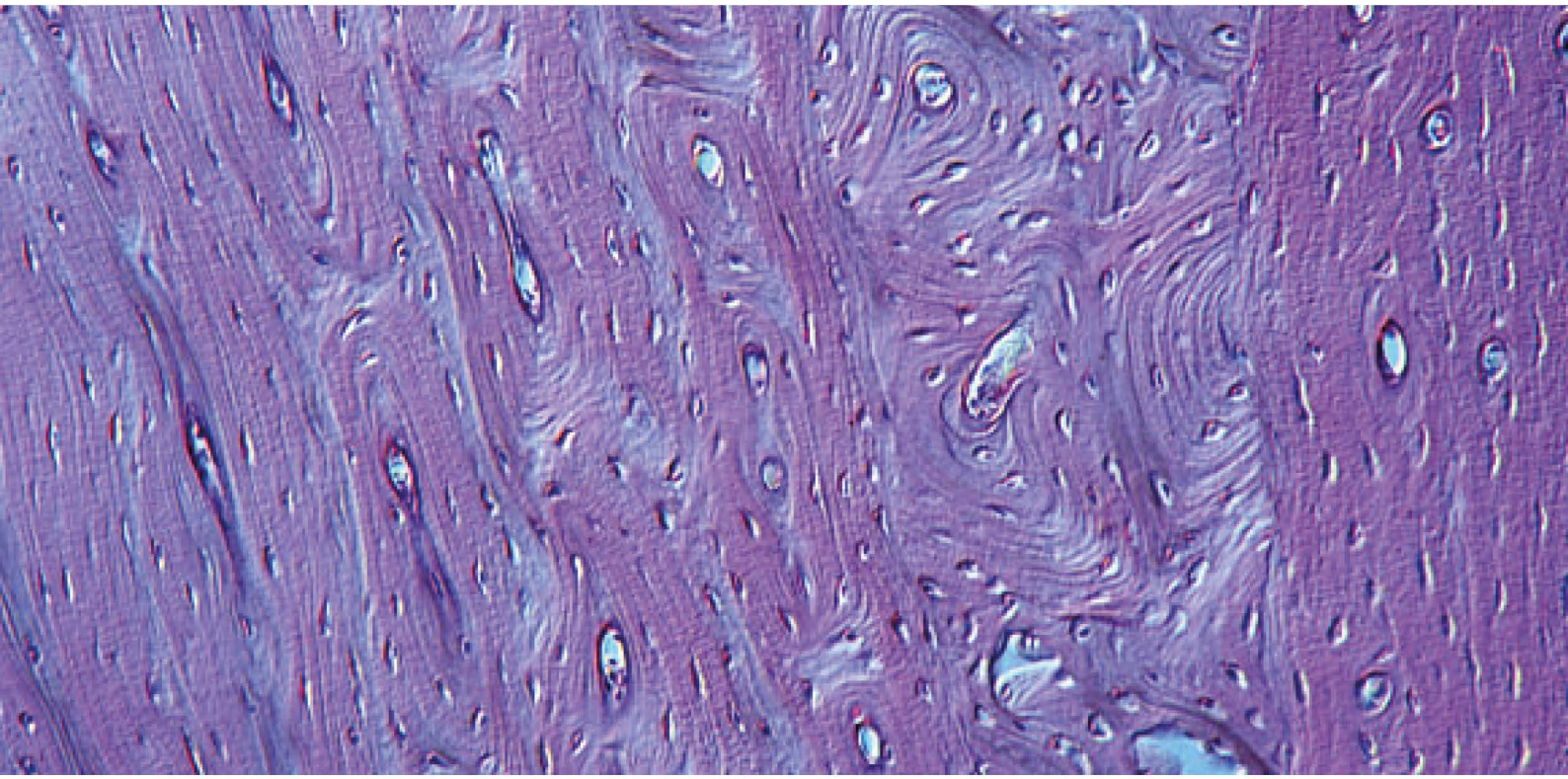

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Enamel-Calibrated Lamellar Bone Reveals Long Period Growth Rate Variability in Humans

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Key Words
Enamel striae of Retzius · Lamellar bone · Growth rhythms

