Introduction: Cementochronology in Chronobiology

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The purpose of this edited volume was to bring together specialists from various fields to present all the information needed to understand and implement cementochronology, the analysis of cementum growth. This interdisciplinary "Cementum Research Program" was initiated in 2010/2011 at a Paris workshop with Jean-Pierre Bocquet-Appel, Joël Blondiaux, Thomas Colard, and me. Initially, the goal was to develop a standardized cementum protocol for age estimation in anthropology (Colard et al. 2015). However, the program's scope expanded rapidly into an first poster symposium held at the 2012 annual meeting of the American Association of Physical Anthropology (AAPA) in Knoxville (USA). With the invaluable support of Jane Buikstra, selected presentations were published in a special issue of the *International Journal of Paleopathology* (Naji et al. 2016).

This event's positive feedback prompted our group to reach out even more broadly to the international community. Our purpose was to connect with zooarchaeologists who were implementing cementum analyses routinely and paleoanthropologists interested in evolutionary processes using dental remains. In collaboration with William Rendu and Lionel Gourichon, the second phase of our research program started in 2015 (Naji et al. 2015) funded by a sizable French grant (ANR CemeNTAA) dedicated to cementum analysis. The results were presented as a second poster symposium at the 2017 annual meeting of the AAPA in New Orleans (USA). This event was an ideal opportunity to meet new colleagues and motivated students. Consequently, prompted by many colleagues' support, in particular Daniel Antoine, we decided to publish our collaborative efforts in this edited volume.

This publication's premise is to reply to one of the first questions anthropologists ask when considering dental cementum as an age indicator: Why should cementum growth follow an annual/seasonal deposition pattern in distinct layers on the roots' surface?

This chapter is a short introduction to the field of chronobiology to present current theories explaining and interpreting cyclic growth marks observed in skeletal and dental tissues, specifically in cementum. The references are by no means comprehensive, but the reader should find adequate primary sources to explore the topic in greater detail in (Dunlap et al. 2003; Foster 2005; Kumar 2017; Lemmer 2009; Lincoln 2019).

Cementochronogly Nomenclature

First, I will briefly discuss the various labels used to name cementum growth deposits to ensure that all scientists, regardless of their field, are talking about the same histological structures. For a detailed history of cementum discovery in biology, see Foster (2017) and Buikstra (Chapter 1). According to the following definitions, we have tried to homogenize the nomenclature relative to cementum growth analysis throughout this volume.

As early as 1887, Black summarized contemporary cementum knowledge, describing its structure as individual "lamellae, layers, or strata" divided by distinct lines called "incremental lines of the cementum" and observing its chronological deposition pattern as "each lamellae being the results of a single period of activity [. . .] each successive lamella is younger than the preceding one" (Black 1887,105–6). Sixty years later, these incremental structures started to be tested for aging animals and humans under several different names describing the contrasting optical layers visible in histological sections.

For animals, Scheffer (1950) and Laws (1952) defined external "growth ridges" and internal "annual growth zones or rings," respectively, for dentin growth in sea mammals, but not cementum. Sergeant and Pimlott (1959) were the first to investigate the principle of growth layers for age estimation in cementum using moose as their study sample. They referred to "cement growth layers" to characterize the annual histological structure composed of the two seasonal "growth zones," one opaque and one translucent. Klevezal' and Kleinberg, in their first seminal review (Klevezal' & Kleinenberg 1967, 67), referenced "annual layers" composed of "bands" and "stripes."

In 1978, the conference on odontocete age estimation (Perrin et al. 1980) proposed standardizing dental growth markers' terminology. Every layer parallel to a tissues' formative surface, contrasting with the adjacent one, was defined as an "incremental growth layer." A repeatable pattern of growth layers counting as a time unit was then termed a "growth layer group (GLG)."

