

ENAMEL MICROSTRUCTURAL SPECIALIZATION IN THE CANINE OF THE SPOTTED HYENA, *CROCUTA CROCUTA*

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Abstract

High stresses that occur in hyena teeth during bone fracturing, together with evidence for extensive microstructural evolution in the enamel of other groups of mammals, predict a high degree of microstructural reorganization in hyena enamel. This study examines the enamel in the canine because the microstructure varies regionally within this tooth, suggesting a causal relationship between variations in stresses and microstructure. Examination of the canine enamel in the spotted hyena (*Crocota crocuta*) by reflected light microscopy and scanning electron microscopy shows a great intensification of folding of Hunter-Schreger bands (HSB; layers of decussating prisms) in the anterolabial enamel of the tooth in contrast with that in other regions. Finite element modeling of the static stresses in the canine under tip loads indicates that the highest tensile stresses occur in the anterolabial enamel and are directed cervico-occlusally there. Premortem cracks are most extensive in the anterolabial enamel and are aligned transversely, confirming the validity of the modeling results. The increased amplitude of vertical folding of the HSB in the anterolabial enamel increases its resistance to fracture under the vertical tensile stresses that are localized in that area. However, the deepest portion of the enamel in this region has HSB with almost no folding, so that the enamel has strong resistance to horizontal tensile stresses as well. The absence of highly folded HSB in any region of the canine of the cougar (*Puma concolor*), in which the stress directions are similar, indicates the evolutionary modifications in *C. crocuta* are related in particular to the high stress intensity in that taxon.

Key Words: Enamel microstructure, dental strength, fracture resistance, stresses, prism decussation, durophagy, Mammalia, Hyaenidae, spotted hyena, *Crocota crocuta*, Felidae, cougar, *Puma concolor*.

Introduction

Bone-eating carnivores may develop the highest dental stresses of any mammal. Among the Carnivora, the Hyaenidae is the best known for the bone-eating habits of most of its living members and the most derived of these is the spotted hyena, *Crocota crocuta* (Werdelin and Solounias, 1991). Savage (1955) reported an experiment in which he fed horse humeri to the spotted hyena and the brown bear (*Ursus arctos*); the bear tried valiantly to crack the bone but was unable to remove more than a few small flakes, whereas the spotted hyena had no difficulty in fracturing the bone. I recently fed a series of vertebrae of domestic cow to several spotted hyenas in the Berkeley hyena colony; none of these individuals required more than three or four minutes to fracture and consume a vertebra.

Structural features of the cranium in the more derived hyaenids, for example, vaulted foreheads and broad palates, have been interpreted as adaptations to high stresses generated during bone eating (Werdelin, 1989; Werdelin and Solounias, 1991). However, the most conspicuous specializations in hyenas are the shapes of the teeth that are used primarily for bone crushing. The premolars in the hyenas are pyramidal in shape, with an unusually broad base and low profile. Similar, though less extreme dental specializations, in combination with heavy dental wear, have been interpreted as evidence of bone-eating behavior in extinct Carnivora such as the borophagine canids (Werdelin, 1989).

Fracturing bones requires the transfer of high stresses at the point of tooth-to-bone contact. If the stresses exerted by bone eaters are higher than those associated with more strictly flesh-eating Carnivora, one would expect the dental materials to have evolved to improve the resistance of the teeth to fracture. As will be shown, even though the canine is not one of the highly modified bone crushing teeth in *Crocota*, it has enamel that differs substantially from that of more strictly flesh-eating Carnivora and appears to have a critical role in withstanding the stresses associated with durophagy.

Enamel strength

Enamel is the most brittle and easily fractured of the dental materials, having in some mammals as little as one-twentieth the work-to-fracture of dentin (Rasmussen *et al.*, 1976). A low work-to-fracture places a limit on the magnitude of stress that a tooth can transmit to a food object and must be a constraining factor in the types of food that a mammal can fragment and readily digest. The function of cusp-shaped dental surfaces is to focus masticatory force in small areas and thereby elevate the stresses that are transmitted from tooth to food. Insectivorous mammals have small masticatory muscles but have sharp-pointed cusps that focus force and develop adequate stresses to penetrate insect carapaces. However, as mammalian herbivores and carnivores emerged from their insectivorous ancestors in the early Cenozoic, jaw muscles increased in size, greatly elevating the maximum stress that could be generated.

Several dental changes in early Cenozoic mammalian teeth appear to have been responses to increased masticatory forces. For example, many taxa acquired cusps with lower profiles and blunter tips than their Cretaceous insectivorous ancestors (Rensberger, 1988), which limited the increase in dental stresses that otherwise would have resulted from increases in the masticatory force.

Accompanying this change, even in the earliest Paleocene (von Koenigswald *et al.*, 1987), were the first appearances of prism decussation in the enamel, that is, prisms in adjacent layers running in alternate directions. These bands of prisms of alternating directions (Hunter-Schreger bands; HSB) can be seen under low magnification reflected light microscopy because prisms inclined toward the surface absorb light and appear darker, whereas prisms parallel to the surface reflect light and are lighter (Fig. 1a). This structure, which increases the resistance of the enamel to fracture by inhibiting crack growth, loosened the constraints on dental shape and thereby opened avenues to new dietary behaviors that are unavailable to taxa with weaker teeth.

Bone-eating taxa test the limits of dental strength. Even enamel with decussation is brittle and susceptible to fracture under tensile stresses, and dental contact with a rigid object concentrates the full load generated by the masticatory musculature in a very small area. The tests by Rasmussen *et al.* (1976), which showed that enamel fractures under relatively low stresses compared to other vertebrate hard tissues, were conducted on human enamel, which has well-developed prism decussation. If there is some further degree of specialization of enamel structure that is both attainable and more resistant to high stresses than the prism decussation characteristic of many mammals, it might be expected to have evolved in hyenas to enable them to safely attain the high occlusal stresses necessary to crack bones.

Effect of decussation plane attitude

Prism decussation optimally strengthens enamel when the decussation planes (the planes bounding layers of prisms of different orientation) run parallel to the direction in which maximum tensile stresses are acting. In cusps of circular cross-section subjected to vertical (orthal) loads, the maximum tensile stresses in the enamel are aligned horizontally and are optimally resisted by horizontal decussation planes (Pfretzschner, 1988; Rensberger, 1992). This is consistent with the combined occurrence of ellipsoidal cusps and orthal chewing directions in association with horizontal prism decussation in many mammals, including humans.

In contrast to many primitive mammals, rhinocerotoids and astrapotheres (a group of large extinct ungulates) have vertical decussation planes in the molariform teeth (Rensberger and von Koenigswald, 1980; Fortelius, 1985; Boyde and Fortelius, 1986). That is, the HSB are similar in form to those in Figure 1a except that the direction of the bands is rotated 90°, making them vertical. The compressed shapes of the cusps of these cheek teeth, combined with a strong horizontal chewing force component, have resulted in maximum tensile stresses aligned in vertical planes (Rensberger, 1992; Rensberger and Pfretzschner, 1992). The 90° difference in direction of the decussation plane shows that decussation planes can assume radically different attitudes in mammals with different stress regimes.

Although it is apparent that the direction of prism decussation in many mammals is locally related to the direction of maximum tensile stresses, less is known about how enamel microstructure varies in relationship to stress intensity. A threshold in stress intensity may initiate the evolutionary appearance of decussation, judging from the absence of decussation in mammals of small size and small jaw muscles and its presence in almost all larger mammals with the exception of those that have secondarily lost chewing function, such as advanced cetaceans (von Koenigswald *et al.*, 1987). Thus far, no one has successfully measured absolute magnitudes of stress within enamel. However, we can gain some initial information about how enamel structure varies in relation to stress intensity by comparing structures in mammals that are predicted to have low stresses with taxa predicted to have high stresses. In a recent study of the primitive perissodactyl *Hyracotherium*, intensity of prism decussation was found to vary regionally within an individual molar in relation to predicted variations in tensile stress magnitude based on finite element modeling (Rensberger, 1993). *Hyracotherium* is a primitive perissodactyl and has only modest development of prism decussation; its dental stresses may be in the lower region of the stress magnitude spectrum among mammals having prism decussation. The present study examines the enamel

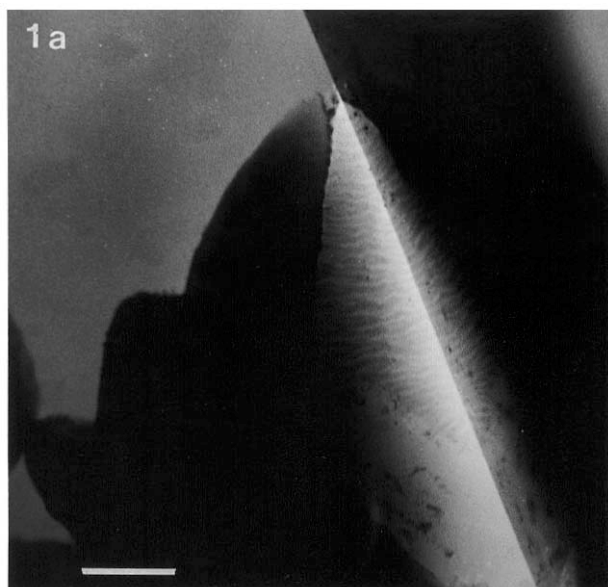


Figure 1a. Hunter-Schreger bands typical of carnivoran enamel under reflected light. Anterior surface of P₃ paracone in domestic cat, occlusal direction toward top; black diagonal bar at right is strip of exposed film blocking light source (see text); HSB visible in enamel in bright area at left of strip and mirrored on film near edge. Vertical crack reflects light from left half of tooth. Bar = 1 mm.