Abstract
Mammalian teeth exhibit incremental structures representing successive forming fronts of enamel at varying time scales, including a short daily increment called a cross striation and a long period called a stria of Retzius, the latter of which, in humans, occurs on average every 8–9 days. The number of daily increments between striae is called the repeat interval, which is the same period as that required to form one increment of bone, i.e. the lamella, the fundamental – if not archetypal – unit of bone. Lamellae of known formation time nevertheless vary in width, and thus their measures provide time-calibrated growth rate variability. We measured growth rate variability for as many as 6 years of continuously forming primary incremental lamellar bone from midshaft femur histological sections of sub-Saharan Africans of Bantu origin and known life history. We observed periodic growth rate variability in approximately 6- to 8-week intervals, and in some cases annual rhythms were visible. Endogenous biological periodicities, cycles manifest in the external environment, and/or perturbations of development are all potentially contained within growth rate variability studies of lamellar incremental patterns. Because lamellae are formed within defined periods of time, quantitative measures of widths of individual lamellae provide time-resolved growth rate variability that may reveal rhythms in human bone growth heretofore unknown.

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Introduction

Cyclic or periodic textures manifest in nature belong to the class we refer to as incremental patterns, which reflect life history attributes and potentially information concerning the mechanisms of pattern formation. Lamellar bone is one such incremental pattern which, if calibrated in time and measured appropriately, might reveal growth rate variability attributable to endogenous and exogenous factors.

Abbreviations used in this paper

CPL	circularly polarized light
PMMA	polymethylmethacrylate
RI	repeat interval
UMCOM	University of Malawi College of Medicine

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Background

Bone-forming cells, i.e. osteoblasts, secrete their organic matrix in discrete tissue patterns which frequently include formation of the lamella, a fundamental microanatomical unit of bone observed in the postnatal tissues of many vertebrate taxa [Enlow, 1975; Enlow and Brown, 1956, 1968; Ricqles et al., 1991]. Though many bone tissue types that are not lamellar exist, to date we have examined growth rate variability from calibrated lamellae in the green iguana, Wistar rat, domestic sheep, macaque and patas monkeys, and humans. The lamella is characterized in histological thin sections by a highly oriented band of collagen separated from adjacent bands by an interlamellar zone of less oriented collagen [Ascenzi et al., 1967], resulting in the discrimination of one lamella from the next in polarized light. Lamellar bone is strikingly incremental in appearance, but the periodic formation of lamellae is the subject of only 2 preliminary reports [Okada and Mimura, 1940; Shinoda and Okada, 1988]. In this early research a 24-hour rhythm was recorded for the formation of 1 lamella in 3 small mammals and a longer but uncertain rhythm in 1 larger mammal.

Fortunately, the possibility now exists to assign a time scale to lamellar bone using calibrated enamel increments that, in mammalian teeth, exhibit incremental features representing successive forming fronts of enamel at varying time scales [Bromage et al., 2009]. There exists a daily period which, by light microscopy, is recognized as a 'cross striation', and there is a long period, i.e. the 'stria of Retzius', that in humans occurs every 8–9 days on average and is measured as the number of cross striations between adjacent striae. The number of daily increments between striae is the repeat interval (RI), which is identical for all teeth of an individual yet variable between and occasionally within a species. Species variability in the RI reflects a statistically significant and positive relationship with body size explained by the discovery that the period responsible for RI formation is one and the same as that required to form one increment of bone, i.e. the lamella, which is the fundamental – if not archetypal – unit of bone [Bromage et al., 2009]. Lamellae of known formation time nevertheless vary in width and thus provide time-calibrated growth rate variability. We aim to apply novel image-analytical methods for characterizing periodic textures manifest in lamellar bone incremental patterns and to illustrate growth rate variability for as many as 6 years of continuously forming primary incremental lamellar bone from midshaft femur histological sections of sub-Saharan Africans of Bantu origin and known life history.