In terrestrial mammals, however, cementum growth layers have a slightly different interpretation: The "growth zones" (Baglinière et al. 1992) represent layers interpreted as a rapid deposition due to increased metabolic activity during the "favorable" season (Demars, Le Gall & Martin 2007, 109). Conversely, annuli (singular, annulus) are slow growth layers formed during a decrease of osteogenic activity. Annuli are thus thinner than zones. Also, in French terminology, "growth rest lines" (*ligne d'arrêt de croissance*) (Castanet 1980) were defined as a very thin structure, highly birefringent and often hypermineralized, that can be found within an annulus or alone and alternating with rapid growth zones (Baglinière et al. 1992, 444).

Finally, in her second seminal review, Klevezal' (1996) proposed a new definition for the cementum growth unit as a "growth layer of the first order" composed of an incremental cementum line (principal element counted for age estimation) and a cementum band (intermediate element).

In humans, the first use of cementum for age estimation specifically was published by Gustafson in 1950 with a nonspecific "cementum apposition" component, representing cementum width, not incremental counts, in his multicriteria dental method (Gustafson 1950). Three decades later, the pivotal publication of Stott and colleagues (1982) used the incremental count of cementum deposits for the first time and described them as "cemental annulations." Since then, cementum annuli (Stein & Corcoran 1994) was used preferentially.

In the influential first large-scale controlled study, Wittwer-Backofen and Buba (2002) labeled the use of cementum as the teeth cementum annulations (TCA) method. The acronym seems to be the most preferred among anthropologists today. However, this name can create confusion with the mammal's slow growth layer's "annulus." In forensic anthropology, Wedel proposed another descriptor, dental cementum increment analysis (DCIA) (Wedel 2007), which is entirely accurate.

Finally, following the 1992 publication of the symposium on vertebrate age estimation using hard tissues (Baglinière et al. 1992), another term, the French "*cémentochronologie*" was proposed to follow the larger context of chronobiology (Grosskopf 1996; Martin 1995). In the English literature, "cementochronology" was introduced during the 82nd annual conference of the American Association of Physical Anthropology in 2013 in a contributed poster symposium titled "Cementochronology," organized by Naji, Colard, and Bertrand (Naji et al. 2013). The purpose of this nomenclature shift was to reflect the broader multidisciplinary approach to a common biological growth process and move away from potentially confusing descriptors.

Chronobiology, the Cycles of Life

The periodicity of growth processes has a deep history, from the ancient Greeks' understanding of daily leaf movement to the first published demonstration of endogenous plants' periodicity in 1832 (cited in Schwartz & Daan 2017). Formally, the field of chronobiology, the study of biological rhythms, can be traced to the 1960 edition of the Cold Spring Harbor Biological Laboratory's annual symposium in Long Island, New York, titled "Biological Clocks" (Lemmer 2009).

Chronobiology rests on the premise that the regular rotation of the earth around its central axis and around the sun produces two fundamental periodicities to which all life, from unicellular organisms to primates, has become adapted. The hypothesis is that circadian clocks govern daily rhythmicity, and circannual clocks provide a seasonal endogenous calendar (Lincoln 2019). The various external stimuli (e.g., light cycle, food availability, temperature variation) provide a template for living organisms to anticipate cyclic environmental events by periodic and predictable internal adjustments in physiology and behavior, even where standard environmental cues are weak or ambiguous (Piccione et al. 2009). The frequencies of these rhythms have evolved to cover nearly every division of time (Lemmer 2009), from *intradian* – less than a day – oscillations of

one per second (e.g., brain waves), or one per several seconds (e.g., heart rate); *circadian*, one within twenty-four hours (e.g., enamel cross-striations, dentine's von Ebner's lines); *multidien*, five-day rhythm (e.g., enamel of domestic pig, Bromage et al. 2016); *circaseptan*, near-seven-day periodicity (e.g., heart rate and pressure, Reinberg et al. 2017); *lunar*, once a month (e.g., ovulation); to *circannual*, one per year (e.g., reproduction, molt, migration, and cementum).