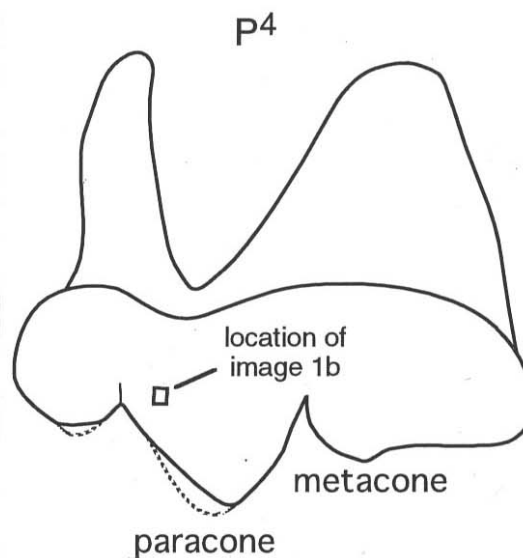
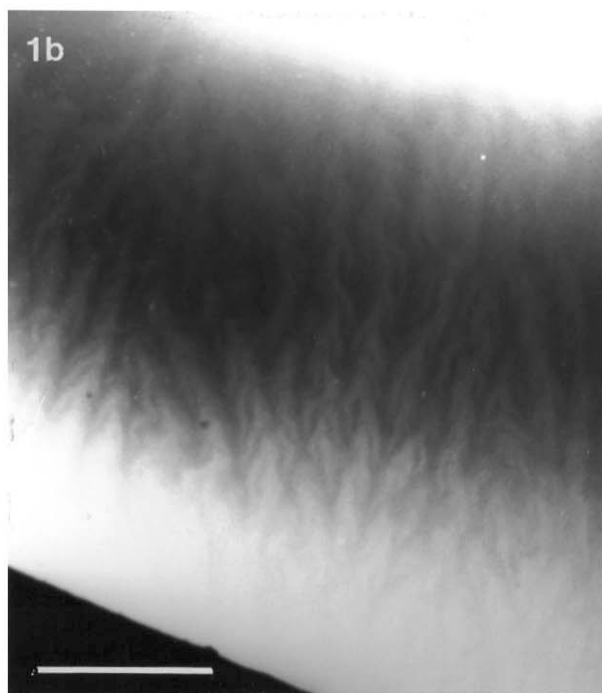


Figure 1b. Hunter-Schreger bands typical of *Crocota* enamel under reflected light. Tangential view of unmodified enamel on left P⁴ paracone, about 7 mm from cervical margin of crown; occlusal direction toward top of micrograph (see sketch for position of image). Transvaal Museum (TV) Zooarchaeology specimen number A3650. Bar = 1 mm.

structure in relation to differences in predicted stresses in a mammal, *Crocota crocuta*, functioning at the opposite extreme of very high dental stresses.

Methods and Materials

The dental adaptations for bone eating in *Crocota crocuta* were studied by: (1) examining whole teeth and sections through them using reflected light microscopy and scanning electron microscopy (SEM) to interpret the enamel microstructure and (2) generating finite element (FE) models to look at stress distribution under different loads.

This paper focuses on the canine of *Crocota crocuta* because this tooth shows a greater regional variation of enamel microstructure than any of the other teeth. The differences found in the canine allow a comparison of within-tooth variation in relative stress intensity with variation in structure. The other cheek teeth have a rather uniformly high degree of specialization of the enamel microstructure throughout the crown. An important differentiation in enamel structure exists in the carnassial teeth (P⁴ and M₁) but will not be considered here. In this paper, the structure in the hyena upper canine enamel is compared with that in another carnivoran (member of the Order Carnivora) of

similar size, the puma (*Puma concolor*), which is not known for bone eating behavior.

The upper canine of one individual of *C. crocuta*,

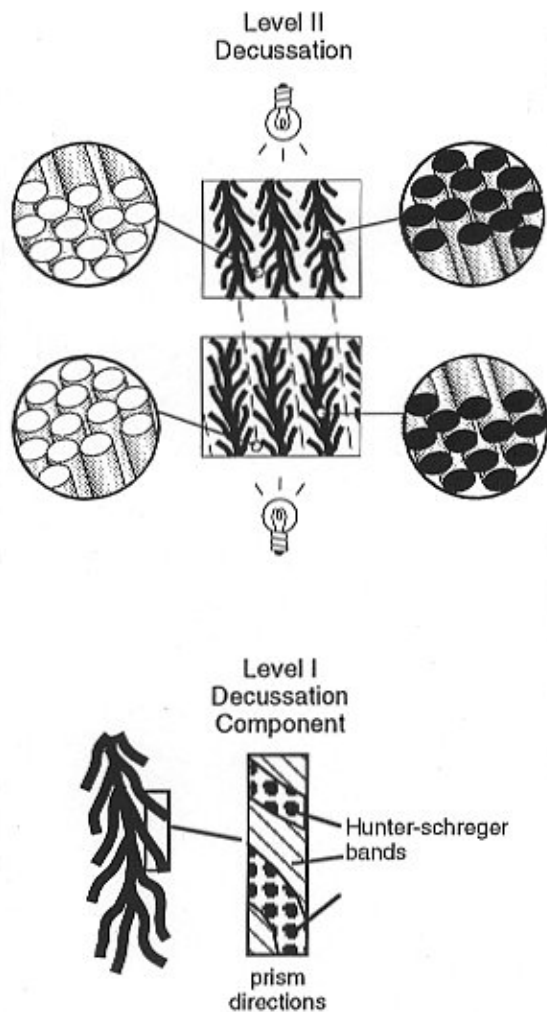


Figure 2. Interpretation of level II decussation and its component level I decussation banding (normal Hunter-Schreger bands) as seen under reflected light. Under vertically reversed light source, the pattern is inverted, owing to reversed prism directions in adjacent columns and differential absorption/reflection of light.

A3650, from the Zooarchaeology Collection of the Transvaal Museum, South Africa, was sectioned vertically, horizontally and tangentially in many planes and examined by SEM. The upper canine from Burke Museum of Natural and Cultural History (UWBM) 27392, *Puma concolor*, was sectioned in the same planes and also examined by SEM.

Upper and lower canines in 17 specimens of *Crocuta crocuta* and 4 specimens of *Puma concolor* were examined with reflected light microscopy for variation in patterns of prism decussation and distribution of premortem cracks.

Microscopy

Light is absorbed more efficiently when it strikes a prism in a direction parallel to the long prism axis than when it strikes normal to the long axis. Where prisms decussate in adjacent layers of enamel, alternating layers reflect light differently, depending upon prism direction, resulting in light and dark HSB. This differential absorption/reflection provides a means of surveying the decussation in large expanses of enamel nondestructively and obtaining an understanding of the distribution of structures before preparing sections and the accompanying loss of dental material.

There are several limitations to interpreting enamel microstructure with low magnification reflected light microscopy. Decussation in most mammals is confined to the deeper parts of the enamel, so that without grinding away the outer enamel, HSB must be viewed through an interval of enamel, which filters out some visual information. Additional disadvantages are that only one direction of view is possible; the decussating prisms tend to interfere with the passage of light, which must pass in and out of the enamel; and the low magnification does not allow direct observation of the pathways of individual prisms. Nevertheless, it is possible to infer the average direction of the prisms locally within the bands of alternating prism direction; observing these differences at low magnification allows one to trace the broad distributions of the structures more readily than is possible at the higher magnifications required to see individual prisms. Furthermore, this procedure does not require preparation of sections and is therefore, both nondestructive and can be accomplished more rapidly, allowing one to verify consistency of morphology across larger numbers of individuals.

The specimens viewed under reflected light were examined using a dissecting microscope and a single light source in a darkened room. Definition of the HSB was further improved by preventing light from directly striking the enamel being viewed. This was accomplished by using an opaque shield, in this case, a strip of exposed and developed photographic film (visible in Fig. 1a) held normal to the surface between the light source and the portion of the surface being examined. When the light source is directed at the enamel surface behind the film, the enamel being viewed receives its illumination mainly from one direction, from the enamel under the edge of the film. Under these conditions, the HSB are best defined in a narrow region of the enamel nearest the edge of the shield.

After examination by light microscopy, sections were prepared for SEM. The structure of one upper canine was studied in a large series of sections. Vertical (radial), transverse (horizontal), and tangential sections, including naturally worn, fractured and ground surfaces, were

examined at various positions from the base of the crown to the tip and around the circumference of the tooth.

Sections were initially prepared by fracturing the enamel. The tooth was first split along pre-existing vertical cracks in the enamel. Those fragments were further fractured by grinding a groove in the dentin, positioning the tooth fragment over a thin rod and manually exerting force on both ends of the fragment to create tensile stress in a direction perpendicular to the groove. Fractured enamel surfaces were initially examined without grinding or polishing; some of these specimens were etched for a few seconds with 10% HCl before sputter coating with gold-palladium to enhance the prism outlines, but some were prepared without etching. After viewing and making micrographs of the fractured sections with the SEM, the fracture surfaces were ground to a flat surface, polished, lightly etched, sputter coated and re-examined with the SEM. Some specimens were prepared by grinding and polishing tangentially through the outer enamel to reach the region of decussation and viewed in this tangential plane. Additionally, the enamel of the natural wear surface in the region of the canine tip was examined because it forms a naturally polished section; in this case, the surface was not ground or polished, but was lightly etched to produce prism relief and then sputter coated.

Fracture surfaces

The same differential crystallite directions that reveal prisms in thin sections of enamel examined under light microscopy produce discontinuities that also respond differentially to fracture, so that fracture surfaces reveal the prism structure. But unlike ground sections, the prisms revealed by fractures along certain planes form a 3-dimensional surface that on a microscopic scale tends to follow the boundaries of prisms. This happens because prism boundaries have a lower work-to-fracture than pathways through the diameters of prisms (Boyde, 1976; Rasmussen *et al.*, 1976). Among the factors that inhibit fracturing is the amount of new surface that is produced in extending a crack over a unit distance. Very rugose surfaces in materials with similar stiffness and distances between atomic planes require more energy to form than smooth surfaces. Also, the extent to which a propagating crack tip encounters prism boundaries running perpendicular to the crack plane determines the ability of the microstructure to reduce the stress concentration at the crack tip and thereby increase the level of applied stress required to fracture the enamel (see Rensberger, 1995a).

Finite element modelling

To understand how the microstructure in *Crocota* is related to the stress distribution in the canine enamel, a 3-dimensional finite element model of the canine was formulated and subjected to tip loads. The elements forming

the outer layer of the model were assigned a stiffer elastic modulus than those forming the interior, to simulate the responses of enamel covering a dentinal core. Although elastic moduli within the ranges of experimentally derived values for enamel and dentine were used, the calculations of static stresses do not provide reliable estimates of the absolute magnitudes because the elastic responses of the enamel components at a microscopic scale cannot be exactly uniform in all directions, owing to the anisotropy of hydroxyapatite at the crystallite level. The complex organization of crystallites in prisms and interprismatic matrix and the complex attitudes of prisms within most mammalian enamels makes it difficult to model the anisotropy at the scale of teeth. Instead, the model allows assessing the effects of gross tooth structure and loading on the distributions of relative stress magnitudes and variations in stress directions over the enamel crown, and making predictions of the positions of maximum stresses and attitudes of planes of prism decussation that optimize resistance to fracture in those positions.