Materials and Methods

Hard-Tissue Specimens and Life History

Midshaft segments approximately 10 cm in length were cut from cadaveric femurs on the right side of 12 people of Bantu origin following the gross anatomy program of the Department of Anatomy of the University of Malawi College of Medicine (UMCOM). One half mandible from the right side was also acquired from 7 of these individuals.

Following cadaver selection, UMCOM staff administered a questionnaire to the next of kin in which medical, social, economic, and life history information was sought. The medical history is particularly relevant to disease risk in Malawi. Social history information relates to living conditions and employment. We also acquired common life history variables, such as age, height, and weight (mass). In addition, questions were developed to solicit information relating to autonomic function.

Specimen Preparation

Nonmineralized organic components of the bone and tooth samples were subjected to daily changes of fresh 1% Tergazyme (Alconox, White Plains, N.Y., USA) enzyme detergent at 50°C until clean [Boyde, 1984]. A circa 1.0-cm block was sawn from the midportion of each femur and subjected to further enzyme detergent treatment until there were no visible nonmineralized organics.

Bone and tooth specimens were subjected to graded ethanol substitution and then 50:50 isopropanol:heptane reflux in a Soxhlet apparatus for 7–14 days followed by polymethylmethacrylate (PMMA) substitution and embedding. Cured PMMA blocks were hand ground on 1,200-grit paper with a Buehler Handimet II (Buehler, Lake Bluff, Ill., USA) along their sides facing midshaft, mounted onto a strain-free Exakt plastic slide (Exakt Technologies, Oklahoma City, Okla., USA), thin sectioned with the Exakt 300 CP Band System, and polished on an automated Exakt 400 CS Grinding System with 1,200-grit paper until plane-parallel at a thickness of circa 50 μm. Mounted histological sections were polished on a Buehler Ecomet 3 to produce a 1-μm surface finish.

Light Microscopy Imaging and Analysis

Histological sections were coverslipped with immersion oil and imaged in circularly polarized transmitted light (CPL) using a Leica-Leitz DMRXE Universal Microscope configured with a Marzhauser motorized stage, CPL filters, and Leica PL Fluotar 40/0.70 (enamel) and PL Fluotar 20/0.50 (bone) objective lenses (Leica Microsystems, Bannockburn, Ill., USA). CPL image montages were acquired using Syncroscopy Montage Explorer software (Synoptics, Frederick, Md., USA). Evaluation of the number of daily increments between adjacent striae of Retzius – the RI – was performed on XY montages of whole sections from 7 of 12 individuals.

Monochrome CPL montages of primary lamellar bone at periosteal and endosteal locations around the femur midshaft cortex were acquired in raster format and rendered as a binary using Adobe Photoshop CS3 (San Jose, Calif., USA) (fig. 1). To formalize the incremental pattern, we applied a discrete model based on the parameterization of the incremental structure [Smolyar and Bromage, 2004]. An in-house program written for Microsoft Excel 2007 (Redmond, Wash., USA) was used to import binary images

