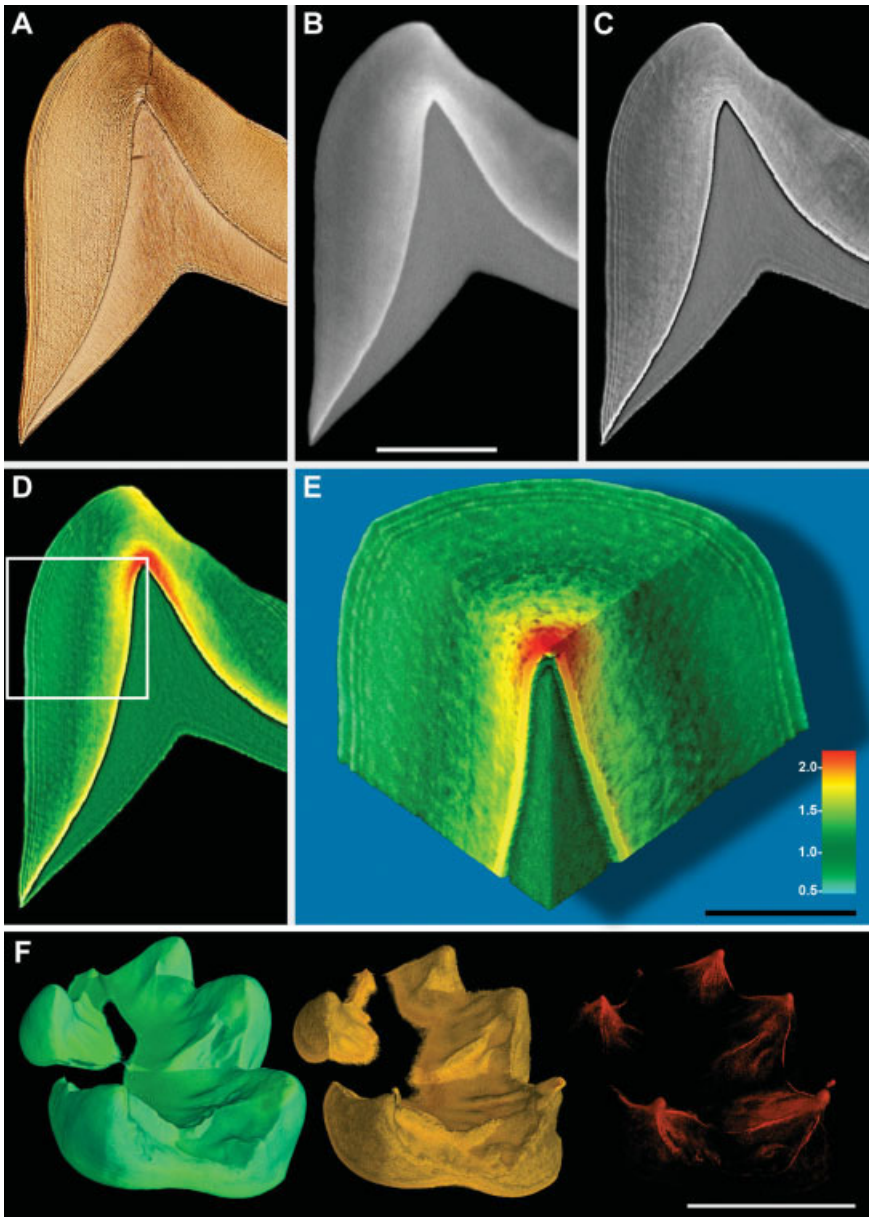

Figure 6. Chimpanzee tooth germ showing integration of multiple technical approaches. Developing chimpanzee cusp after (A) and before (B–F) physical sectioning. A) Light micrograph of 100-μm-thick histological section. B) Synchrotron microtomographic virtual 50.6-μm thick section (derived from 3D data with an isotropic voxel size of 5.06 μm) imaged in monochromatic absorption mode, showing the quantitative degree of mineralization. C) Virtual 50.3-μm thick section (derived from 3D data with an isotropic voxel size of 5.03 μm) imaged with propagation phase contrast mode, illustrating incremental lines in the enamel and dentine. D) Virtual color-coded absorption and phase contrast composite; area enlarged in E) indicated in white box. E) Three-dimensional model showing enamel mineralization and developmental pattern. Scale indicates quantitative mineral density in hydroxyapatite g/cm^3. F) 3D rendering of isodensity segments extracted from the absorption scan data showing the 3D mineralization pattern of the enamel. Left, densities from 0.5 to 1.3; middle, densities from 1.3 to 1.8; right, densities from 1.8 to 2.2. Scale bar in B) is 1 mm, 0.5 mm for E), and 10 mm for F).

are lacking for early and middle Pleistocene *Homo*.