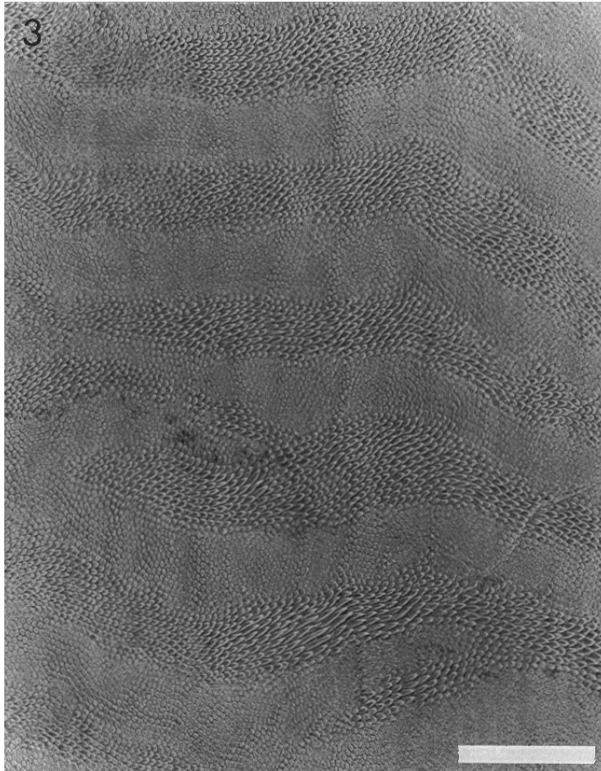
Results

Structure under light microscopy

The HSB in the canine of *Crocota* are bent in complex wavelike shapes of varying amplitude. Wavelike HSB have been reported in a few other mammals, including the canine enamel of an extinct group of South American ungulates, the *Astrapotheria* (Rensberger and Pfretzschner, 1992). Undulating HSB also occur in the enamel of other carnivorans.

The structure in *Crocota* is distinctive in several regards. In certain regions, the amplitude of the waves attains a much greater magnitude (Fig. 1b) than is seen in most carnivorans (Figs. 1a and 3). Where the amplitude is high the wave crests and troughs of adjacent HSB are vertically aligned through many vertically successive bands; in other Carnivora, vertical alignment rarely extends beyond several adjacent bands and is less regular. The waves in *Crocota* tend to be spaced rather evenly in the circumferential direction, whereas the spacing is irregular in other mammals. Furthermore, the crests and troughs in *Crocota* coincide with circumferentially alternating, vertically oriented light and dark bands. These vertical bands resemble vertical HSB, which are known in some large ungulates (Rensberger and von Koenigswald, 1980; Fortelius, 1985; Boyde and Fortelius, 1986). However, the vertical bands in *Crocota* are unique in that they are of greater thickness and spacing than either horizontal or vertical HSB in other mammals.

The fine structural pattern of the HSB in *Crocota*, as seen in tangential view under light microscopy (Fig. 1b), resembles a "fir tree" in which branches at the margins



(Figures 4-7 on facing page)

Figure 4. Worn tip of *Crocuta* left upper canine, occlusal view of anterior side, showing deeply folded decussation planes. D = dentin; E = enamel; A = anterior direction; L = lingual direction. Scanning electron micrograph; enamel unground, lightly etched. TV A3650. Bar = 1 mm.

Figure 5. Worn tip of *Crocuta* left upper canine; occlusal view of anterior enamel at higher magnification than in Figure 4. Scanning electron micrograph. L = lingual; A = anterior direction. Note discontinuities in individual HSB near centers of crests and troughs of folds. Unground natural wear surface, lightly etched. Ground, polished and etched vertical radial section visible at lower left. TV A3650. Bar = 100 μ m.

Figure 6. Radial section of Figure 5, with oblique view of adjoining naturally worn tangential section (right). Occlusal direction toward top. TV A3650. Bar = 100 μ m.

Figure 7. Transverse section of labial enamel 3 mm from worn tip of *Crocuta* upper canine, occlusal view (looking cervically). Enamel-dentin junction at upper left corner of micrograph. Section ground, polished and etched. Prisms decussating in horizontal plane near EDJ, but decussating in vertical radial planes away from EDJ. Occlusal view. TV A3650. Bar = 100 μ m.

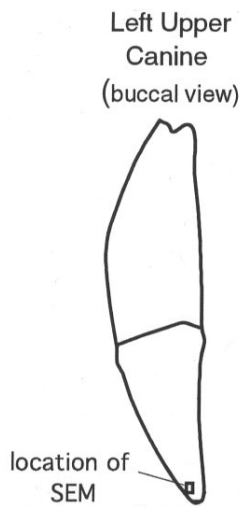


Figure 3. Scanning electron micrograph of tangential enamel section of near tip on buccal side of upper canine in *Puma concolor* (UWBM 27392). Bar = 100 μ m. Occlusal direction toward top (see also diagram).

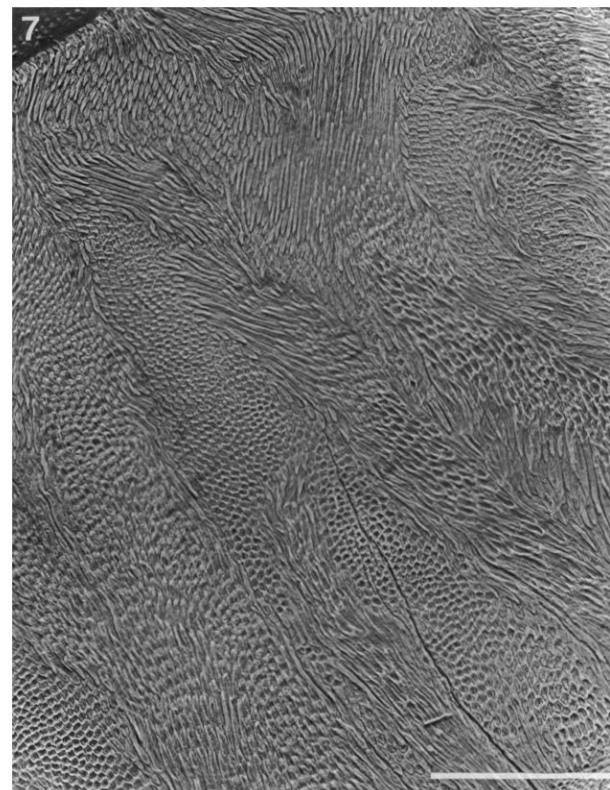
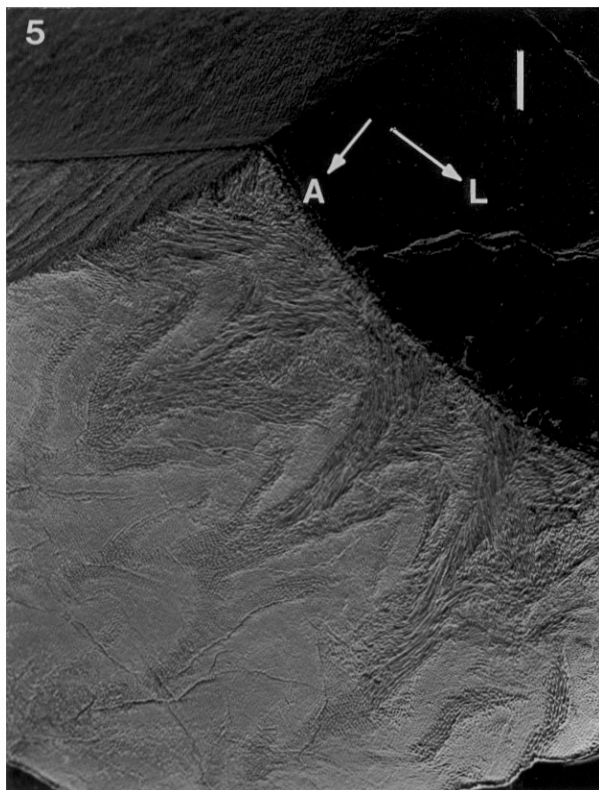
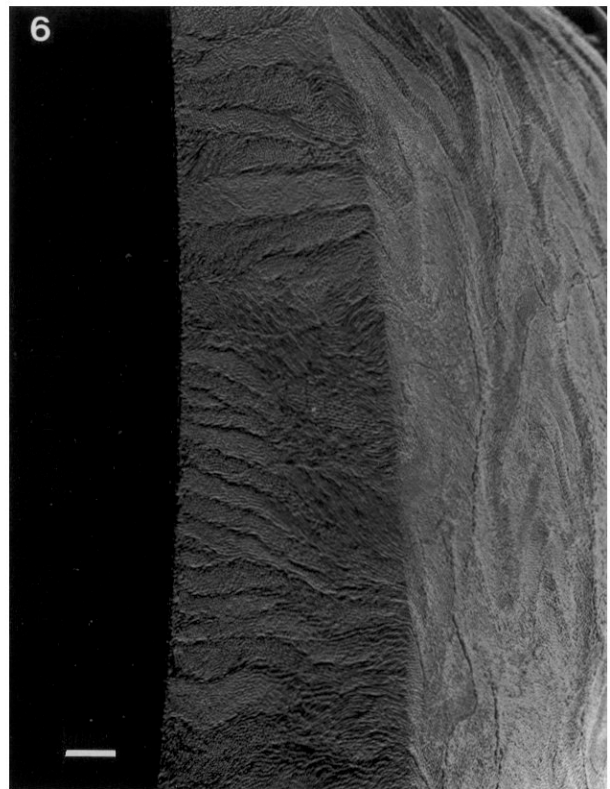
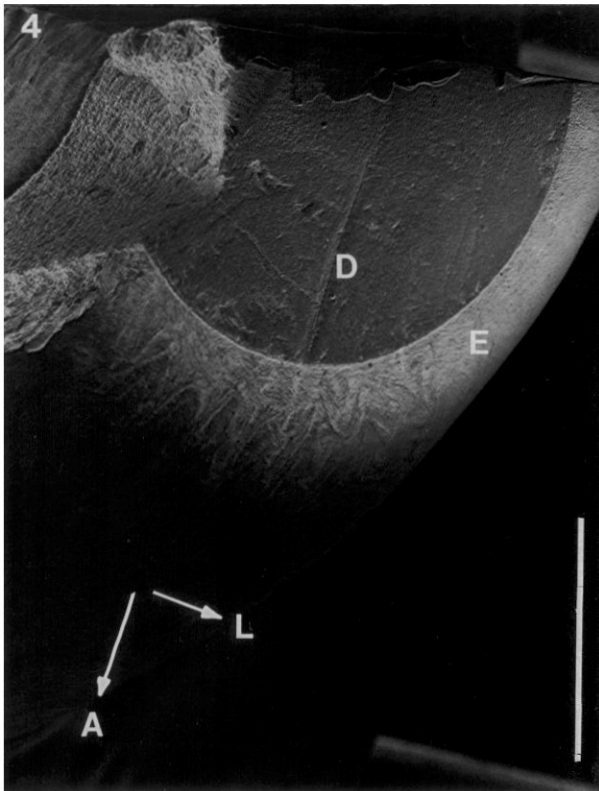
descend at a steep angle and curve outward in tapering filaments of smaller light and dark bands of the size and spacing of normal mammalian HSB. The marginal light-colored “filaments” are absent in the centers of the dark zones. Similarly, the centers of the light zones are devoid of

dark bands but are bounded laterally by the alternating light and dark HSB that bound the dark zones.

