Growth variability, dimensional scaling, and the interpretation of osteohistological growth data

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Osteohistological data are commonly used to study the life history of extant and extinct tetrapods. While recent advances have permitted detailed reconstructions of growth patterns, physiology and other features using these data, they are most commonly used in assessments of ontogenetic stage and relative growth in extinct animals. These methods have seen widespread adoption in recent years, rapidly becoming a common component of the taxonomic description of new fossil taxa, but are often applied without close consideration of the sources of variation present or the dimensional scaling relationships that exist among different osteohistological measurements. Here, we use a combination of theoretical models and empirical data from a range of extant and extinct tetrapods to review sources of variability in common osteohistological measurements, their dimensional scaling relationships and the resulting interpretations that can be made from those data. In particular, we provide recommendations on the usage and interpretation of growth mark spacing/zonal thickness data, when these are likely to be unreliable, and under what conditions they can provide useful inferences for studies of growth and life history.

1. Background

The use of osteohistological data, derived from thin-sectioned bone cross-sections, to characterize the development, growth (via change in body mass), and life history of extinct tetrapods has seen widespread adoption and advancement in recent years. Building off earlier foundational work (e.g. [1–4]), new methods have been developed in recent decades that include age-retrocalculation, model-based growth estimation, estimates of sexual maturity and several physiological proxies [5–13]. Nevertheless, the most common use of osteohistological data in extinct tetrapods remains the assessment of relative ontogenetic stage and skeletal maturity based on largely qualitative or simplistic assessments of bone apposition rates up to the time of death. This is often performed through examinations of collagen fibre orientation and complexity, vascularity, counts of annual growth marks (e.g. lines of arrested growth or...
LAGs), thickness of growth zones/spacing between growth marks and the presence or absence of an external fundamental system or EFS (also known as an outer circumferential layer) [14–16].

Changes in growth zone spacing/thickness throughout the bone cortex are commonly used to infer the ontogenetic stage of individual organisms [17,18]. This approach has even been used to hypothesize novel taxa on the basis of putatively distinct growth rates (defined here as mass-specific growth rate) or adult body sizes (defined here in terms of body mass) when compared to known taxa in palaeo-ecosystems [19–21]. While in general terms growth zone spacing will decrease from the inner to outer cortex over the total ontogenetic period in tetrapods [4,11,22,23], this pattern varies and depends on functional and allometric patterns that are specific to the bone and taxon being analysed, as well as the ability to resolve patterns due to growth stage and individual growth variation. All of these factors may also be dynamic and shift through the ontogeny of an individual, as it encounters changes in resource availability and environment (e.g. [11]), biomechanical changes through ontogeny [24], reproduces [25,26] and/or responds to pathology [27].

In particular, selection of bones for sectioning is often predicated on factors such as availability or curatorial permission rather on their utility in assessing growth, even though different bones vary in their overall growth pattern in terms of the number, symmetry and width distribution pattern of growth zones between LAGs or other age markers. Horner et al. [14] demonstrated differing numbers of LAGs between elements in a hadrosaurid skeleton, while Waskow & Sander [28] noted a significant discrepancy in the number of preserved LAGs between ribs versus limb and girdle elements in a sauropod skeleton. Recent histological examinations of extant taxa have found that LAG counts can differ from absolute age and vary between elements, sexes and depending on the method of section preparation [26,29]. A taxonomically broad study employing inter-elemental sampling across theropods by Cullen et al. [30] noted only minor discrepancies in growth mark numbers between elements in some juvenile specimens, but found profound differences with respect to zonal widths and the development of an EFS between bones. Most notably, they found that bones that bear less weight (e.g. ribs, fibulae and gastralia) exhibit evidence for an earlier onset of growth cessation than do major weight-bearing bones such as the femur (supporting predictions made by Padian et al. [31]). Relatedly, some bones can act as a good proxy for overall somatic growth (e.g. primary weight-bearing bones, such as femora, in bipeds [30,32]), while, as a result of allometric and other effects, other bones may be more difficult to relate back to the growth patterns of the whole organism (e.g. metatarsals, ribs and gastralia [14,30,33,34]). Similar scales of allometric differences in bone development have been observed in extant birds, with markedly different relationships, and sources of variation apply to a wide range of extinct and extant taxa, and (III) examine additional case study data on whole-organism growth, interpretations of these smaller scale patterns of growth zone spacing or thickness within the cortex have been used to draw a variety of sometimes disparate conclusions concerning the relative growth patterns of individual organisms and species. For example, some describe patterns of evenness or steady decreases in zone thickness as an indication of slow or slowing growth rates (e.g. [38–44]), whereas others describe even or stable growth zone thicknesses as an indicator of consistent and/or fast growth rates (e.g. [45,46]). It is also relatively common to infer a linear relationship between growth zone spacing/thickness and overall growth rate/pattern (e.g. [38,47,48]). Potentially driving some of these interpretive differences may be the confounding of patterns observed in individual bones (or parts of bones) as being proxies to overall somatic growth of the whole organism (i.e. body mass), rather than being treated as a bone-specific property [49].

