Structure of White Rhinoceros (*Ceratotherium simum*) Horn Investigated by X-ray Computed Tomography and Histology With Implications for Growth and External Form

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ABSTRACT The nasal and frontal horns of two individuals of *Ceratotherium simum* were examined by x-ray computed tomography (CT scanning), gross observation of sectioned horn, and light microscopy of histological sections of the horn tissue. CT scans of both sets of horns reveal a periodic banding pattern that is evident upon gross observation of sections as darker bands of tissue. The overlap of these bands in both histological and CT slices suggests the presence of both a photoabsorbent component (melanin) and a radiodense component (calcium phosphate salts, most likely hydroxyapatite or octocalcium phosphate). The distribution of these two components in the horns is hypothesized to contribute to the differential wear patterns that produce the characteristic sweeping conical shape of rhinoceros horn from what otherwise (in the absence of wear and UV exposure) would be cylindrical blocks of constantly growing cornified papillary epidermis. Although extant rhinocerotids are unique in possessing a massive entirely keratinous horn that approximates the functions of keratin-and-bone horns such as those of bovid artiodactyls, the tissue structures that make up the horn are strikingly convergent with other examples of papillary cornified epidermis found in horses, artiodactyls, cetaceans, and birds. J. Morphol. 267: 1172–1176, 2006. © 2006 Wiley-Liss, Inc.

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Rhinoceros horns are unusual among the horns of ungulates in that they lack a bony horn core. Instead, the horns are anchored to the dermis covering the frontal and nasal bones, and are associated with pronounced bony rugosities in most individuals (Hieronymus and Witmer, 2004). The true "horny" part of rhinoceros horn is an epidermal derivative, consisting of keratinized tubules of cells set in an amorphous keratinized matrix. The tubules comprise ~40 lamellae of squamous cells and range from 300–500 μm in diameter (Ryder, 1962). The amorphous matrix is made up of keratinized fusiform interstitial cells (Lynch, 1973). Each tubule grows from a generative layer of epidermis (stratum germinativum) covering a dermal papilla. The amorphous matrix is grown from the stratum germinativum of the epidermis between dermal papillae. As the epithelial cells of the horn are dead upon the completion of keratinization, all growth in rhinoceros horn takes place at the base.

Rhinoceros horns, as structures formed of cornified papillary epidermis, are part of a phylogenetically diverse assemblage of convergent cornified epidermal appendages, including the cornified sheaths of pecoran artiodactyl horns, bird beaks, turtle beaks, amniote claws and hooves, and baleen (Homberger, 2001). The independent origin of each of these examples provides a basis for identifying convergent morphologies, which in turn may shed light on functional aspects of cornified papillary epidermis (e.g., resistance of tubules to bending, preferential tearing directions). Here we report on previously undescribed aspects of melanization and calcification in the horns of white rhinoceros, *Ceratotherium simum*, and discuss the impact these that features may have on the growth and shape of the horn.

MATERIALS AND METHODS

The horns examined in this study came from two individuals, a 32-year-old female (Ohio University Vertebrate Collection [OUVC] 9541) formerly housed at The Wilds (Cumberland, OH) and a 41-year-old male (OUVC 9754) formerly housed at the Phoenix Zoo (Phoenix, AZ). Both animals died for reasons unrelated to this study.