As in the case of typical HSB (Rensberger and von Koenigswald, 1980), when the source of illumination is in the occlusal or cervical direction (parallel to the axes of the “fir trees”), then is shifted 180° to the opposite pole of the occlusal-cervical direction), the “fir tree” pattern exactly reverses itself: dark “trees” change to inverted light “trees” and light “trees” become inverted dark “trees” (Fig. 2).

The absence of light-reflecting enamel in the centers of the large dark bands (near the centers of the dark “fir trees”) indicates that the prisms there are all aligned in one direction, toward the light (Fig. 2), and the absence of light-absorbing enamel in the centers of the light “fir trees” indicate that the prisms there are all aligned perpendicular to the direction of the light. In other words, the individuality of the small HSB at the margins of the “fir trees” and their disappearance near the centers of the “fir trees” indicates that the prism direction in each HSB must change along the course of the band as it nears the center of a vertical dark or light zone. Although some change in prism direction is frequently evident along an individual HSB in other

Crocuta canine enamel structure



carnivorans (Fig. 3), there the change is random in its occurrences. The structure in *Crocota* is unique in that the change (1) involves specifically a reversal with respect to the vertical axis of the tooth and (2) occurs near the crests and troughs of the HSB.

The alternating light and dark bands of the filamentous margins of the vertical zones have the size and features of the HSB in other medium-sized mammals. They differ, however, in the directions of the bands, which form angles with the horizontal occlusal plane of 70 to upwards of 80 degrees, instead of the low angles characteristic of most mammals with wavy HSB (Figs. 1a and 3).

When the light source is in a direction parallel to the decussation planes, so that light enters from a direction perpendicular to the axis of the tooth, the vertical light and dark bands of the “fir tree” pattern disappear and are replaced by horizontal HSB with only moderately steep folds. This 90° change in apparent alignment of the structure does not occur in the HSB typical of other mammals, except for certain regions of astrapothere and rhinocerotoid cheek teeth (Rensberger and Pfretzschner, 1992; Rensberger, 1995b) where HSB of two distinct orientations occur at different depths through the thickness of the enamel.

Considered together, the data from reflected light microscopy suggests that the structure in *Crocota* enamel involves (1) extremely steep folding of HSB; (2) enhanced vertical alignment of the crests (occlusal bends) and troughs (cervical bends) of the folds; and (3) along each HSB, the directions of the prisms make a 90° or greater rotation between the crest and the trough of each fold, so that at the crests, the prisms are all directed occlusally and outward, and at the troughs, the prisms are all directed cervically and outward. These changes combine to create a higher level of prism decussation superimposed on the primitive decussation. This higher level of decussation is characterized by (1) a much wider spacing of the decussation zones than in the primitively horizontal HSB of Carnivora and other mammals and (2) by vertical orientation of the decussation planes, at right angles to the decussation planes of most other mammals.

Except for the vertical attitude of main decussation planes, their wider spacing and the presence of small peripheral HSB, these structures have all the characteristics of what are traditionally called HSB. To distinguish these structures from traditional HSB and simultaneously recognize their similar function, appearance and undoubted phyletic derivation from normal HSB, the “fir tree” structure will therefore be called level II HSB or level II decussation. These structures have also been described as folded HSB (Rensberger, 1995a) and zigzag HSB (Stefen, 1997); the term level II emphasizes the historic components and functional re-organization that the enamel prisms have undergone.

(Figures 8-11 on facing page)

Figure 8. Adjoining right angle sections: radial (left facet), transverse (right facet) in posterolabial enamel of *Crocota* upper canine. Occlusal view. TV A3650. Bar = 100 μm.

Figure 9. Center of edge between radial section and transverse section of Figure 8 at greater magnification. TV A3650. Bar = 100 μm.

Figure 10. Radial section with unpolished fracture surface in labial enamel of *Crocota* upper canine. Outer surface at upper left, occlusal direction toward lower left. Note rugose surface and varying directions of decussation planes. TV A3650. EDJ = enamel-dentin junction. Bar = 100 μm.

Figure 11. Radial fracture surface of labial enamel in *Crocota* upper canine at higher magnification. Orientation as in Figure 10. TV A3650. Bar = 100 μm.

The appearance of less extremely folded bands when the light source is shifted laterally from a vertical position indicates that the folding of the decussation planes beneath a given locus on the tooth surface is not constant through the thickness of the enamel and that less extremely folded HSB exist either behind or in front of more highly folded HSB. This complex structure was found under reflected light microscopy in each tooth of each individual examined and, except for the canine, is essentially distributed throughout most of the enamel crown.

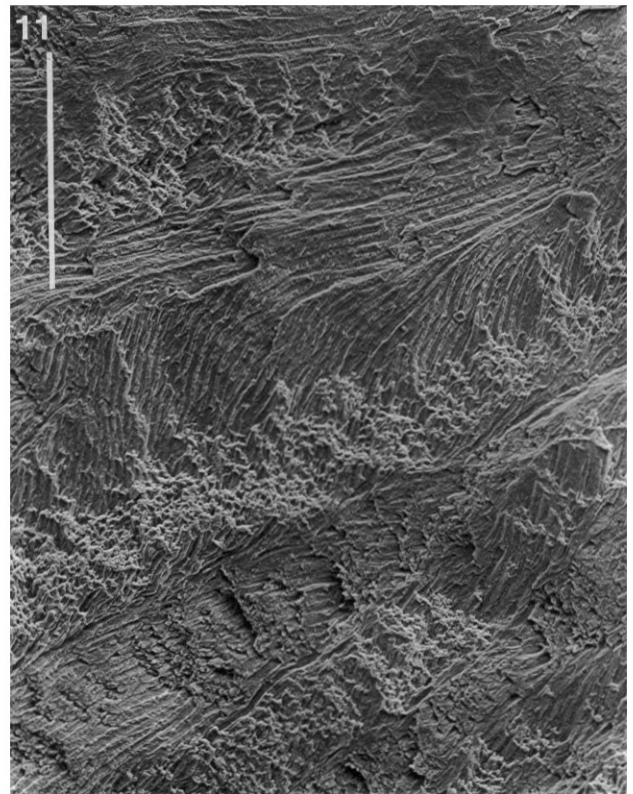
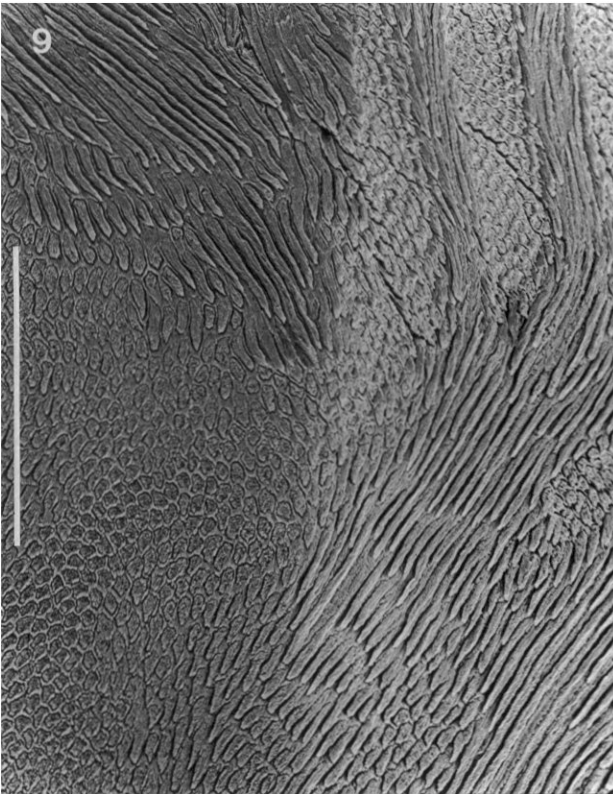
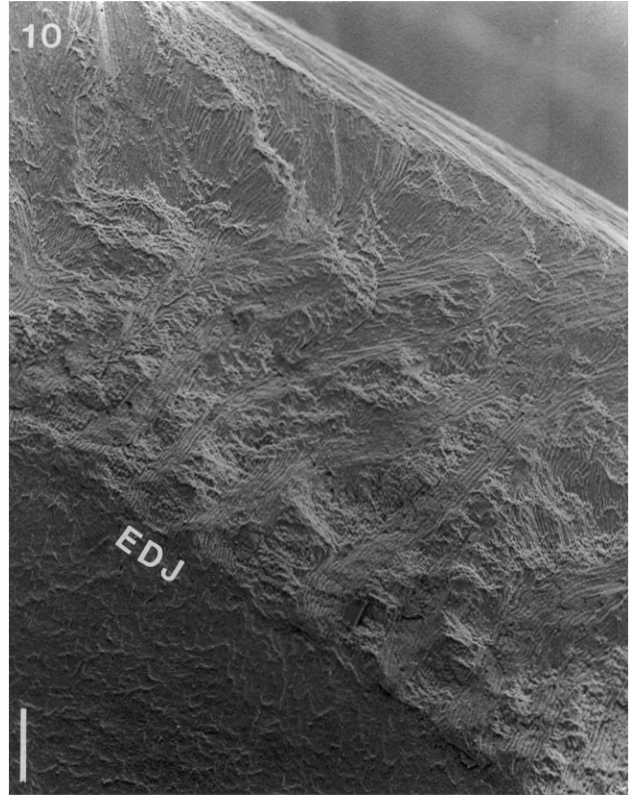
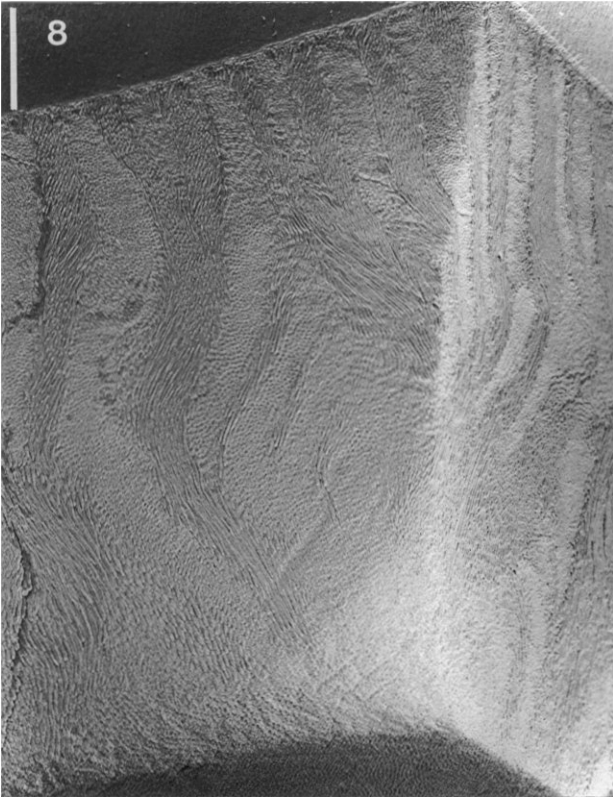
Structure under SEM