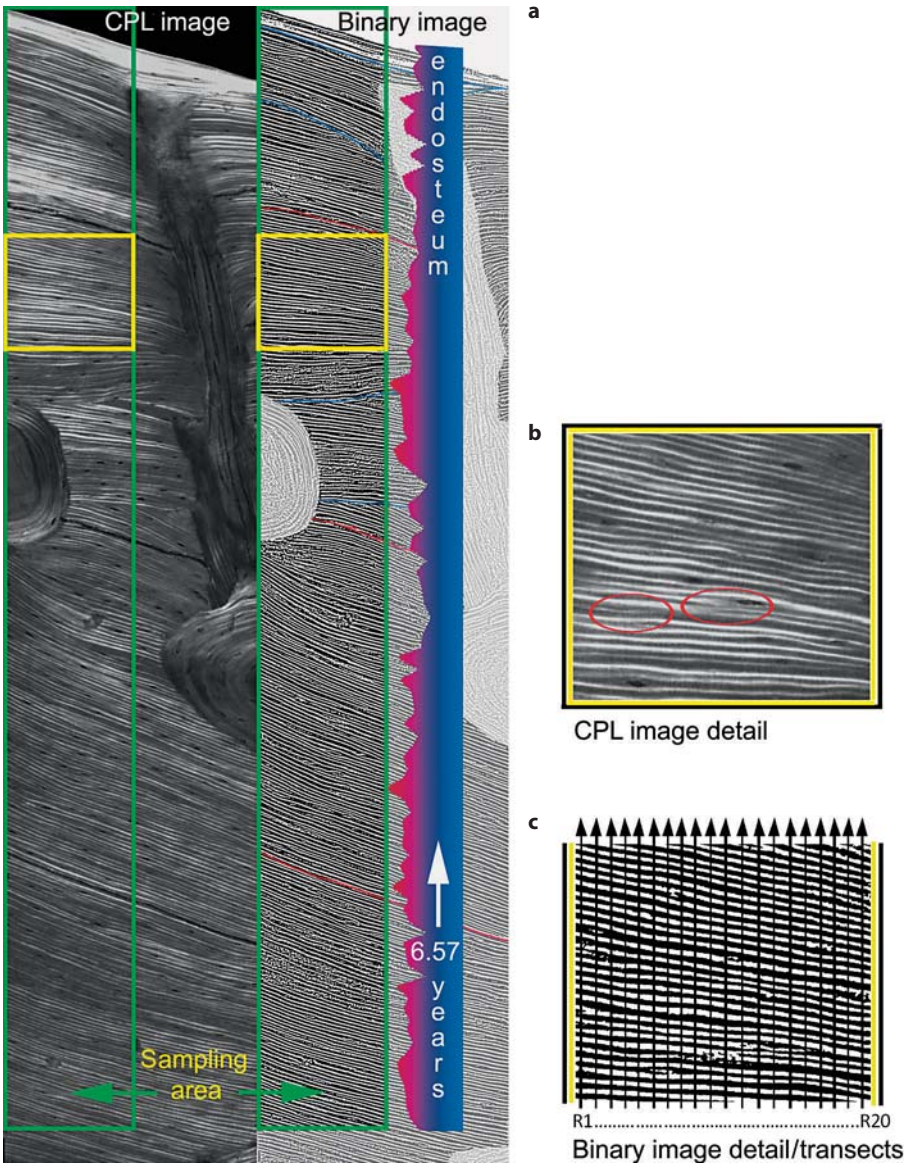


Fig. 1. a CPL and binary images of the primary lamellar bone of individual 08-02, a businessman (table 1) (field width circa 200 μm for each image). The areas analyzed for growth rate variability are enclosed within green insets. Red lines on the binary image are dehydration cracks, while blue lines represent instances in which a cessation or reversal in growth occurred. To the right of the binary image is a colored diagram of growth rate variability in which growth rate peaks to the left and decreases to the right from the bottom to the top (endosteum) of the image for a duration of over circa 6.5 years. **b** CPL image detail from the yellow inset in **a**, in which anisotropic lamellae are illustrated in marked red oval areas. **c** Binary image detail with transects overlain in preparation for the measurement protocol.

and to semiautomatically plot transects perpendicular to the direction of growth. Transects generated intersections with lamellae, each of which was given an XY coordinate. Measurements were then made (in μm) between all adjacent coordinates along transects. The impact of anisotropy on the accuracy of measurements and, hence, evaluations of growth rate variability was assessed by calculations of entropy and structural anisotropy, which we accomplished by evaluating all alternate possible relationships between lamellae crossed by adjacent transects [for a complete description of the method see Smolyar and Bromage, 2004] (for examples of lamellar bone anisotropy see fig. 1).