The nasal and frontal horns of OUVC 9541 (Fig. 1A) and the frontal horn of OUVC 9754 were bisected in the midsagittal plane for gross anatomical observation. A longwave ultraviolet lamp (Ultra Violet Products UVL-26P, Upland, CA) was used to examine fluorescence in the epidermal horn (Fig. 1A). The right half of the nasal horn of OUVC 9541 and the entire frontal horn of
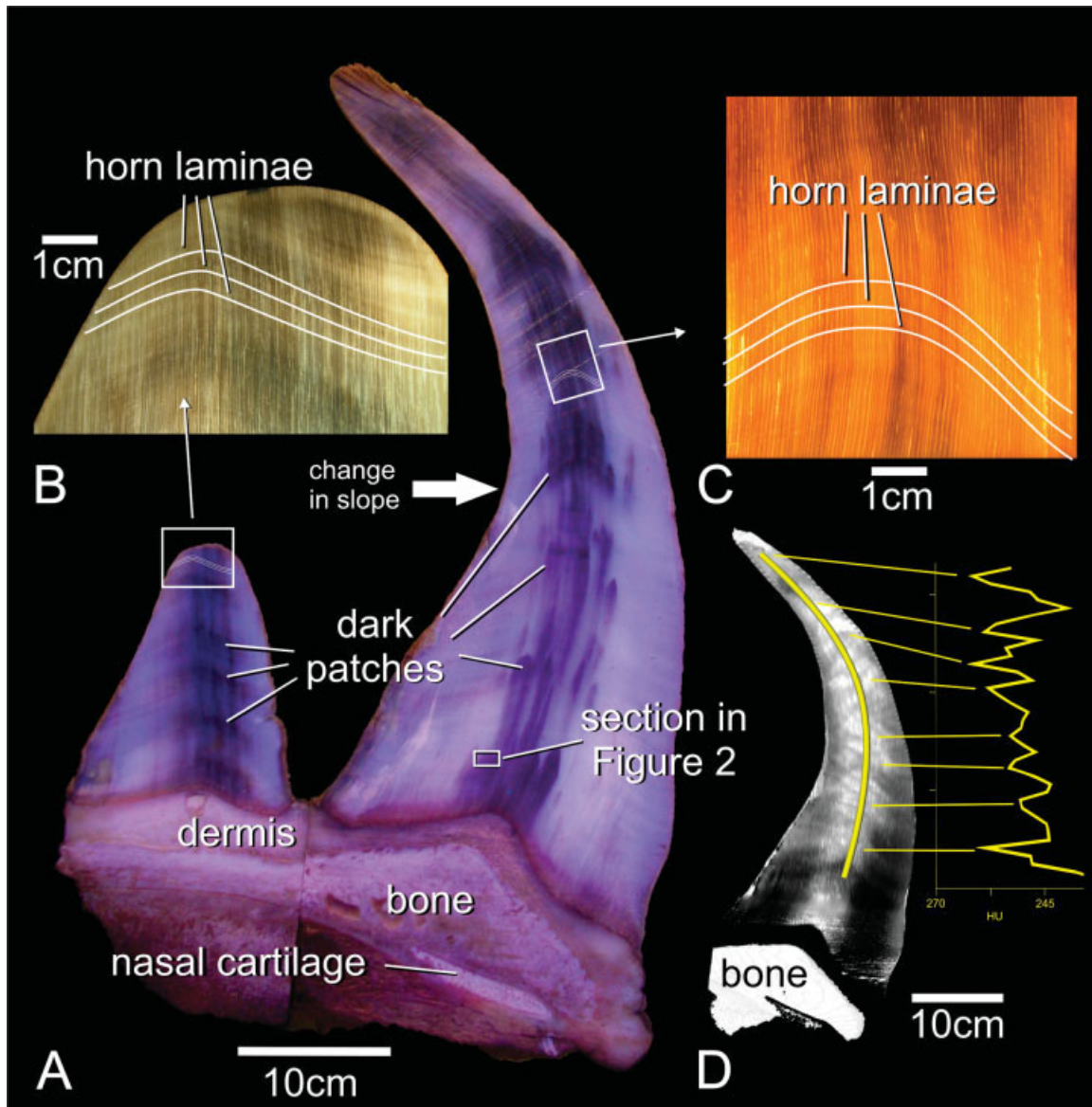
Fig. 1. Nasal and frontal horns of white rhinoceros (*Ceratotherium simum*, OUVC 9541) in sagittal section, as viewed under fluorescent light (**A**), white light (**B,C**), and by CT (**D**). Note the fine horn lamellae (**B,C**) as well as the periodic alternation of dark patches (**A**). Small box in **A** shows area sampled for histological section in Figure 2. Arrow in **A** shows a break in the curvature of the nasal horn, most likely related to a decreased rate of wear of the more calcified and melanized horn. Strong overlap exists between the melanized dark patches (**A**) and radiodense patches in CT scan (**D**). The line probe next to the CT scout in **D** shows this change in radiodensity in Hounsfield units.