Verifying the structural interpretation based on reflected light microscopic examination and determining the 3-dimensional distribution of the structures through the thickness of the enamel required examination of sections with SEM. The observations made during the SEM study correlated well with the reflected light observations, justifying nondestructive light microscopic sampling of other individuals to supply statistical breadth to the interpretations.

Worn canine tip Scanning electron micrographs of the structure on the worn surface of the tip on the anterolabial side of the upper canine of *Crocota* reveal details of the high amplitude folds in the HSB (Figs. 4–6). The curvature of the worn enamel edge at the tip of the canine presents a smooth transition between an almost horizontal section at the enamel-dentin junction (EDJ) and a vertical tangential section at the outer margin of the tooth.

The wide and regular spacing of the waves in the decussation planes can be seen in Figure 4. At a higher

Crocuta canine enamel structure



magnification (Figs. 5 and 6), the continuity of individual HSB is seen to be lost near the centers of the crests and troughs of the deep folds where the prisms of adjacent HSB assume a common direction, confirming the interpretation made under light microscopy. The decussation planes are strongly vertical, especially in the mid-region of the enamel thickness.

It is clear that the vertical decussation does not reach the EDJ, however, because crossing sets of prisms more or less confined to a horizontal plane occur near the EDJ. This can be seen in Figure 5, where prisms near the EDJ are running dominantly in a direction parallel to the surface, which is horizontal in that region. The alternate directions of prisms within the horizontal plane near the EDJ can be seen more clearly in a ground horizontal section (Fig. 7). At short distances away from the EDJ toward the outer surface, some bundles of prisms still run horizontally, but in adjacent areas, the prisms are perpendicular or strongly oblique to the surface. The boundaries (the decussation planes) between these bundles are vertical and run radially (normal to the EDJ) toward the outer surface. However, the prism directions within the bundles of horizontal prisms in this section can be seen to vary considerably, and this is true generally of the bundles of both the vertically and horizontally directed prisms.

Because the layers of decussating prisms are strongly folded, sections taken at right angles to one another tend to be self-similar. A pair of intersecting sections (Fig. 8) shows sets of prisms whose decussation planes intercept the horizontal section (top of micrograph) and the extensions of those decussation planes also intercept the vertical section (bottom of micrograph) at a high angle. The central part of the horizontal section (Fig. 8) shows filamentous HSB bifurcating from and merging into larger HSB. An enlargement in the region of the center of the intersection of these sections (Fig. 9) shows similar structures in the vertical section: two filamentous HSB uniting in a single larger HSB. This is quite unlike the regularity of HSB in other mammals (e.g., rhinocerotoids) that also have strongly reinforced enamel; there decussation planes intercept only a vertical or a horizontal section, not both.

Roughness of fracture surfaces Radial (vertical) fracture surfaces near the tip of the canine are rugose (Figs. 10 and 11) and indicate considerable structural resistance to fracture.

Transverse (horizontal) fractures are exceedingly rugose. As seen in occlusal view (Fig. 12a), the horizontal fracture surface of the anterolabial enamel consists of prominent, regularly arranged ridges running in a radial direction through the enamel. In an enlarged view of the same surface (Fig. 12b), the middle to outer portion of the enamel (central to upper left in micrograph) is seen to consist

(Figures 12-13 on facing page)

Figure 12. Unground transverse fracture surface of anterolabial enamel in *Crocota* upper canine near tip. Cervical view, looking occlusally. (a) Low magnification; note the lesser rugosity of adjoining radial fracture surface (R) at lower right. Naturally abraded outer enamel surface visible to left of R. E = enamel; D = dentin. Bar = 1 mm. (b) Higher magnification of transverse enamel section of Figure 12a; note the fractured ends of vertical prisms at crests of ridges. EDJ = enamel-dentin junction. Bar = 100 μ m. (c) Normal view of fracture surface of Figure 12a, showing transition from horizontal decussation (top) to vertical decussation (middle region). EDJ is slightly off image at upper left corner. Bar = 100 μ m. TV A3650.

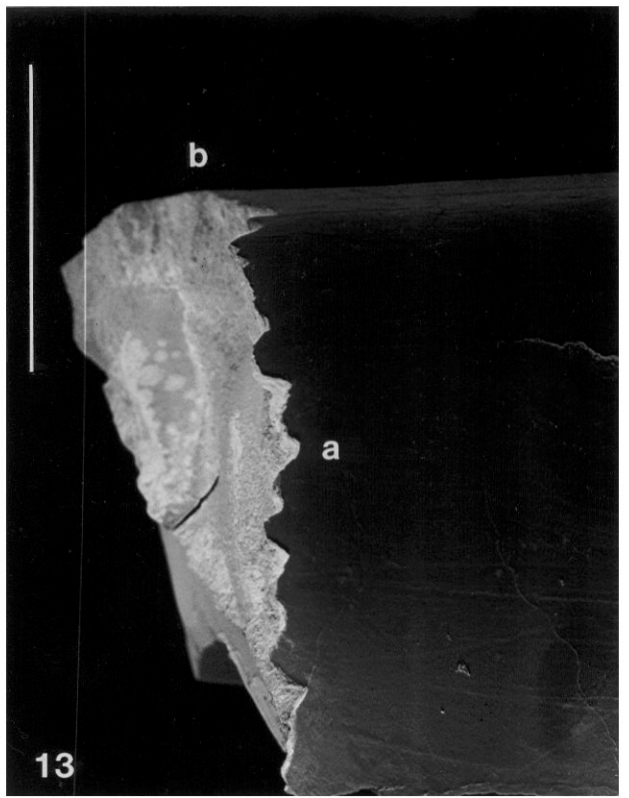
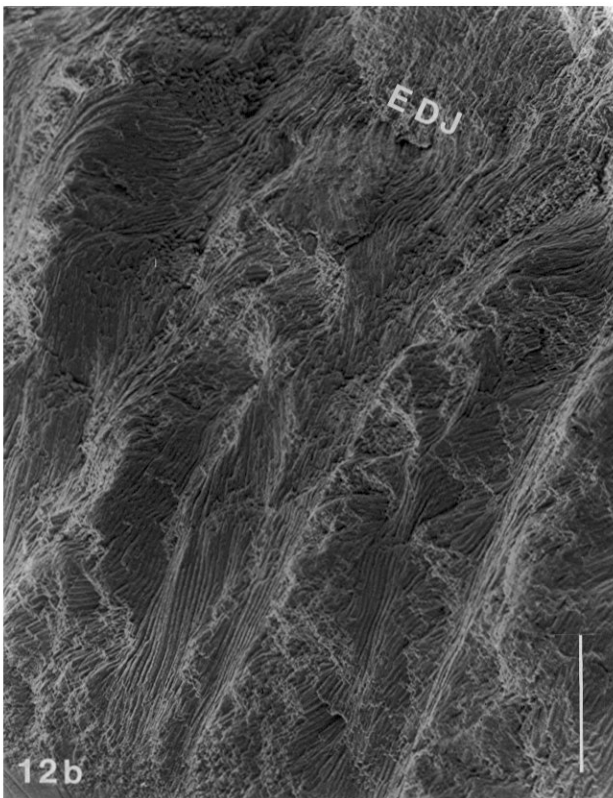
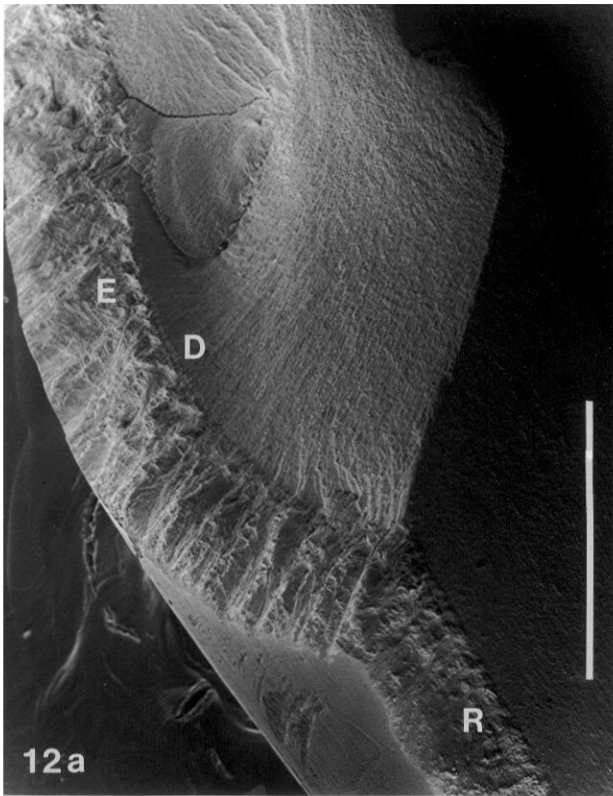
Figure 13. Profile of enamel fracture surface on anterior side (a) and smoother surface on lingual side (b) of *Crocota* upper canine. Occlusal direction toward left. Bar = 1 mm.

of layers of horizontal prisms (prisms parallel to the plane of the micrograph) adjacent to bundles of prisms running vertically at the centers of the ridges. Decussation planes are by definition parallel to both sets of prisms and therefore are vertical and running toward the outer surface. The horizontal fracture surface follows to a considerable extent the prisms running more or less horizontally. Prisms are most easily fractured parallel to their boundaries (Boyde, 1976; Rasmussen *et al.*, 1976); the intervening vertical prisms add resistance to the fracture because the propagating crack must pass through their diameters in forming the horizontal fracture.