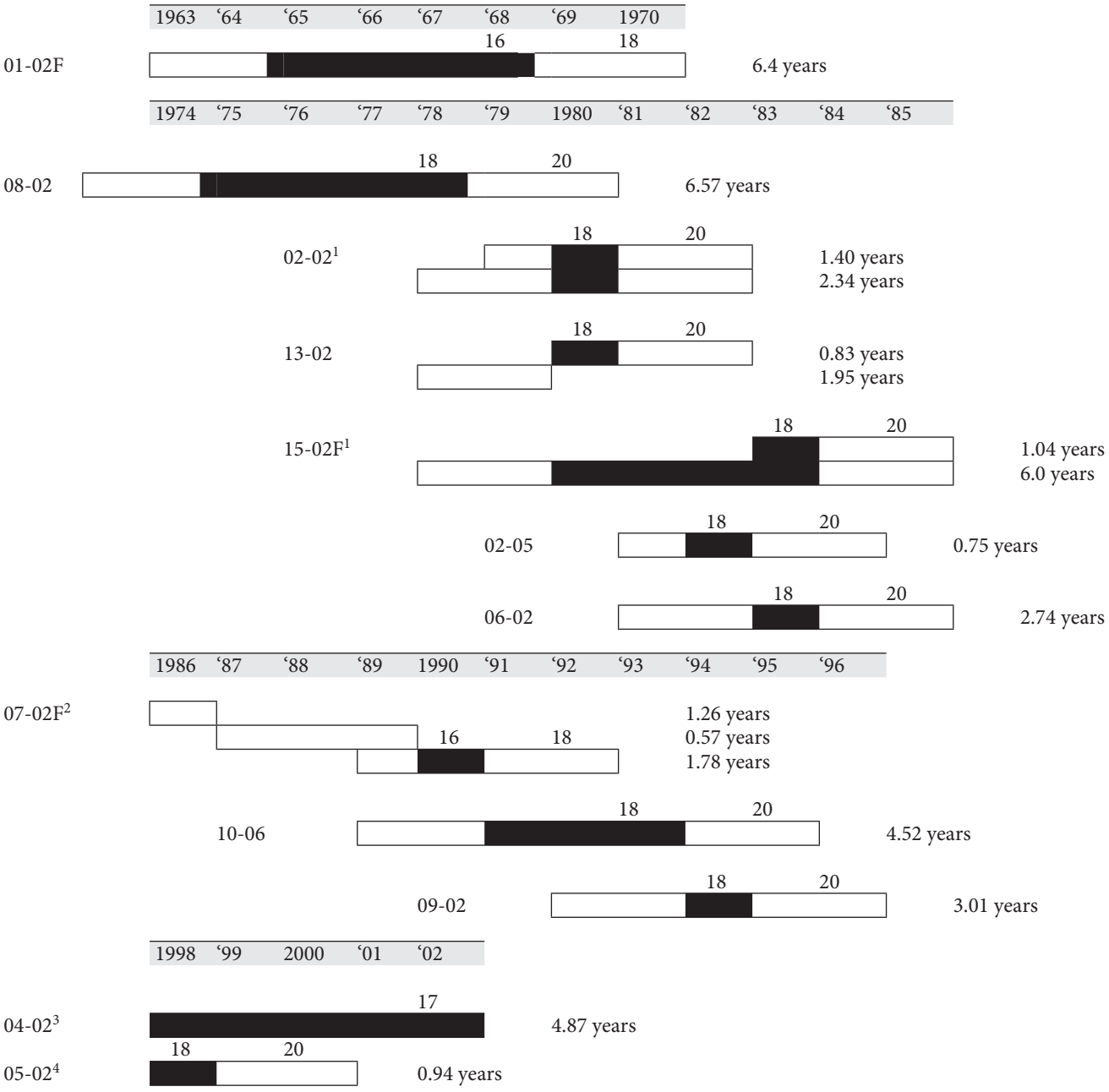
Duration of Lamellar Development

We used the RI to calculate the total duration of lamellar development for each CPL montage. There were 5 individuals in the study sample that did not have enamel specimens, and thus for them we used calculations of average RI values obtained from a large sample of teeth from people of sub-Saharan African origin.

Because bone lamellae and/or their CPL montages are frequently anisotropic, it is not possible to calculate an unbiased number of lamellae and, thus, the duration of lamellar bone development. However, in the absence of an accepted protocol, we subjectively counted the number of lamellae as best we could considering the anisotropy we could see and believing our calculations to be close (e.g. 5%) to the real time elapsed.