Today, chronobiology is incorporated into practically all fields of human and nonhuman endeavors, including ecology, biology, sociology, and psychology (Reinberg et al. 2017) to optimize sleep, diet, immune system response, or performances, among other factors.

Mechanisms Responsible for Annual Cycles

We will focus here on the mechanisms and consequences of the annual cycles (For a review of circadian cycles, see Panda et al. 2002; Weinert & Waterhouse 2017). Annual rhythms can be classified into three types (Lincoln 2019; Zucker et al. 1991).

Type 1 trans-generational annual rhythms are mostly observed in short-lived species with multiple generations throughout the year. The innate circannual timing mechanism passes from individual to offspring across the year and may be expressed at only one phase of the life cycle (Lincoln 2019). The presence of annual environmental cues such as annual variations in temperature and photoperiod influences the annual neuroendocrine rhythms via hormonal maturation, for example, puberty and reproduction (Zucker et al. 1991). This is a population/cohort response because an individual cyst only hatches once. A variant is a modular rhythm for longer-lived insects that transform from the egg, through larval instars, to pupation and hatching of the sexually mature adult (Lincoln 2019). Again, this rhythm is a population/cohort event because each animal pupates only once.

Type 2 seasonal (circannual) progressive rhythms recur under constant conditions with a period between ten and twelve months, more typical of long-lived species, including primates. The progressive development from juvenile to adulthood to old age and circannual timing generates cycles in multiple aspects of physiology and behavior (e.g., gonad size, body weight, food intake, gut morphology, immune function, molt, thermoregulation, hibernation, and migration) (Lincoln 2019). Type 2 persists in the absence of periodic light input (Zucker et al. 1991) but varies among and within individuals (Piccione et al. 2009). Animals have an individual annual chronotype (e.g., early rutting/late rutting). This circannual chronotype has been observed in humans with a seasonal affective disorder that could be interpreted as a natural adaptation to winter, where a change in appetite and increased body weight in autumn and the development of withdrawal behaviors in winter was once an advantage for our hunter-gatherer ancestors (Lincoln 2019). Also, mRNA expression levels indicated in one study that 23 percent of the genome showed significant seasonal differences with two distinct antiphase patterns: One set of genes up-regulated in summer and the other, approximately equal, up-regulated in winter (Dopico et al. 2015).

Type 3 annual rhythms are found in animals living in unpredictable environments (desert/equator) where the cue is rainfall and plant growth. In other words, type I rhythms are evoked by environmental cycles, while types II and III are synchronized to environmental cycles (Piccione et al. 2009).

The current working hypothesis to explain circannual cycles assumes that this timing first evolved in free-living eukaryote cells as an adaptation to survive the winter (Lincoln 2019). These organisms alternate between seasonal growth and dormancy across their life-history, which requires a genetically regulated, cell-autonomous, and transgenerational mechanism. Switching between growth in the circannual "summer" and dormancy in the circannual "winter" is a highly adaptive strategy that has thus been conserved in our evolution and is observable today in five of the eight eukaryotic kingdoms (Helm & Lincoln 2017).

The Clock-Shop Model: Combining plant models with animal models provides a general theoretical mechanism linking circadian (daily) modification to circannual rhythm timescale (Schwartz & Daan 2017). Knowledge of the physiological pathways governing time measurement mechanisms has been unfolding rapidly in the past decades through comparative studies that have uncovered the photoperiodic signal transduction cascades in birds, fish, and mammals (Foster 2005). These studies revealed the universality and diversity of photoperiodic mechanisms, such as the fact that molecules involved are conserved while the tissues responsible for these mechanisms are species-specific (Ikegami & Yoshimura 2017). The highly adaptive strategy of annual growth, irrespective of an organism's size and longevity, has determined the evolution of innate, genetically regulated timing processes, broadly encompassed by the clock-shop model (Lincoln 2019). In mammals, in particular, we now understand that the cyclic rhythms are controlled by the body's "central clock" located in the brain at the suprachiasmatic nuclei and supported by "peripheral clocks" located in several other tissues (Liu & Panda 2017; Zheng et al. 2014).