Crucially when considering the use of data from isolated bones, it should be noted that patterns of spacing can vary from one side of a bone when compared to another (e.g. [19] versus [50]), as well as between bones of the same individual [14,30,33,34,49]. This can be further complicated by differences in interpretation related to the assessment of ‘double/multiple’ growth mark complexes (e.g. [30,51]). Variation in growth zone spacing/thickness can also be problematic for some retrocalculation methods that use spacing to estimate missing LAGs (e.g. [52]). Several studies have previously discussed intra-skeletal and intra-bone variability in growth zone spacing/thickness, and the potential for subjectivity that can exist in inferring ontogenetic stage and growth pattern from zonal spacing changes (e.g. [14,30,33,34,50]). Others still have gone further and argued that growth zone spacing/thickness patterns cannot be used reliably under any circumstance in skeletally immature specimens (e.g. those lacking an EFS) [51].

Given these interpretive differences of organismal mass-specific growth and various proxies measured from osteohistological cross-sections, as well as the previously documented concerns related to the variability in these proxies and their use in relative ontogenetic inferences, our goal here is to (I) review theoretical models of bone apposition and growth, and the resulting dimensional scaling relationships between various measures in each and overall body mass growth in the whole organism, (II) test which interpretations best fit quantitative histological data for each of these measures obtained from a range of extinct and extant taxa, and (III) examine additional case study data on sources of intra-element variation in growth mark spacing and when these indicators can be of value as a relative growth indicator. While the majority of the data examined here are derived from dinosaurs, these patterns, scaling relationships, and sources of variation apply to a wide range of extant and extinct tetrapods and should be considered in any study reconstructing growth patterns using bone histology.

2. Methods

Three conceptual models with differing underlying growth assumptions were generated using a combination of Microsoft Excel and the R programming suite [53], visualizing in each case the expected bone cross-section (idealized as a circle for a
cylindrical bone shaft) and relative patterns of growth zone radius (R), delta growth zone radius (∆R; equivalent to LAG spacing), growth mark circumference (C), growth zone area (A), delta growth zone area (∆A), body mass (BM; approx. R² in weight-bearing limbs, see Campione et al. [54,55]), and delta body mass (∆BM) over the endosteal to periosteal record at the point of minimum circumference of an individual bone growing roughly symmetrically, such as the diaphysis of a femur or tibia. Following prior work [5], we assume that body mass can be inferred from the geometry of the bone cross-section as in the styloplodial bones of amniotes [54] and femora of bipeds [55]. To approximate true limb bone growth, our models include a central region free of LAGs to simulate the absence of early LAGs due to remodelling and/or medullary cavity resorption. Here, we are mainly concerned with how each variable changes relative to the others. As such, the presented curves are dimensionless, being scaled to a percentage for each variable for ease of comparison and visualization. The actual units would be millimetres (radius and circumference), square millimetres (area) and kilograms (body mass). We provide plots (with dimensions) for each specimen, model and measure, which can be found in the electronic supplementary material, data S1 and S2.

In Model 1, the pattern produced is based on an assumption of constant widths in growth zone radii (AR; LAG spacing) across the section (i.e. an endosteal to periosteal transect). This can be expressed as ∆R = k. In Model 2, the pattern produced is based on an assumption of constant growth zone area (∆A) across each zone in the section, expressible as ∆A = k. In Model 3, the pattern produced is based on an assumption that change in growth zone area (∆A) decreases at a scale of 0.8/LAG across the section, to simulate a constantly decreasing growth rate. This is expressed as ∆AN = 0.8 ∆AN−1 (where n > 3, n represents an ordinal number of the LAG, and A1 and A2 are arbitrary positive values). Other models using different scaling factors were also evaluated and yielded comparable results; model data can be found in the electronic supplementary material, data S1.