In addition to this source of resistance to crack propagation, other impediments are created by prism decussation {see pp. 154-157 of Rensberger (1995a)}. There are tensile stresses aligned at right angles to the nominal stress a short distance ahead of the tip of a propagating crack. When a crack that is following prisms aligned parallel to its plane and a layer of prisms running normal to that direction are situated ahead of the tip, the tensile stresses ahead of the tip will be acting in a direction parallel to the prisms ahead of the crack. Although these stresses are lower than the stress acting normal to the crack plane at its tip, they tend to initiate small cracks parallel to the axes of the crossing prisms ahead of the tip and at right angles to the main crack plane. When the main crack tip reaches that area, the small oblique cracks in effect enlarge the diameter of the main crack tip and thereby reduce the magnitude of the stresses concentrated at the tip that are acting to propagate the crack.

It can be seen in these micrographs that some of the

Crocuta canine enamel structure



vertical prisms at the crests of the ridges sweep upward from their horizontal attitudes nearer the EDJ (lower right of Fig. 12b, lower left of Fig. 12c), encouraging the path of the crack to turn vertically. The effect of this microstructure is to produce a wavy crack plane of high amplitude (Fig. 13) and large surface area. The formation of larger crack surface area absorbs additional energy, diminishing the amount of energy available to propagate the crack.

Owing to the presence of horizontal decussation near the EDJ, the ridges of the horizontal fracture surface are absent in that region (lower left of Fig. 12c) and there the main resistance is to vertical cracks. However, the ridge and valley system dominates most of the horizontal section, indicating overall strong resistance to horizontal fracturing.

Difference in structure around tooth circumference

The rugose horizontal fracture surface described above characterizes the anterobuccal to anterolingual sides of the canine but this rugosity diminishes on the lingual and posterior sides of the tooth. This can be seen in the almost flat fracture surface on the posterolingual side of the tooth (near "b" at right side of Fig. 13, behind the serrated region). This flattening of the fracture surface occurs because the prism decussation planes become more nearly horizontal, allowing the fracture to follow a flatter path within the horizontal plane. This lateral change in structure can be traced around the crowns of unmodified canines using reflected light microscopy and was observed in all of the upper and lower *Crocota* canines examined. The strongest development of level II decussation occurs on the anterior side, and extends farther toward the posterior on the buccal side than on the lingual side.

Structure away from canine tip The amplitude of folding of the decussation planes diminishes toward the base of the crown (Fig. 14). This micrograph is of a section of anterolabial enamel about 14 mm cervically from the worn tip of Figure 4. The tangential section in the upper (lighter) part of this micrograph shows folds of a much lower amplitude than occurs near the tip. Furthermore, the individual HSB are seen to be continuous across the crests and troughs, which lack the unification of prism direction in the vertically adjacent bands that is characteristic of level II decussation. In the horizontal section (darker lower section of the micrograph), the prisms are mainly horizontal (parallel to the plane of the section) and show only horizontal decussation within this plane, unlike the horizontal sections near the tip where strong vertical decussation is evident. There is no clear differentiation of structure through the thickness of the enamel in the mid-region of the crown, except a tendency for decussation to diminish and prisms to become more nearly parallel near the outer surface, a condition that occurs in the enamel of most mammals. The decussation structure at this distance from the tip is not differentiable from the structure in many

(Figures 14-17 on facing page)

Figure 14. Tangential (v) and transverse (h) ground and polished sections in the labial enamel of the *Crocota* upper canine, 18 mm cervical from occlusal surface shown in Figures 4 and 5. Slightly oblique occlusal view (looking cervically). Outer two-thirds of enamel thickness removed. TV A3650. EDJ = enamel-dentin junction. Bar = 100 μm .

Figure 15. Unground transverse fracture surface of anterior enamel near upper canine tip in *Puma concolor*. EDJ = enamel-dentin junction. Bar = 100 μm .

Figure 16. Transverse ground and polished section of anterior enamel near tip of upper canine in *P. concolor* (compare with Fig. 7). EDJ = enamel-dentin junction. Bar = 100 μm .

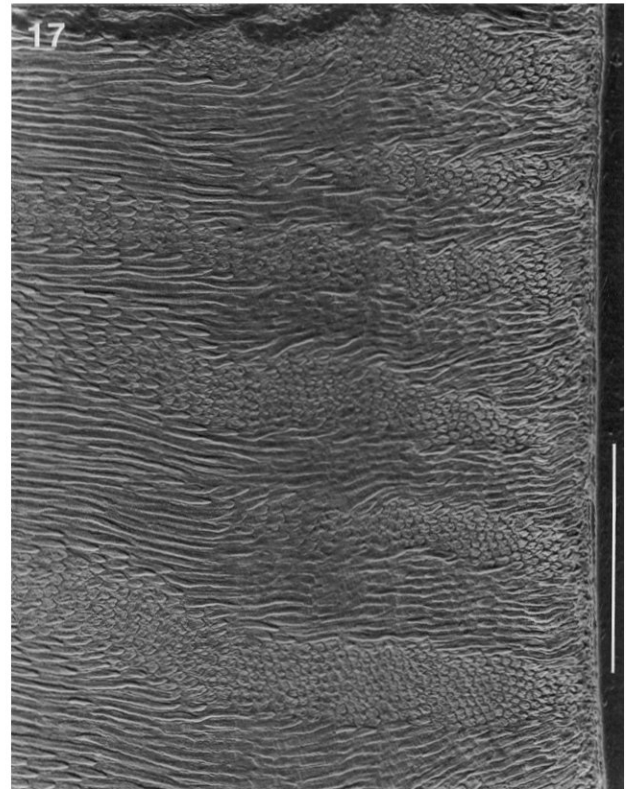
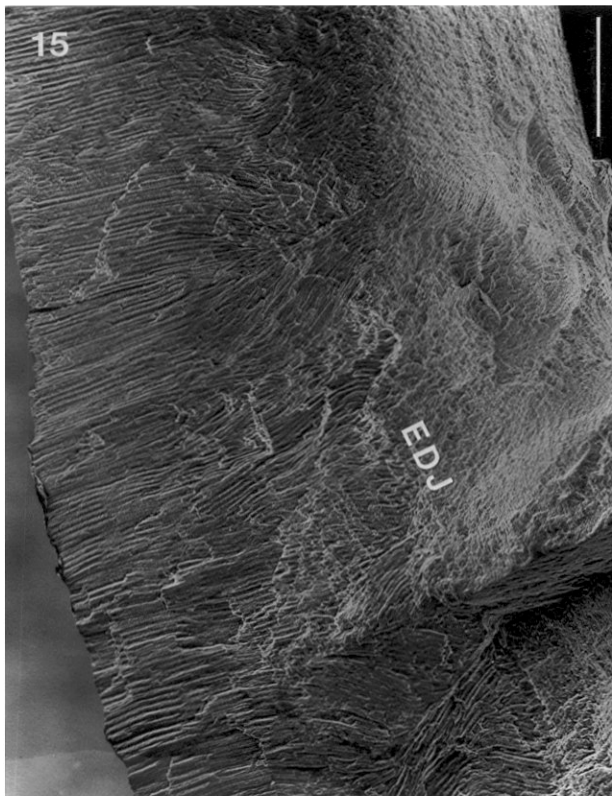
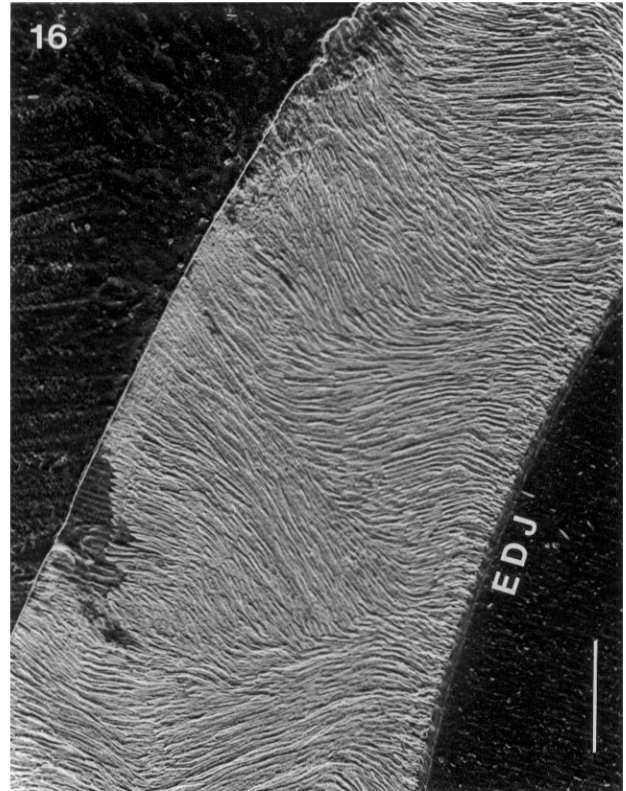
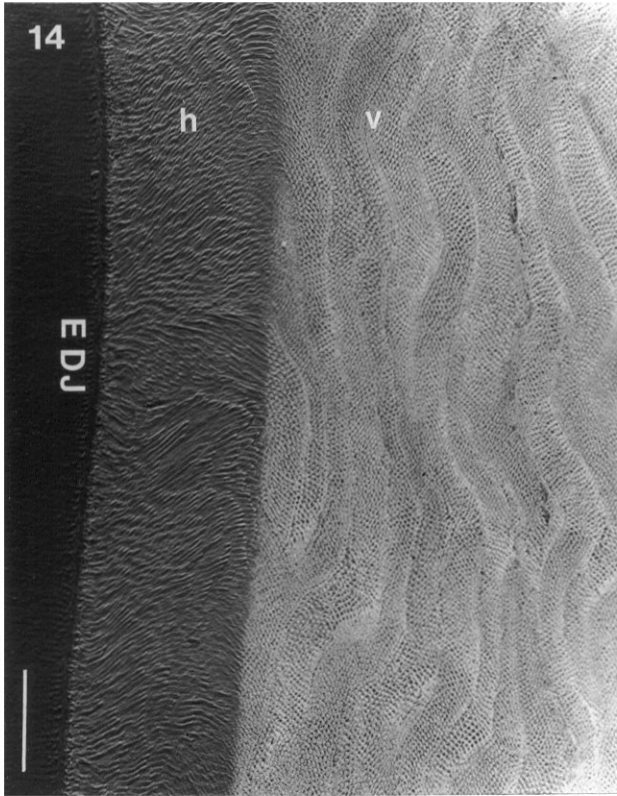
Figure 17. Ground and polished radial section of anterolabial enamel in upper canine of *P. concolor*, 15 mm from cervical margin of crown. EDJ at right. Bar = 100 μm .

mammals and is typical of level I HSB. Closer to the tip of the *Crocota* canine this structure grades irregularly into level II HSB.