The clock-shop model proposes that environmental signals, notably photoperiod, are relayed by the sensory systems to the central pacemakers to synchronize physiology with the seasons using, among other mechanisms, melatonin-responsive thyrotropic cells in the *pars tuberalis* of the mammalian pituitary gland (Ganguly & Klein 2017). The conjecture is that long-term timing mechanisms reside in all tissues but with dominant pacemaker systems in the brain and pituitary gland orchestrating the circannual phenotype (Lincoln 2019). Circadian and circannual timing systems thus share formal properties: ancestry, cell autonomy, innateness, entrainment, temperature compensation, and ubiquity (Lincoln 2019).

At a molecular level, the favored model for circannual timekeeping proposes that the long-time domain is generated by the cyclical epigenetic regulation of chromatin structure (DNA and histone proteins), determining whether specific circannual timer genes are transcriptionally active or not. This regulation drives the oscillation between the two stable, operational states of subjective summer and subjective winter (Lincoln 2019; Stevenson & Lincoln 2017).

The consequence is that circannual timing mechanisms are more flexible and reprogrammable in the long term, which means if a consistent seasonal change is

observed in any organism, it is most likely to be regulated by an endogenous timing mechanism rather than by a passive response to the environment.

Several cyclic mineralization pathways have been identified for bones, suggesting that bone deposition and mineralization are under direct circadian controls (Zheng et al. 2014). For example, the diurnal variation in the synthesis of type I collagen and osteocalcin is under a local circadian oscillator mechanism. Also, analyses in sheep and humans suggest that a single biological rhythm governs all lamellar bone formation within a given taxon (Bromage et al. 2009).

For teeth, there are daily variations in the rate of production and secretion of enamel proteins between early morning and late afternoon, suggesting that enamel protein secretion is under circadian control and that enamel matrix production and maturation are closely controlled by selectively regulating key enamel matrix proteins encoding genes (Zheng et al. 2014). Like enamel, dentin is formed incrementally, indicating the involvement of a circadian clock mechanism during dentinogenesis. This cyclic growth has been demonstrated using proline tracers that labeled collagen during dentin formation and showed that twice as much collagen is secreted during the daylight twelve hours as during the nocturnal twelve hours. These studies suggest that dentin, similar to bone and enamel, is controlled by a circadian clock mechanism (Zheng et al. 2014).

Unlike the other dental hard tissues, cementum does not seem to be controlled by circadian mechanisms but more likely by circannual ones. Stock and colleagues (2017) first identified second-order lines in Beluga whales between first-order (annual) increments in contrasting Ca and Zn variations. Dean and colleagues (2018) have also identified an average of twelve second-order increments (monthly), interpreted as menstrual within chimpanzee samples. Recent work on cementum presented in this volume (Chapter 1, Chapter 6, Chapter 7, Chapter 14) is starting to document specific pathways (e.g., pyrophosphate regulation, vitamin D absorption, hormonal variations) that might be involved in mammals' annual cycles, specifically.

Linking circadian mechanisms to annual ones across mammalian species of various body mass is a complex question. Lincoln argues that circannual rhythms are independent and cannot be explained by frequency demultiplication of circadian rhythms, although changes in the circadian system occur in parallel with the circannual cycle and are the basis of photoperiod entrainment of the circannual clock (Lincoln 2019, 4).

However, Bromage and colleagues (2016) have proposed a hypothesis that "a periodic rhythm longer than the daily biological clock regulates some aspects of metabolic variability that contribute to variability in body size and the pace and pattern of life" (Bromage 2016, 19). Their metabolome and genome analyses from blood plasma in thirty-three domestic pigs revealed that blood plasma metabolites and small noncoding RNA (sncRNA) strongly oscillate on a five-day multidien rhythm, as does the pig enamel.