To place the duration of lamellar bone development in approximate real time, we extrapolated the time to include known ranges of ages at skeletal maturity from studies of physical development in people of sub-Saharan African origin [Loder et al., 1993; Lewis et al., 2002]. These studies provide information useful for

Table 1. Lamellar bone development and approximate alignment in real years



Bars/boxes for each individual in the study sample appear below the years (shaded). Specimen numbers to the left of the boxes/bars include an F for females; otherwise individuals are male. Black bars indicate the duration of lamellar development for the years most likely to be represented. Age ranges at skeletal maturity are given above the bars/boxes, and the total lamella formation time is given on the right for each histological section evaluated based on the 9- and 8-day lamellar formation rate for females and males, respectively. Open boxes to the left and right of the black bars indicate the duration of lamella formation if maturing early or late, respectively.

[1] Two CPL montages were assessed for this individual.

[2] Cement lines indicate arrested growth for 2 periods of lamellar deposition indicated as open boxes of 1.26 and 0.57 years' duration.

[3] This individual was still growing at the time of death.

[4] This individual was still growing at the time of death if late maturing.

estimating the average age of skeletal maturity among Malawians, which for girls is between 16 and 18 years and for boys is between 18 and 20 years. The alignment of lamellar bone development in real time included the possibility that any one individual may have matured either early or late with respect to age ranges at skeletal maturity for their sex (table 1). An assumption that we must naturally make, and which we agree needs testing, is that the last lamella formed at periosteal or endosteal margins in the principal direction of cortical growth (i.e. drift) is at or near to the time of skeletal maturity.

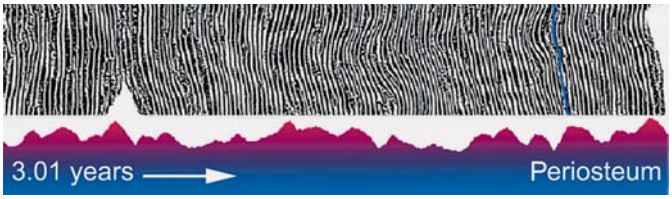


Fig. 2. Binary image of the primary lamellar bone of individual 09-02, a fisherman (table 1) (field height circa 200 μm). The growth rate is shown on the Y-axis while time is represented on the X-axis. Three cycles of growth occur in 3 years.

Results

Seven individuals of the 12 in our study sample included teeth for obtaining their RI. Six males were observed to have an RI of 8 days (02-02, 06-02, 08-02, 09-02, 10-06, and 13-02) and 1 female exhibited an RI of 9 days (15-02) (table 1). To estimate the RI for the remaining 5 specimens in the study sample, we calculated average values for a sample of 244 South Africans of African origin of known age and sex described previously [Reid and Dean, 2006]. Raw data for this sample kindly provided by Don Reid indicate that, while the sample range was 6–12 days, female RI tends to be longer (mode = 9 and mean = 9.2) than the RI of males (mode = 8 and mean = 8.6); this is in agreement with the suggestion of a sex difference in this sample by Smith et al. [2007] and with the values observed for the teeth in our study sample. Thus, for determinations of the duration of lamellar bone development from all CPL montages, 9 days/lamella was used for females and 8 days/lamella was used for males.

Most individuals in the study sample provided subjective periodic growth rate variability in the approximate range of 6–8 weeks; for instance, individual 08-02, a businessman, has a roughly 8-week rhythm (fig. 1). However, attention to the characteristics of the original CPL and binary images for this individual reveals several discontinuities; red lines on the binary image are dehydration cracks and have no timeline significance, while blue lines represent instances in which a cessation or reversal in growth occurred for some unspecified period of time. This means that assessment of the duration of lamellar bone development for this image represents a minimum period of time.

Subjective annual periods are also revealed in some cases. Individual 09-02, a fisherman, illustrates a low frequency rhythm containing 3 increases in 3 years (fig. 2).