Empirical osteohistological growth data relating to the above measures were then also plotted for eight taxa and compared to the models. Data were obtained from the literature and collected by the authors. Samples represent a range of ontogenetic stages, taxonomic groups, and include both extinct and extant taxa. Data of the growth mark circumferences, growth zone area, inferred body mass, and changes in growth mark spacing/thickness were plotted for each specimen using the R programming suite and Microsoft Excel, and collated together as image plates using Affinity Designer. Detailed specimen and measurement data can be found in the electronic supplementary material, data S2.

Additional comparisons were performed to examine intra-bone and intra-individual (i.e. inter-bone) variability in growth zone spacing/thickness, as well as interpretive issues related to the presence of double/multiple growth mark complexes. Data from Fowler et al. [50] and Sereno et al. [19] were used to examine growth mark circumferences of the femur of LH PV18 (‘Raptorex kriegsteinii’) and measure growth zone spacing/thickness changes across endosteal to periosteal transects at four locations on the bone cortex in order to quantify variation in this metric within a single bone. Data from Cullen et al. [33] were used to provide an example of growth zone thickness differences between bones of the same individual (CMN FV 12086, cf. Dromiceiomimus brevitiertius (sensu [56])). Lastly, data from Cullen et al. [30,34], based on data originally published in Woodward et al. [51], from the tibia of a juvenile tyrannosaurid (BM0P 2006.4.4, cf. Tyrannosaurus rex) were used to illustrate double/multiple growth mark complexes, their variation across the cortex, and the potential impacts of differences in interpretation of these features. Measurement data for these comparisons can be found in the electronic supplementary material, data S3.

3. Results

Under Model 1 (figure 1a), where ∆R (i.e. LAG spacing) remains at a constant rate, the other measures show an increasing rate due to dimensional (i.e. geometric) scaling, in which a circle’s area increases by the square of the radius, and corresponding mass increases by the cube (e.g. A = πR², C = 2πR, BM = ∼R³). Consequently, this cannot be interpreted as total body growth rate remaining constant or consistently fast/slow. Due to these dimensional scaling relationships and the resulting nature of bone growth (a variation of the square-cube law), the rate of growth must increase in order for LAG spacing to remain constant (a well-documented mathematical property appreciated by many biologists and palaeohistologists, e.g. [23,31,57–59]). Relatedly, the pattern illustrated in Model 2 (figure 1b), where ∆A (change in zonal area; i.e. annual tissue apposition) remains at a constant rate, the radius will continue to change (R = √A/π), resulting in a pattern of steady decreasing rate in ∆R (LAG spacing). Such a pattern of consistent reduction in LAG spacing could be superficially interpreted as a decrease in growth rate, yet as this model demonstrates, it is actually representative of a constant rate of new bone tissue apposition where it is being measured. Body mass, scaling more closely to the cube of radius, would show an even stronger pattern in the deviation from linearity than appositional area (figure 1).

While Model 1 and/or Model 2 present LAG spacing patterns similar to those observed in some bone cortices or segments of bone cortices (usually ones with incomplete growth records of juvenile individuals, which do not include the growth inflection point, such as figure 1d,e), they are not representative of the pattern typically recorded when a majority or near-majority of the overall ontogenetic trajectory is preserved (figure 1f–k). Model 3 closely approximates this as a general pattern, operating under a pattern of zonal area decreasing at a rate of 0.8A/LAG. It is reflective of the universal rather than taxon-specific nature of these dimensional scaling relationships, that Model 3 approximates the pattern observed in small and large extinct non-avian theropods (figure 1g,i), ornithischians (figure 1h,i), extant mammals (figure 1j) and extant birds (figure 1k).

The immature theropod specimens (figure 1d,e) illustrate the difficulties of using LAG spacing for relative growth inferences. Although their overall circumference, area and body mass estimation patterns present a relatively consistent trend and are likely comparable to mature specimens of the same taxon as part of a broader growth assessment, their LAG spacing patterns are highly variable and likely prone to edge effects due to their relative nature and the short period recorded. A pattern of decreasing LAG spacing can, depending on whether growth is defined in terms of linear, area or volume, varyingly represent an increasing, steady/consistent, or decreasing rate of total body growth, making statements about decreasing LAG spacing alone of limited significance.