The adaptive benefits for circannual timekeeping, especially for larger vertebrates, include two critical features. The first is its predictive power to anticipate and prepare for upcoming seasonal changes in the environment (Ball et al. 2017). In a highly seasonal habitat, where changes in food supply and other selective pressures can be

predicted through photoperiod, the timing mechanism allows for precise regulation of the timing of cycles in physiology and behavior of fundamental significance in evolution (Helm & Lincoln 2017). The second is organisms' ability to express robust annual cycles, which at specific phases override the effects of proximate cues, including photoperiods, such as in cross-equatorial migratory birds or hibernating species (Helm & Lincoln 2017).

Circannual Rhythms Validation Studies

Even though, in theory, circadian and circannual rhythms are relatively well understood from an evolutionary perspective, seasonal rhythms are expressed at the individual level. Therefore, these cyclic growth markers still need to be identified by repeated measurements on documented individuals to understand the mechanism's geographical and temporal variability. Circannual rhythms can be observed in many biological or behavioral mechanisms (Ball et al. 2017). In wildlife biology or anthropology, identifying a seasonal pattern in hard tissues to define a precise and accurate marker to estimate age and season at death or any life event represents a powerful tool to explore topics such as demography and mobility patterns robustly.

To demonstrate hard tissue seasonal growth rate in animals, including humans, three lines of evidence can be sought from documented subjects: (1) Long term capture–recaptures and sampling; (2) chemical labeling; and (3) empirical identification (Baglinière et al. 1992). We will see that all three have been successfully used in skeletal and dental tissues to support the hypothesis of circannual incremental deposits.

Bone and Other Hard Tissues

Sclerochronology is the method that describes elapsed time from recorded hard tissue (Baglinière et al. 1992). Otholometry studies fish's otolith (inner ear bone), composed of accretion of calcium layers with two periodicities: a daily cycle influenced by water temperature and an annual period (Baillon 1992; Kimura 1977; Kimura & Chikuni 1987). The annual periodicity of growth has been validated using both injection of fluorochrome calcein and empirical observations (Baillon 1992; Mounaix 1992). Similarly, fish scale growth scalimetry has been empirically observed to have an annual deposition rhythm in various species (Mounaix 1992).

Skeletochronology is the use of periodic incremental growth structure in bones for age estimation studies. It has been applied in dinosaurs, reptiles, and mammals (Castanet et al. 1977; Woodward et al. 2013) as early as the 1930s (Clerc 1927). Long-term capture–recaptures have demonstrated the annual growth of one layer of new bone every year through successive amputations of the same limb in amphibians such as toads (Hemelaar 1985). The annual growth pattern has also been tracked in vivo bone labeling of fluorescent marker calcein or tetracycline and recaptures in crocodiles (de Buffrénil & Castanet 2000). For example, in the dermal scutes of Nile crocodiles, the laminae (the bone tissue between successive growth marks) are deposited by

accretion/resorption phases so that the presence of one zone and one annulus marks the passage of one year (Woodward et al. 2013). Also related, cartilaginous shark bones present comparable circannual growth layers (Baglinière et al. 1992).

Mammals: The annual periodicity of bone growth has also been tested in mammals. An extensive study of the mouse lemur *Microcebus* assessed the number of annual growth layers in captive individuals of known age across the skeleton (Castanet et al. 1993). More recently, circannual cycles have also been empirically observed in forty species of ungulates of varied size, diet, and habitat from the Equator to near the Poles environments (Köhler et al. 2012). Results showed that lamellar bone growth is arrested during the unfavorable season and accompanied by decreases in body temperature, metabolic rate, and bone-growth-mediating plasma insulin-like growth factor-1 levels. This "growth arrest" forms part of a plesiomorphic metabolic strategy for energy conservation (Köhler et al. 2012). Conversely, at the beginning of the favorable season, phases of intense tissue growth coincide with peak metabolic rates and correlated hormonal changes, indicating an increased efficiency in acquiring and using seasonal resources (Köhler et al. 2012). These results from tachymetabolic mammals show unequivocally that annual growth layers are a universal pattern of homoeothermic endotherms and should not be regarded as anything more than endogenous markers of annual rhythms (Padian & Lamm 2013).