Structure in *Puma concolor* compared Horizontal fracture surfaces in *Puma* enamel from the canine tip region lack the high relief pattern of ridges and valleys characteristic of *Crocota*. The enamel structure on these fracture surfaces (Fig. 15) indicates that the fracture is able to follow an almost horizontal plane because the prisms are dominantly parallel with that plane. A ground horizontal section of the anterior enamel shows that decussation occurs largely within the horizontal plane (Fig. 16). One of the characteristics of the level II decussation in *Crocota* is that the lines of intersection of decussation planes with the plane of a radial section bend (Figs. 6 and 10), which adds to the multidirectional resistance to stress. In contrast, the decussation planes intercepted in radial sections in *Puma* are relatively straight in their course from the EDJ to the outer surface (Fig. 17).

The microstructural characteristics of the canine tip region in *Puma* extend essentially throughout the canine. *Puma* not only lacks level II decussation, but the wavy level I decussation is relatively uniform, with only minor variations in its morphology in different regions of the canine.

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Regional Difference in Stresses

The growing body of evidence showing close relationships between the geometry of enamel decussation and stresses in mammalian teeth raises the question whether the discontinuous distribution of the level II decussation in the *Crocota* canine is related to regional differences in stresses.

The analysis shows, however, how the direction of loading and the shape of enamel and dentinal parts of the tooth combine to affect the distributions of stress magnitudes and stress directions and that these effects are sufficient to explain the regional differentiation of the microstructure. This is probably true in part because the diverse prism directions in enamel with extensive decussation makes the enamel much less anisotropic in stiffness than individual crystals of apatite, and in part because under bending deformation from large loads it is difficult for microstructures to alter the dominant stress directions and regions of concentration.

The model was loaded in different directions. Two directions, one parallel to the axis of the tooth at its base, and another parallel to the axis at the tip, though differing by only 17°, result in greatly different distributions of stress, one of which closely matches the observed pattern of cracks in the enamel.

When the load is parallel to the basal axis of the tooth, the maximum tensile stresses are concentrated on the anterior and anterolabial surfaces (Fig. 18). The magnitude of the maximum tensile stresses diminishes around the circumference of the tooth and only compressive stresses occur on the posterior side. The directions of the maximum tensile stresses are vertical in the region of the tooth where they have the highest magnitude, and become more horizontal toward the base and around the circumference of the tooth (Fig. 18) where the tensile stresses are low. This prediction of vertical tensile stresses in the anterobuccal side of the tooth is supported by the occurrences of cracks in *Crocota* canines. Almost all of the canines of *Crocota* examined had more extensive horizontal premortem cracking in this region than in any other, indicating vertical tensile stresses. Cracks were identified as premortem by the presence of crack edges with occlusal polishing (Rensberger, 1987). The most extensive premortem cracking was on the anterior and anterolabial sides. In one individual, small horizontal cracks were also abundant in other areas of the tooth, but those on the anterior and labial sides were distinguishable by their larger size and wider spacing.

In the other case, when the tooth is loaded in the direction parallel with the axis at the tip, the region of maximum tensile stress shifts to the posterior side of the tooth near its base (Fig. 19). This shift occurs because the

axis of the canine at the tip is directed about 17° more anteriorly than at the base. When the load is parallel to the axis at the base, the anterior convexity of the axis causes the tooth to bend, creating high tensile stresses on its anterolabial side. In contrast, when the tooth is loaded parallel to the axis at the tip, this vector is not parallel to the basal axis and the tooth is caused to rotate anteriorly, setting up high tensile stresses at the posterior region near the base where it is anchored. In life, this rotation would cause these stresses to be distributed through the extensive area of posterior alveolar bone that surrounds the large root and therefore would be much lower. When the tooth is loaded during biting in a direction more nearly parallel to the basal axis, the convex axis causes the tooth to bend and the highest tensile stresses to be aligned vertically and concentrated on the anterolabial side; it is there that the enamel is maximally reinforced by dominantly vertical decussation planes that parallel and therefore resist these potentially dangerous stresses.

Stress magnitude in *Crocota* and *Puma*

The relative stress magnitudes and stress directions calculated in the canine model can be considered applicable to *Puma concolor* as well as *Crocota crocuta*. Although the canine in *Crocota* is thicker in diameter, during construction of the canine model it was found to be similar to that in *Puma* in size as well as details of the 3-dimensional curvature of the axis. Therefore, directions and distribution of the stresses in the *Puma* canine are predicted to be the same as in *Crocota*. In other words, the fundamental differences in the mechanics of the canine in these two taxa can be narrowed down to simply the differences in the magnitudes of the loads experienced.

Because the canine is located anterior of the origins and insertions of the masticatory muscles, it cannot achieve stresses as high as those occurring more posteriorly in the tooth row. Nevertheless, empirical evidence indicates that the canine in *Crocota* is subjected to higher occlusal stresses than are the canines of other carnivorans. Hyenas have higher frequencies of broken teeth compared to felids and canids, and the most frequently fractured tooth in hyenas is the canine (Van Valkenburgh, 1988). Additional evidence for unusual loading forces on the *Crocota* canine is the greater depth of striae gouged in the enamel surface (Fig. 20a) than on canines of other carnivorans of similar size, such as *Puma concolor* (Fig. 20b).

Discussion

Enamel prism decussation occurs most commonly in relatively flat, tabular units. In the Carnivora, the premolars, canines and molar cusps frequently have a modified form of horizontal decussation in which the decussation planes form wavelike surfaces (Figs. 1a and 3). Since

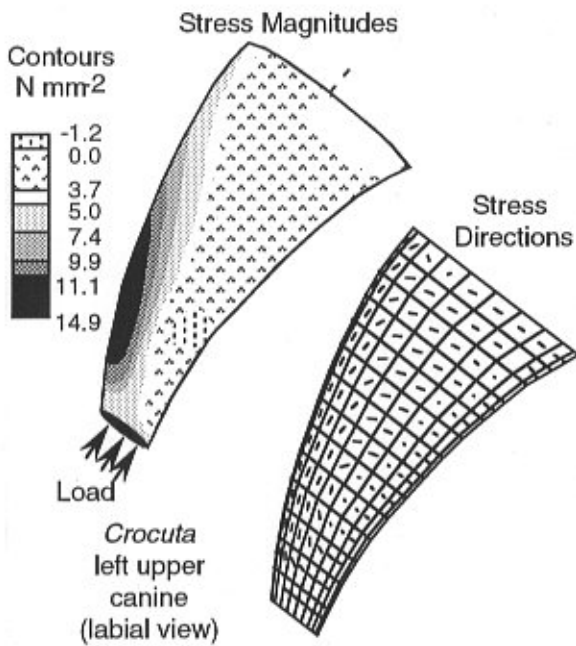


Figure 18. Maximum tensile stress magnitudes and directions developed in a finite element static analysis (FEA) 3-D model of *C. crocuta* left upper canine under load parallel to central axis of tooth at base of crown. Darker shades represent higher stresses.

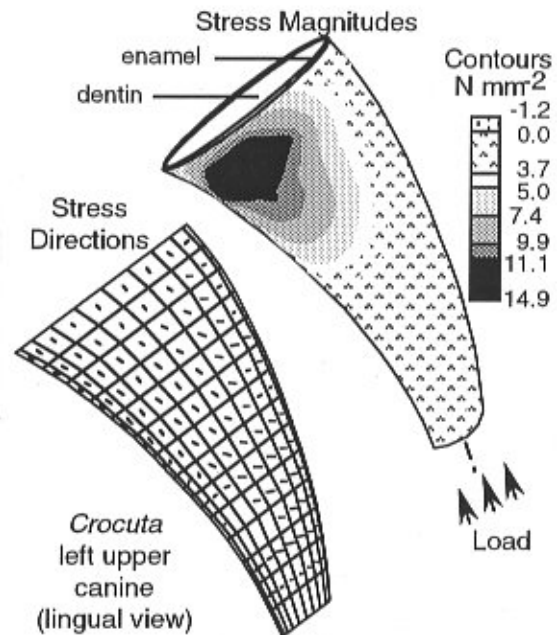


Figure 19. Maximum tensile stress magnitudes and directions developed in FEA 3-D model of *C. crocuta* left upper canine under load parallel to central axis of tooth at tip of crown. Darker shades represent higher stresses.

decussating enamel optimally resists tensile stresses acting parallel to the decussation plane, enamel with wavy decussation planes would in theory have improved resistance to stresses that are less consistently aligned in a single direction. Although carnivores move their mandibles in an orthal direction, the teeth may be loaded in varying directions when they kill prey or tear away parts of prey items. In addition, because bone surfaces are hard and nonconforming, striking bone can produce large loads at unpredictable locations and directions on the sides of cusps during uniformly orthal cusp movements. Such differences in loading direction and locus would produce strongly varying stress directions in the enamel. This may be the source of selection for the wavy decussation planes in carnivores, especially in the canine enamel. The cheek teeth in extinct astrapotheres, which functioned to masticate vegetation in a uniform translatory motion, have flat decussation planes, but in the astrapothere canine tusk the decussation planes are wavy, consistent with the more varied directions in which these mammals loaded the projecting tusk (Rensberger and Pfretzschner, 1992).