OUVC 9754 were scanned on a GE HiSpeed FX/i Helical CT scanner at O'Bleness Memorial Hospital (Athens, OH). Slice thickness and spacing was 1 mm. Scanning parameters for OUVC 9541 were 120 kV and 150 mA, whereas those for OUVC 9754 were 120 kV and 120 mA. The field of reconstruction was 278 mm for OUVC 9541 and 282 mm for OUVC 9754 for 512 × 512 pixels using a bone algorithm. CT data were compiled in the Amira 3.1.1 (Mercury-TGS, San Diego, CA) and eFilm 2.0 (Merge-eFilm, Toronto, Canada) software packages for analysis and 3D reconstruction.

Portions along a medial parasagittal section of the horn of OUVC 9541 (Fig. 1A) were embedded in EpoThin epoxy (Buehler, Lake Bluff, IL), mounted on plastic slides, and ground to ~2 mm thickness. This set of unstained sections was examined by trans-mitted light microscopy to determine melanin distribution within the horn.

RESULTS

Horn is deposited dorsoventrally in successive sheets (here termed horn laminae) with irregular layers of ~1.0–2.0 mm. Each lamina represents a presumably coeval period of growth of horn tubules and intertubular matrix. In sagittal or transverse section, horn laminae appear as bands (Fig. 1B,C). The horn laminae fluoresce under UV light, aiding
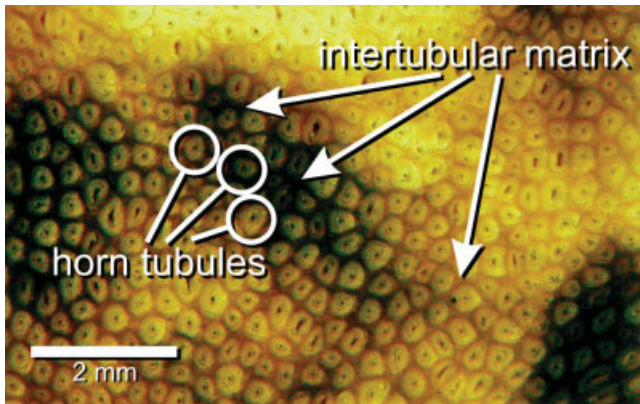
Fig. 2. *Ceratotherium simum*, OUVC 9541. Transmitted light view of a thick section (200 μm) of white rhinoceros horn from the center of a dark patch, showing areas of melanized intertubular matrix surrounding lighter horn tubules.

in their delineation. The color value of each lamina varies across its lateral extent, such that the central part of each lamina is darker in color than the periphery. This central dark patch is not uniform along the length of the horn, but rather shows pulses of darker horn interspersed with lighter horn. These dark patches alternate at an ~6-cm interval (Fig. 1A). The pattern of dark patches is also visible in CT as alternating radiolucent and radiodense bands (Fig. 1D). Gross examination of the frontal horn

shows a similar pattern of periodic dark patches at an ~2-cm interval (Fig. 1A) and horn laminae that alternate irregularly at ~0.5–2.0 mm (Fig. 1B).

Histological examination of thick sections shows that within dark patches more heavily pigmented cornified epidermal tissue is restricted to the intertubular matrix (Fig. 2). The horn tubules themselves retain a similar light color from the edge of the horn to its center. Rhinoceros horn can thus be viewed as a composite material, with tubules of keratinocytes forming "fibers" that are embedded in a matrix of varying composition (Fig. 3).