Human Studies: In humans, the use of bone remodeling patterns in age estimation is limited to growth stages of life because intracortical remodeling usually prevents any meaningful analysis in senescing individuals. In forensic contexts, however, the histological determination of adult age in cortical bone employs remodeling activity through the evaluation of osteon density, the number of primary vascular canals, the amount of unremodeled lamellar bone, the percent remodeled bone, and the average size of secondary osteons or Haversian canals (Streeter 2011). However, the correlation between observed age and estimated age in adults is poor at best since not all growth marks reflect environmental cues on growth. Some marks reflect temporary realignments of internal bone structure such as cortical drift; others may directly reflect environmental stresses (Woodward et al. 2013).

The timing of lamellar growth rates was measured using fluorescent labeling in the bones of rats, monkeys, sheep, and humans and revealed that the number of days needed to form one lamella is species-dependent: seven days for rats, twenty-eight days for macaques, thirty-five days for sheep, and fifty-six days for humans (Bromage et al. 2009). Further research based on the histological analyses of skeletal remains of twelve Bantu individuals of known sex and life-history discovered that incremental lamellar bone is deposited with long-period (five to six weeks) growth rate variability previously unobserved in humans (Bromage et al. 2011). Of greater interest to us, potential annual growth deposits have also been observed in some of the individuals (Bromage et al. 2011, 505).

Overall, there is ample evidence that skeletal growth markers that are periodic often occur annually (Woodward et al. 2013). It now also appears that in most vertebrates, including dinosaurs and mammals, cyclical growth markers resulting from the temporary cessation of growth simply reflect internal hormonal cues rather than direct environmental influence (Woodward et al. 2013).

Teeth

Odontochronology: With the advent of more powerful microscopes, dentin and enamel have also been explored to identify growth cycles (Hogg 2018) and, ultimately, cementum during the fifties (Chapter 1).

Enamel and Dentine: Mammalian teeth exhibit microanatomical incremental features representing successive forming fronts of enamel and dentine at varying timescales (Bromage et al. 2009; Dean 2006). The outcome is visible in light microscopy as a daily "cross-striation" and as a long period, "stria of Retzius," measured as the number of cross-striations between adjacent striae and is thus reported in units of whole days. The number of daily increments between striae is identical for all teeth of an individual yet variable between and occasionally within a species that reflects a positive relationship with body size (Padian & Lamm 2013).

Incremental dentin lines are termed von Ebner's lines, which delineate the amount of mineral deposited in a single day. The circadian mineralizing lines in dentine are distinguished by their characteristic appearance, where small spheres of mineralizing dentine increase in size until they eventually coalesce (Dean 2006). Using a biomarker on macaques, Bromage (1991) confirmed the daily rate of dentine von Ebner's lines formation. Later, Dean and Scandrett (1996) also used biomarkers on humans to correlate dentin and enamel formation. However, enamel and dentine have not been linked to any circannual growth patterns in mammals but have been in some species of toothed fish, where the annual growth lines were demonstrated by tetracycline labeling (Day et al. 1986).

Cementum: The benchmark of experimental studies involves using fluorochrome dyes to record cementum growth at precise intervals. In bears, epi-fluorescent photomicrography was used to date a chemical biomarker's exact position in cementum increments to the nearest year (Matson & Kerr 1998). Bosshardt and colleagues (1989) used tetracycline labeling and fluorescence mapping of acellular and cellular cementum in one macaque to demonstrate that acellular cementum formation is a tightly controlled biological phenomenon that occurs with the same regularity and speed wherever this type of tissue is needed.