We have made 2 other observations of note. Our first observation is that all individuals in the study sample

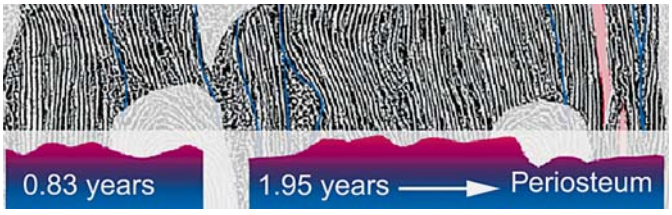


Fig. 3. Binary image of the primary lamellar bone of individual 13-02, a farmer (table 1) (field height circa 200 μm). The growth rate is shown on the Y-axis while time is represented on the X-axis. The low amplitude of growth rate variability may reflect that this individual was a farmer who experienced a severe drought during the time represented by the primary lamellar bone deposition.

maintained their pattern of periodic growth rate variability to the very last lamella to be deposited. There is no decrease in growth rate at the completion of cortical bone development, contrary to what one might expect from the decelerating body mass or height velocity curves of developing children. Our second observation is that the growth rate variability for 2 subsistence farmers is subjectively more flat, lacking growth spikes and relatively abrupt growth decelerations characterizing the other individuals in the UMCOM sample (e.g. individual 13-02; fig. 3) comprising fishermen, businessmen and women, sex workers, and the unemployed.

Discussion

Information about biological periodicities, environmental and/or physiological cycles, and growth perturbations are all potentially contained within growth rate variability studies of lamellar incremental patterns. Because lamellae are formed within defined periods of time,

quantitative measures of widths of individual lamellae provide time-resolved growth rate variability with the potential to reveal bone growth rhythms.

Previously we observed a circa 28-day rhythm in lamellar bone growth from an early human ancestor [Bromage et al., 2009], a result that we also obtained in preliminary analyses of a human female of Anglo-Celtic origin living in Melbourne, Australia (results not reported here). In the present study of humans of Bantu origin, for the first time we have observed striking lamellar growth rate rhythms revealing heretofore unknown cycles at various length scales. The 6- to 8-week rhythm frequently identified (fig. 1) does not presently align with any known endogenous physiological or exogenous environmental rhythm, while the occasional observance of an annual rhythm (fig. 2) is known to occur in seasonal environments that experience yearly oscillations in resource availability [Klevezal, 1996].

The lack of subjective evidence for decelerating growth rate variability during the last 6 months to 1 year of cortical development is interesting. On the one hand, analysis of the ontogeny of the section modulus – a measure of bone strength depending on the tissue mass and distribution of the bone cross section – in a longitudinal sample of people of mostly northern European origin found no clear lag in the temporal association between growth velocity of bone strength and body weight/bone length measures [Ruff, 2003]. Thus we may be confident that the last bone lamella formed was at or near the time of skeletal maturity; indeed, the longitudinal analyses by Ruff [2003] clearly show velocity curves for femoral strength indices that mirror those of whole body mass and height velocity curves. This is likely because increases in height necessarily accompany an increase in weight and increased bending under a load, both of which the midshaft femur is sensitive to. We thus suspect that primary lamellar bone deposited on endosteal surfaces facing the direction of growth should be more or less synchronous with periosteal deposits on the contralateral cortex during cortical drift and size/shape change at the midshaft femur; we acknowledge that this relationships needs testing.

That our results do not indicate a deceleration in growth velocity may in part be due to a difference in the way European vs. African-Bantu achieve their femoral bone cross section properties. The difference may also reflect the comparison observed between industrialized and developing nation peoples of sub-Saharan African origin. In a study of Kenyan Turkana pastoralists, a marked difference in the pattern of growth was revealed; while African-Americans are consistently heavier at all ages, the Turkana pastoralists nevertheless reach the same height at maturity [Little et al., 1983]. What is more, the Turkana reach this height via a more consistent and prolonged period of growth than do African-Americans, and as such they do not have the discernible inflection point toward asymptotic growth moderation in their distance curve (i.e. the accumulated growth curve) at adolescence.

We are also fascinated by the low amplitude of growth rate variability characterized by the 2 subsistence farmers contrary to the increased variability observed for people in other occupations. Both of these farmers had been depositing primary lamellar bone while Malawi experienced a severe drought (individuals 02-05 and 13-02 during the 1980–1981 drought; table 1) in which conditions are recognized to have deleteriously affected the small farmer [Vogel et al., 1999]. We provisionally suggest that diminished growth rate variability is a characteristic of farmers prone to fluctuations in climate and who are less capable of ameliorating shortfalls and food instability.