DISCUSSION
Periodic Banding and Annual Growth

The 6-cm periodicity of the radiodense dark patches in the nasal horn corresponds very well with annual growth rates of white rhinoceros nasal horn in wild populations (~5 cm/year per Pienaar et al. [1991]; 5–6 cm/year per Rachlow and Berger [1997]; both rates were measured from internal landmarks in the horns and represent tissue turnover rather than whole-horn elongation). The 2-cm periodicity of the frontal horn reflects its relatively slower growth, which is also consistent with the findings of Rachlow and Berger (1997). The periodicity of the horn laminae is much more irregular. Color value changes
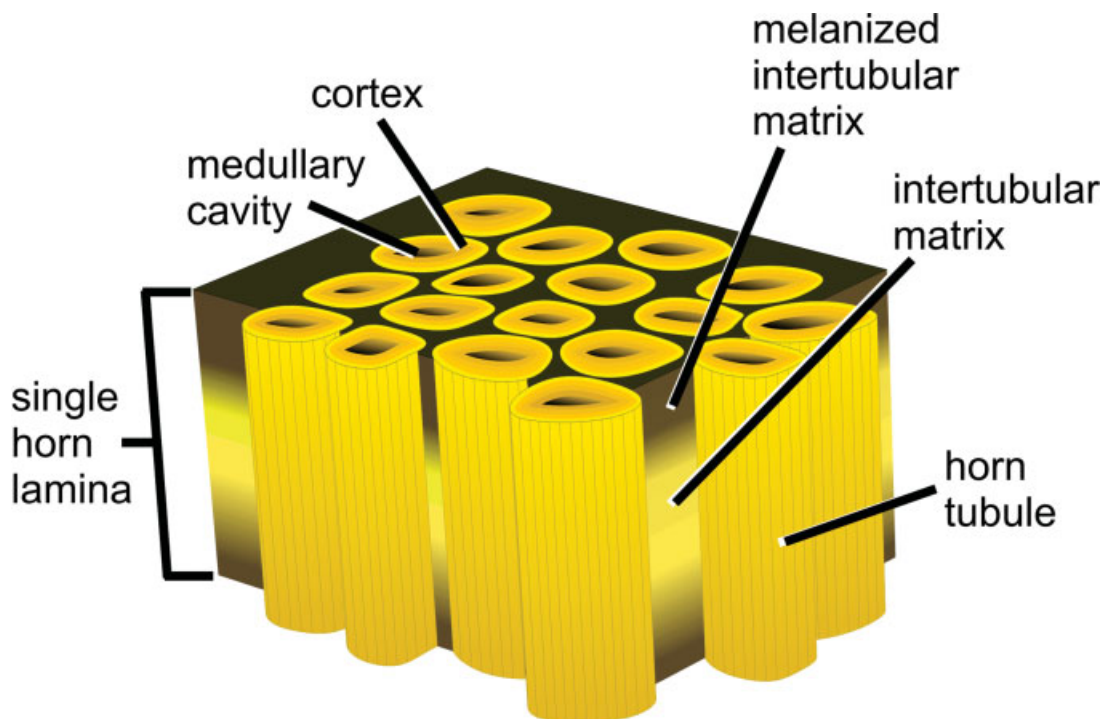

Fig. 3. *Ceratotherium simum*. Schematic of white rhinoceros horn showing horn tubules, each with cortex and medullary cavity, as well as melanized and nonmelanized intertubular matrix, together composing one horn lamina.

between adjacent horn laminae may be more akin to fault bars in feathers, which are caused by changes in keratinization due to external factors (mechanical damage, diet, etc.) during feather growth (Prum and Williamson, 2001).

Seasonal variation in the growth rates of other keratinized tissues such as the claws of sheep and cattle have been variously linked to changes in photoperiod and changes in temperature (Clark and Rakes, 1982; Hahn et al., 1986). OUVC 9541 spent the entirety of its life outside of the climate and historical latitudinal range of naturally occurring white rhinoceros populations (~40° N in Ohio, USA, compared to a probable historical range in Africa of ~33° N to 33° S as per Groves [1972]). OUVC 9754, however, lived in an environment (Arizona, USA) that is quite similar to the northern- and southernmost extent of the African range. As both of these specimens show similar periodic structures in their horns, we are confident that this horn morphology is not simply an artifact of unusual environments.

Co-occurrence of Radiodense Features and Dark Periodic Bands

The intensity of the dark patches suggests that there are differences in the rate of melanin deposition during the process of horn growth. Although this satisfactorily explains the gross observation results, melanin itself is not radiodense enough to produce similar patterns in a radiograph. The difference in contrast in radiography can be attributed to higher concentrations of calcium salts accompanying melanin deposition in the dark patches. Co-occurrence of melanin and calcium (as octocalcium phosphate) has been noted in the horns of saiga (*Saiga tatarica*) (Hashiguchi et al., 2001). The presence of higher concentrations of calcium can be interpreted as a primary mechanism and not a pathological finding, as several other forms of horny tissue aside from rhino and saiga horn also contain appreciable portions of hydroxyapatite or octocalcium phosphate (Arnott and Pautard, 1968; Pautard, 1970; Hashiguchi et al., 1995).