Empirically, the presence of an endogenous growth rhythm based on growth mark formation in vertebrate skeletal tissues has been proposed by many researchers in a diversity of environments (Grue & Jensen 1979; Klevezal' & Kleinenberg 1967). For example, hibernating mammals form an annual increment despite being prevented from hibernating (i.e., in the absence of typical environmental influences) (Perrin et al. 1980). Also, Grue (1976) noted that mink raised on farms where they were fed a steady diet reduced their food intake during winter months. Likewise, cyclical cementum incremental growth is visible in tropical vertebrates and cannot be ascribed to marked seasonal fluctuations (Klevezal' 1996, 93). Logically, the early hypotheses formulated to interpret these observations included the presence of a genetic component for increment formation (Grue & Jensen 1979).

Finally, age and seasonal data obtained through the analysis of incremental growth structures corroborate similar data obtained through various other morphometric

methods (e.g., teeth attrition) in living specimens. In many cases, these provide more accurate and precise age estimates (Matson et al. 1993; Miller 1974; Stallibrass 1982).

Overall, the annual cementum growth cycle has been observed in control groups of known age and season of death of terrestrial (Klevezal' & Kleinenberg 1967; Lieberman 1993) and marine mammals (Klevezal' & Myrick 1984; Perrin et al. 1980; von Biela et al. 2008). Additionally, cementum has been found in fossils and extant animals outside of the mammalian phylogeny, including reptiles (Enax et al. 2013; Luan et al. 2009), ichthyosaurs (Maxwell et al. 2011), mosasaurs (LeBlanc et al. 2017), dinosaurs (García & Zurriaguz 2016), and toothed fossil birds (Dumont et al. 2016). Similarly, in humans, more than thirty-five studies of known-age individuals have been repeatedly successful in demonstrating the strongest correlation between acellular cementum increment number and chronological age (for a full summary, see Chapter 1; Naji et al. 2016; Naji & Koel-Abt 2017).

Biological Aging and the Tempo of Senescence

Today, biologists have a strong hypothesis for interpreting cyclic growth patterns. The evolutionary clock-shop model is gaining rapid empirical validation in chronobiology, even though tissue-specific molecular/physiological pathways are not fully understood yet, and intermediate mechanisms between circadian and circannual patterns may still need exploring (Bromage et al. 2016).

For cementum, the annual/seasonal periodicity has been demonstrated repeatedly in documented collections by chemical labeling studies and the most extensive cross-species empirical validation tests probably ever produced for an age indicator (Chapter 1). Also, there is some evidence that the antiquity of the thecodont tooth attachment system that includes the alveolar bone, a periodontal ligament, and cementum has been hypothesized to be a plesiomorphic shared feature of all amniotes for the past 290 million years (LeBlanc et al. 2017; Newham et al. 2020) and is thus probably under tight genetic control (Chapter 3, Chapter 4, Chapter 16).

Not knowing the precise molecular and physiological mechanisms for cementogenesis should not lead anthropologists to dismiss cementochronology entirely. This is not a failure of the method; this is simply the state of our current knowledge. Today, we cannot explain most of the adult standard anthropological aging methods we have been using for more than a century. Fundamental research and validation studies have allowed us to tease out some variables responsible for the skeletal and dental changes we observe and use to model biological age.

For example, in skeletal estimators based on the ilium's auricular surface, the sternal rib end or the cranial sutures, the "age" component is the weakest to explain observed changes. Biomechanical wear and tear are the principal components of change for the first two, and we still do not fully understand the processes for the latter. Infections (e.g., septic arthritis), mineralization defects (e.g., osteoporosis), or genetic syndromes (e.g., craniosynostosis) will also have a compounding or leading influence in any degenerative age indicator. Of course,