Level II decussation, in which HSB are still more intensely folded and modified in other ways, occurs in the region of the canine in which the maximum tensile stresses

are vertical. In Level II decussation, the main decussation planes are vertical, owing to the extreme vertical attitudes of the bent HSB as well as the unification of prism directions in the crest or trough of the fold to a single direction. From this it appears that level II HSB represent a response to vertical tensile stresses, which they maximally resist, and provide a functional replacement for horizontal decussation, which maximally resists horizontal tensile stresses and which dominates other regions of the canine.

Whereas rhinocerotoids and astrapotheres were able to acquire vertical level I prism decussation, hyenas presumably have attained the same function by progressively increasing the amplitude of primitively wavy HSB until the vertical components dominated in regions where the stresses are dominantly vertical, and by eventually aligning prisms in the crests and troughs in vertically reversed directions.

However, another aspect of level II decussation is the retention of horizontal HSB in part of the enamel thickness, giving the enamel continued resistance to horizontal tensile stresses as well. This, and the existence of wavy HSB in carnivorans in general, suggests that this enamel is fundamentally an adaptation to resist varied stress directions resulting from non-constant loading conditions

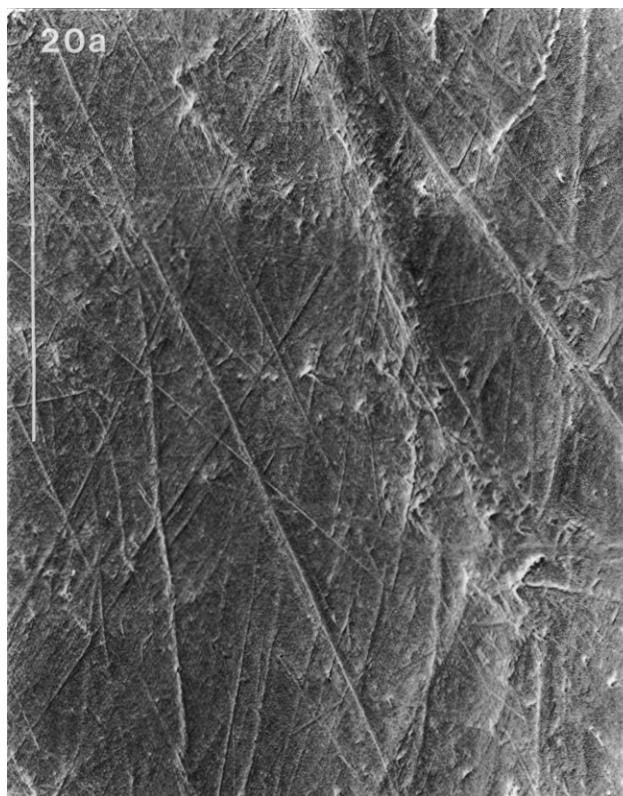


Figure 20 (at left). Differences in stria depth and width in worn surfaces of upper canines of *C. crocuta* (TV A3650) (a) and *P. concolor* (UWBM 27392) (b). Scanning electron micrographs at homologous positions and same orientation on anterolabial enamel near tip of tooth. Occlusal direction toward top, anterior toward right. Bar = 100 μm .



that are probably characteristic of carnivory. Level II HSB are differentiated functionally from the primitive level I HSB from which they are derived by the superimposition of resistance to vertical tensile stresses caused by axial bending of cusps, whereas level I HSB in less derived taxa are only resistant to horizontal tensile stresses generated by vertical compression of ellipsoidal structures. Yet off-axis loads causing at least some axial bending of cusps must occur throughout most carnivorous mammals. This predicts the existence of some threshold level of bending magnitude beyond which the enamel and/or cusp is subject to fracture.

The occurrence of extensive level II HSB in hyenas, which habitually crack bones, indicates an association with very high stresses. Distinct regional differentiation of wave amplitude does not occur in the canine enamel of *Puma concolor*, even though the canine is similar in shape to that in *Crocuta* and therefore probably develops maximum (though lower) tensile stresses that act in similar directions in the same regions. The moderately wavy HSB in *Puma concolor* are apparently sufficient to inhibit fracturing under the levels of maximum tensile stresses attained in this taxon. The factor accounting for the greater intensity of HSB folding in the canine of *Crocuta* must be the magnitude of the stresses attained in those regions. An alternative evolutionary strategy that would have reduced the likelihood of canine fracture would have been a change in shape to a smaller length/ diameter aspect ratio. However, such a change would reduce efficiency of the canine in its primary function - deep penetration for purposes of killing or holding prey or for defense.

Level II decussation, which is regionally limited in the canine, is much more extensive in the postcanine teeth, which have generally been assumed to provide the bone crushing function. However, the presence of level II decussation in the canines of *Crocuta* suggest that the canine also is used in bone eating and this seems to be confirmed by recent observations of feeding individuals by Van Valkenburgh (personal communication).

The attainment of an evolutionary modification of enamel, the most brittle of the dental materials, permitted hyenas to acquire the advantages of bone-eating and still retain almost full functionality of the tooth without an inordinate increase in fracture frequency.

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Discussion with Reviewers

B. Van Valkenburgh: Wolves and other large canids are also known for moderate amounts of bone consumption, but unlike hyenas, they usually use their rearmost molars to crack bones. As the molars are farther away from the canines, it might be expected that wolf canines would be more similar to those of pumas than spotted hyenas. Have you looked at any of the larger canids?

Author: Level II HSB appear under reflected light microscopy to be absent in the canines of large domestic *Canis*. However, it is visible near the tips of the larger cusps of the premolars and molars, though irregular or

absent in the small cusps, for example paracone and metacone of M² and paracone of P¹.

W. von Koenigswald: Stefen and Rensberger (unpublished results) introduced the term “zigzag HSB” for the specific modification of the Hunter-Schreger bands with “level II decussation.” Using the term “zigzag HSB” for this enamel type would strengthen continuity in the nomenclature.

Author: The term “zigzag” roughly describes the shape of the folds in hyena HSB. However, in a phyletic and functional context, a more discriminating term is useful to emphasize the uniqueness of this structure compared to zigzagging HSB that characterize the enamel structure in other taxa, including the pattern in the tusks of astrapotheres (Rensberger and Pfretzschner, 1992). Beyond the zigzag shape of the HSB that occur in other taxa, the folded HSB that characterize hyena cusps have prisms at the crests or troughs aligned in a single (and reversed) direction to form a second level of prism decussation of larger scale that is superimposed on the primitive decussation from which it was derived and which characterizes most mammals. The designation Level II decussation or Level II HSB calls attention to this reorganization and emphasizes the functional implications of the structure and its derivation.

B. Van Valkenburgh: It is possible (but unlikely) that the remarkable Level II decussation observed in the teeth of spotted hyenas is a family characteristic and unrelated to diet. Have you been able to examine the canine teeth of the less durophagous members of the Hyaenidae - the aardwolf and striped hyena?

Author: Based on reflected light microscopy, the structure in the canine of the striped hyena is similar to that in the spotted hyena. I haven't examined the aardwolf, but Stefen (1997) found only the undulating HSB characteristic of many carnivorans in its dentition, consistent with the hypothesis that Level II decussation is not simply a family characteristic.

B. Van Valkenburgh: It would be fascinating to track the evolution of bone cracking teeth from the extinct species Hyaenidae to the modern forms in terms of both macro- and microstructure. From this perspective, have you had a chance to examine any extinct species of hyaenids? Or the extinct North American borophagine dogs, which are well known for their convergence in craniodental structure with modern hyenas?

Author: Level II HSB are extensively developed in the borophagines (personal observation), consistent with the other convergences on hyenas. Clara Stefen has traced the distribution of enamel types in the fossil and Recent species of the Hyaenidae; the data will be published soon.

J. Thomason: What sorts of modifications are necessary to the cellular mechanisms causing the enamel to lay prisms down in complex versus simple patterns? The context of the question is that complex enamel seems to be available on demand; animals specializing on hard food seem to be able generate complex HSB structure. This implies that the changes to the production mechanism do not in themselves represent a major adaptation.

Author: The prism directions follow the paths of the ameloblasts as they travel through the thickness of the enamel from the EDJ to the outer surface. The HSB result from groups of ameloblasts that travel as a unit, pass near other groups moving in a different direction and change directions in their courses at certain loci. What mechanisms control the directions of ameloblasts and orchestrate the changes in direction are not known. Level I prism decussation appears early in the Paleocene, as soon as small carnivorous mammals (arctocyonids) were emerging, and indeed indicates that Level I decussation can evolve rapidly.

Although the diversity of structures, the phyletic changes that must lead to these complex patterns and the functionality that has been found seem to argue for “availability on demand,” there appear to be constraints. For example, the vertical prism decussation that characterizes the enamel in rhinoceroses and astrapotheres is functionally optimal in the flat parts of the lophs of the cheek teeth but not in the regions where the enamel bends around cusps; in the latter regions the vertical decussation still dominates but horizontal decussation is developed in the middle of the enamel thickness there; owing to the horizontal direction of the dominant tensile stresses in the convex enamel, the optimal direction of the decussation plane in those regions would be horizontal. The actual structure that exists in a given taxon may be constrained by pre-existing microstructures, competition between different decussation mechanisms that may be optimal in different dental regions, and the degree of modification of the factors controlling ameloblast paths that is required for a particular solution.

J. Thomason: Is it possible to test the mechanical properties of enamel (ultimate stress and its anisotropy, in particular), and has this been done? Such data are obviously desirable for your FE models.

Author: There have been a number of studies of the material properties of enamel (including that of Rasmussen *et al.*, 1976). The resulting values for some of these properties, for example the elastic modulus, have varied considerably, probably because of the anisotropy in enamel and the difficulty of preparing samples of thin materials of irregular shape for testing without modifying the mechanical properties in the sample.

C. Wood: How does structure on single, conical cusps in the postcanine dentition fit in with this analysis? That is, would the model hold for those cusps?

Author: Level II HSB are present in the conical cusps of the other teeth. This structure is also visible in dogs near the tips of the larger cusps of the premolars and molars, though irregular or absent in the small cusps, for example paracone and metacone of M^2 and paracone of P^1 . The same stress relationship described for the canine holds for other conical cusps in any mammal that chews with vertical jaw movements. The criterion for Level II HSB is the magnitude of the vertical load, so that many mammals with cusps like these do not require such specialized structures because the loads are lower.