Another recent application of computed tomography is the characterization of primate tooth roots. Wood, Abbott, and Uytterschaut[88] originally demonstrated the taxonomic value of hominin mandibular postcanine root number, size, and morphology using physical specimens and radiography. Nondestructive micro-CT imaging can now be applied to visualize tooth roots *in situ*, permitting identification of postcanine root number, shape, and size (linear dimensions, surface area, volume) in primate fossil material.[46,81,89,90] This approach may serve in further investigations of reported differences in root lengths between Neanderthals and contemporaneous modern humans.[91] Tomographic studies of living human and primate root size have also demonstrated a correlation with functional demands.[90,92] Kupczik and Dean[90] recently used computed tomography to document the relatively large postcanine root surface areas of *G. blacki*, which they suggest may have been required to sustain large forces during mastication of highly resistant food items such as bamboo. Experimental and descriptive microtomographic studies are just now beginning to investigate phylogenetic, taxonomic, and functional aspects of primate tooth roots.

Enamel-Dentine Junction Shape

Studies of dental morphology have long regarded the internal interface between coronal dental tissues, the enamel-dentine junction (EDJ), as a highly conserved and developmentally informative character. This interface represents the initial position of the cells that are responsible for enamel and dentine secretion and is believed to be heavily influenced by embryological enamel knots that form before hard-tissue secretion (reviewed in Avishai and colleagues[93] and Skinner and colleagues[94]). Olejniczak and colleagues,[85,95–97] have demonstrated that the primate EDJ has a taxon-specific shape, despite some intra-taxon variation, and that components of this shape, such as relative dentine horn height, may serve to group members of the Ponginae. Before the recent application of micro-CT (Fig. 5), EDJ shape was assessed from impressions of enamel caps,[98] acid etching of complete teeth,[99] or physical sections.[95,96]

Skinner and colleagues[94] recently considered the EDJ from a nondes-

tructive 3D perspective, demonstrating that dental traits commonly identified on the outer enamel surface may result from different EDJ configurations. It appears that in some cases these traditional "discrete traits" may not be developmentally homologous.[94] This study also demonstrated another potential advantage of micro-CT studies: Teeth that show marked attrition that obscures or obliterates external morphology can still be included in analyses of EDJ shape, underscoring the value of nondestructive studies of internal tooth structure. Similar research on external dental tissue structure may one day allow worn teeth to be rebuilt volumetrically to better understand their original form, as well as the complex process of tooth attrition.

A WAY FORWARD: INTEGRATION OF TEMPORAL, CHEMICAL, AND STRUCTURAL INFORMATION

One challenge of having new tools is learning how to best apply them. It is clear that decades of studies on tooth development, chemistry, and structure have provided valuable insight into the evolution of primate life history, paleodiets, and taxonomy. Future studies of dental tissues will undoubtedly benefit from increased technological efficiency in chemical sampling, tomographic scanning resolution and sensitivity, and increased computing power for data processing. We suggest that additional advancements may be found through the synergy of these seemingly disparate research areas. For example, studies of tooth chemistry may be better informed by complementary applications of nondestructive microtomography to characterize mineral density and the duration of mineralization[8] (Fig. 6). Furthermore, absorption micro-CT imaging of teeth before sampling may yield information about internal diagenetic modification, leading to more efficient selection of material for chemical analyses. Similarly, the integration of incremental development, maturation patterns, and isotopic sampling strategies may facilitate more precise assessments of dietary or environmental change while providing the potential for identifying life history events such as birth or weaning.[8,16,17]

Other examples of this synergistic approach include studies of hominin dental development that have used micro-CT imaging to characterize aspects of internal structure or the developmental status of unerupted teeth *in situ*.[10,47,87] Structural features of developmental significance, such as the thickness of cuspal enamel, the length of the EDJ, and the length of tooth roots, can be accurately characterized nondestructively. Data obtained from conventional or virtual histological investigations can then be used to estimate the duration of formation along these virtual growth axes, leading to more precise estimations of tooth growth and age at death in rare fossil juveniles.

A final example of research integration is the quantification of volumetric growth rates, which can be calculated as overall crown averages using knowledge of incremental development and 3D dental tissue metrics. For example, a chimpanzee first molar tooth was subjected to micro-CT scanning before histological sectioning and analysis (Figs. 1, 2, 4), yielding the volume of enamel (178.6 mm^3) and dentine (293.3 mm^3) and the maximum formation time of each tissue (722 and 1427 days, respectively). This information was used to estimate minimum average volumetric crown growth rates (247.4 μm^3/day for enamel and 205.5 μm^3/day for dentine), along with the minimum average coronal extension rate (260.4 μm^2/day, using a measured EDJ surface area of 188 mm^2). Volumetric rates of primate tooth growth can be compared to rates of brain growth[100] or other aspects of somatic development, as well as to characterize volumetric increases in the formation of the deciduous dentition or the successive formation of the permanent dentition. This analysis can also be extended to volumetric quantification at the microstructural level with propagation phase contrast X-ray synchrotron microtomography, as prisms may be virtually extracted in 3D and the rate of secretion determined along the prism length from the cross-striation spacing. These examples of research integration represent a few promising means to advance our understanding of primate developmental biology, and to initiate the formulation of more refined areas of dental tissue research over the coming years.

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