Potential concerns with using LAG spacing data for relative assessments of ontogenetic stage and growth rate are further underscored by comparisons of LAG spacing at varying positions within a single bone cortex (figure 2n). Depending on the position within the sampled bone cross-section, the observed LAG spacing patterns can differ considerably (particularly when comparing I–I’ to the other three transects). Similarly, LAG spacing patterns may vary substantially between bones of the same individual (figure 2b),
4. Discussion

Our models and empirical case studies clearly illustrate the quantitative relationship between organismal growth (via change in body mass) and various proxies measured from potentially complicating relative growth and maturity assessments. Lastly, the presence of double or multiple growth mark complexes (figure 2c) can frustrate attempts to count the number of growth marks and consequently the related zonal thickness/spacing.

Figure 1. Theoretical schematic models of bone apposition and related empirical data, showing relative scaling of several osteohistological parameters. (a) Model 1 assumes constant widths in successive growth zone radii (ΔR; LAG spacing) across the section. (b) Model 2 assumes constant growth zone area (ΔA) across each successive zone in the section. (c) Model 3 assumes steadily decreasing growth zone area (ΔA) at a scale of 0.8/LAG across successive LAGs in the section. Scaling of osteohistological parameters in empirical data (histological bone cross-sections) of (d) immature theropod ‘Raptorex kriegsteini’ (LH PV18), (e) immature specimen of theropod Gorgosaurus libratu (FMNH PR 2211), (f) near-mature specimen of the theropod Ratitaves evadens (ROM VP 1790), (g) a skeletally mature specimen of the theropod Tyrannosaurus rex (FMNH PR 2081), (h) a near-mature specimen of the ornithischian Centrosaurus apertus (TMP 2013.021.0002), (i) a near-mature specimen of an indeterminate hadrosaurid (TMP 2011.050.0122), (j) a near-mature specimen of the extant cervid mammal Odocoileus virginianus (ROM R 9188) and a skeletally mature specimen of the extant palaeognath bird Apteryx mantelli (ROM 159612). Models of bone apposition (a–c) based on a circle cross-section with an equal growth in all directions outward. For (a–c) the upper panel illustrates bone cross-section (and inset), while the lower panel illustrates relative changes in various osteohistological parameters. In each panel, the horizontal axis represents individual LAGs (from endosteal to periosteal) and the vertical axis illustrates the relative scaling of the various osteohistological parameters (parameters presented as dimensionless and equally scaled to show relative change as a percentage of the maximum). Abbreviations: A, area; EFS, external fundamental system; k, constant; LAG, line of arrested growth; PM, periosteal margin; R, radius.
osteohistological sections (e.g. zone radii/LAG spacing, zone area, LAG circumferences, etc), as well as the scaling relationships between those latter proxies. Interpretations of LAG spacing (ΔR) by itself appear to be frequently inconsistent with organismal growth and vary from study to study. While previous research has illustrated some of the issues inherent in using LAG spacing in single-element sampling (e.g. [33]), these interpretations have remained prevalent in the literature, and the broader concerns remain. Our results demonstrate that when LAG spacing is equal to sub-equal, it does not represent a steady/consistent rate of total body growth but rather an increasing rate (figure 1a). Similarly, consistent small-scale reductions in LAG spacing do not represent decreasing rates of body mass accumulation, but rather a steady/consistent growth rate in this regard (figure 1b). However, major reductions in LAG spacing can be consistent with decreases in total somatic growth rate (figure 1c), and an overall shift from greater LAG spacing to lesser LAG spacing.
should be expected over the duration of the ontogenetic period, based on our empirical data from multiple terrestrial amniote groups (figure 1f–i). Confidence in such an interpretation is bolstered if the diminishing zonal width culminates in an EFS effectively demonstrating cessation of bone apposition.

We also demonstrate the variable nature of zone radii/LAG spacing throughout osteohistological sections, and the potential for subjectivity that can arise from inconsistencies in their use. Our example to illustrate intra-element variability (figure 2a), LH PV 18 ('Raptorex kriegsteini') was chosen because previous studies have drawn nearly opposite conclusions on the relative maturity of this specimen based on examination of LAG spacing patterns on one side of the preserved bone cortex [19] versus the other [50]. An additional source of variability concerns intra-skeletal variation and the choice of which element to examine. It has been previously documented that growth mark counts can vary between bones of the same individual (e.g. [14,15,33]), and that LAG spacing patterns can similarly vary between sampled bones (even those with equal LAG counts) as a result of allometric differences across the skeleton. An example of this can be seen in figure 2b, where the tibia and pedal phalanx of this individual ornithomimid provide two distinct patterns of LAG spacing, with the former tracking closer to Model 3 and the latter being perhaps closest to Model 2. Interpretive differences could easily occur if sampling isolated elements, particularly if relying on bones whose growth trajectories are not closely constrained to tracking overall somatic growth of the individual (e.g. non-weight bearing) [30,33,60,61].