Horn Growth and Shape

The generalities of rhinoceros horn morphology have been fairly well understood for quite some time (Boas, 1931), but the mechanism by which horns maintain this morphology has received little attention. The variations in melanin content and calcification described here provide a mechanistic basis for controlling horn shape by differential wear.

Melanin has been variously implicated in increasing the hardness and strength (Bonser and Witter, 1993; Bonser, 1996b) as well as the long-term resistance to wear (Averill, 1923; Bonser, 1996a) of cornified epidermal structures at a gross level. However, a number of studies have shown

no quantifiable increase in work-to-fracture or hardness (stiffness) associated with melanin in cornified epidermal tissues such as horse hoof wall and feather barb (Bertram and Gosline, 1986; Douglas et al., 1996; Butler and Johnson, 2004), thus refuting a mechanically significant role for melanin in these systems. Keratins are substantially weakened by prolonged exposure to UV light (Marshall, 1986), and melanin may act to reduce the degree of wear by absorbing light entering the tissue (Jimbow et al., 1986). Although melanin itself does not appear to contribute to increased work-to-fracture or hardness, it is highly probable that calcification accompanying melanization (as shown by Hashiguchi et al. [2001] and this study) changes the hardness or compressional modulus of these tissues. The co-occurrence of calcification in melanized cornified epidermis may be responsible for the equation of hardness and melanization reported in other systems (Bonser and Witter, 1993; Bonser, 1996a).

The higher concentration of melanin and calcium salts in the center of white rhinoceros horn is likely to play a role in determining the overall conical shape of the horn. Healthy horn grows at a nearly constant rate throughout its areal extent. In the absence of any wear or keratin degradation, growing rhinoceros horn would form a gently curving cylinder. Three major factors combine to remove material from the horns by abrasion and wear: 1) UV-induced keratin degradation (Marshall, 1986); 2) reduced work-to-fracture as the horn tissue desiccates (Bertram and Gosline, 1987; Kitchener, 1987); and perhaps most importantly 3) stereotypical behavioral use patterns, such as scraping and "horn-wiping" on the ground, vegetation, or bars in an enclosure, and horn-clashing between individuals (Bigalke, 1945; Kingdon, 1979; Owen-Smith, 1988; Dinerstein, 2003). Progressive wear on older (i.e., more distal, dehydrated, and UV-damaged) portions of the horn produces the characteristic conical horn shape. The fact that mature males engage in more frequent bouts of scraping and horn-clashing than females may thus explain their slightly shorter horns (Kingdon, 1979).

The horns of many rhinos are not uniformly conical, but rather show a marked change in slope, such that the base forms a squat cone and the distal part continues as a more tapered cone. This change reflects the rate at which softer outer horn is worn away to expose more resistant material in the center. The change occurs near the point where the more heavily melanized and calcified tissue nears the external wear surface (arrow in Fig. 1A). The difference between the concentration of melanin and calcium salts in the intertubular matrix of the horn and the tubules themselves suggests that the intertubular matrix is responsible for these differences in hardness.

CONCLUSIONS

Rhinoceros horn provides an independently derived example of a cornified papillary epidermal appendage. The concentration of melanin and calcium salts in the core of rhinoceros horn varies annually, and appears to play a role in maintaining characteristic horn morphology. Local differences in melanin content and calcium salts reflect changes in the composition of the intertubular matrix, without necessarily involving the tubules of the papillary dermis.

Although the specific disposition of melanin and calcium salts in rhinoceros horn is perhaps unique among cornified papillary epidermis, the general tissue structure that forms rhinoceros horn is strongly convergent with many similar tissues, such as ungulate hoof wall (Nickel, 1938), bovid artiodactyl horns (Trautmann and Fiebiger, 1952:368), baleen plates (Lambertsen et al., 1989), and the papillary horn of cockatoo bills (Homberger, 2001). Comparative studies that take advantage of this convergence may shed light on phylogenetic and functional controls on cornified epidermis morphology.

ACKNOWLEDGMENTS

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