Our preliminary research on 12 individuals of Bantu origin from Malawi indicates that long period growth rate variability assessed by analyses of incremental lamellar bone is a common feature of human development. Our research on this topic will be fueled by 5 years of systematic collecting of up to 100 individuals which is to commence in 2011, and our hope and expectation is that future studies will link observed long period growth rate variability to real physiological and environmental rhythms.

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References

Ascenzi, A., E. Bonucci, D.S. Bocciarelli (1967) An electron microscope study on primary periosteal bone. J Ultrastruct Res *18:* 605–618.

Boyde, A. (1984) Methodology of calcified tissue specimen preparation for SEM; in Dickson, G.R. (ed): Methods of Calcified Tissue Preparation. Amsterdam, Elsevier, pp 251–307.

Bromage, T.G., R.S. Lacruz, R. Hogg, H.M. Goldman, S.C. McFarlin, J. Warshaw, W. Dirks, A. Perez-Ochoa, I. Smolyar, D.H. Enlow, A. Boyde (2009) Lamellar bone is an incremental tissue reconciling enamel rhythms, body size, and organismal life history. Calcif Tissue Int *84:* 388–404.

Enlow, D.H. (1975) Handbook of Facial Growth. Toronto, Saunders.

Enlow, D.H., S.O. Brown (1956) A comparative histological study of fossil and recent bone tissues. Part 1. Tex J Sci *8:* 405–443.

Enlow, D.H., S.O. Brown (1957) A comparative histological study of fossil and recent bone tissues. Part 2. Tex J Sci *9:* 186–214.

Enlow, D.H., S.O. Brown (1958) A comparative histological study of fossil and recent bone tissues. Part 3. Tex J Sci *10:* 187–230.

Klevezal, G.A. (1996) Recording Structures of Mammals: Determination of Age and Reconstruction of Life History. Rotterdam, Balkema.

Lewis, C.P., C.B.D. Lavy, W.J. Harrison (2002). Delay in skeletal maturity in Malawian Children. J Bone Joint Surg Br *84-B:* 732–734.

Little, M.A., K. Galvin, M. Mugambi (1983) Cross-sectional growth of nomadic Turkana pastoralists. Hum Biol *55:* 811–830.

Loder, R.T. et al. (1993). Applicability of the Greulich and Pyle skeletal age standards to black and white children of today. Am J Dis Child *147:* 1329–1333.

Okada, M., Mimura, T. (1940) Zur Physiologie und Pharmakologie der Hartgewebe. 4. Mitteilung: Tagesrhythmus in der Knochenlamellenbildung. Proc Jpn Pharm Soc 95–97.

Reid, D.J., M.C. Dean (2006) Variation in modern human enamel formation times. J Hum Evol *50:* 329–346.

Ricqles, A.D., F. Meunier, J. Castanet, H. Francillon-Vieillot (1991) Comparative microstructure of bone; in Hall, B. (ed) Bone-Volume 3: Bone Matrix and Bone Specific Products. Boca Raton, CRC Press, pp 1–78.

Ruff, C. (2003) Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. Bone *33:* 317–329.

Shinoda, H., M. Okada (1988) Diurnal rhythms in the formation of lamellar bone in young growing animals. Proc Jpn Acad *Ser. B, 64:* 307–310.

Smith, T.M., D.J. Reid, M.C. Dean, A.J. Olejniczak, R.J. Ferrell, L.B. Martin (2007) New perspectives on chimpanzee molar crown development; in Bailey, S., J.J. Hublin, (eds): Dental Palaeoanthropology. Berlin, Springer, pp 177–192.

Smolyar, I., T.G. Bromage (2004) Discrete model of fish scale incremental pattern: a formalization of the 2D anisotropic structure. ICES J Mar Sci *61:* 992–1003.

Vogel, C., M. Laing, K. Monnik (1999) Drought in South Africa, with special reference to the 1980–1994 period; in Wilhite, D.A. (ed): Drought: A Global Assessment. London, Routledge Press.