Perhaps more complex is the issue of double or multiple growth mark complexes (figure 2c), also referred to as 'split LAGs'. These structures have been reported fairly widely among extinct and extant tetrapods, being noted, for example, in amphibians [4,62,63], lepidosauroids [64,65], turtles [66], crocodylomorphs [67], mammals and other synapsids [68,69], non-avian theropod dinosaurs [13,30,33,34,70,71] and birds [72]. Although widespread, the occurrence of these structures does vary, with them being particularly abundant in some theropod dinosaur specimens (e.g. FMNH PR 2211, *Gorgosaurus*, see [30]), while being rare in ornithischians (pers. obs.). Double or multiple growth mark complexes are typically considered to occur within one year of growth, being attributed to aestivation or environmental stressors [4,64,66]. Identifying these structures and distinguishing them from a series of distinct annual growth marks can be difficult and can introduce subjectivity and interpretive differences (particularly as age, zonal spacing and growth estimates become compounded across comparisons and multi-individual studies). However, these structures can generally be distinguished by using full-section samples and tracing the circumference of the marks in question, as such growth mark complexes will typically merge, diverge and re-merge at multiple points over their circumference (e.g. figure 2c: insets I, II and III), in contrast with a single LAG which can be traced as a single line around the circumference. The outermost of the multiple LAG set can then be measured, representing the closest equivalent to a typical LAG and related annual growth cycle indicator.

At a broader level, these model results allow the assessment of prior arguments made concerning the utility (or lack thereof) of LAG spacing as a tool for relative ontogenetic assessment. We recommend that caution be taken in making inferences from LAG spacing generally, and particularly in cases where results may be equivocal in terms of their direct support for one growth/ontogenetic hypothesis versus an alternative. In this, we largely agree with the concern expressed by Woodward et al. [51] with respect to the use of LAG spacing for relative ontogenetic assessments (i.e. that it is sufficiently variable as to be unreliable in skeletally immature specimens), but would temper that position to suggest that the use of LAG spacing may be warranted so long as (1) a sufficient amount of the overall growth pattern is preserved in the bone cortex to characterize the most likely stage of the overall growth pattern to which it belongs and (2) that we understand how the growth of the sampled element relates to the total body growth in that taxon (i.e. midshaft circumference of the femur is a good proxy for whole-organism body mass).

To the first point, a 'sufficient amount of the overall growth pattern' could include either end of the growth period and/or the area around the growth inflection point, and ideally at least two of these, as these provide sufficient information to characterize other major aspects of the overall growth pattern. This can be seen in figure 1f–i, where LAG spacing patterns are broadly consistent with the overall trend observed in other metrics (accounting for their differing scaling relationships) in specimens where a sufficient proportion of the growth record is preserved (though only one is skeletally mature). However, when these sections of the growth record are absent (typically in juvenile specimens), the preserved pattern may be too brief (relative to the total) to facilitate a rigorous assessment of ontogenetic stage and growth rate and in particular may present a LAG spacing record that is very sensitive to short-term variability in growth pattern and which deviates strongly from the overall record preserved via zonal area and growth mark circumference (as illustrated in figure 1d,e). In such cases, the histological growth record of a specimen may be at best equivocal in terms of providing an assessment of a particular ontogenetic stage, or supporting alternative hypotheses related to the taxonomic validity of a specimen versus its status as an earlier ontogenetic stage (e.g. Gorgosaurus) of a known taxon. This issue may be potentially alleviated through comparisons to a broader range of specimens representing additional ontogenetic stages (i.e. a growth series), and/or the integration of model-based growth estimation approaches. Many studies use additional skeletochronological markers including fusion between elements such as at neurocentral sutures or between girdle or appendicular bones to bolster interpretations of growth stage based on histology, but the variability and timings of such skeletochronological markers vary between and within taxa and are only quantified for a few taxa [73–76].

Related to the second point, concerning how growth of the sampled element relates to total body growth in that taxon, the element under examination must be carefully considered when assessing if LAG spacing patterns should be used. While the spacing and other histological patterns in a femur or tibia may be a good proxy for somatic growth in a biped, the LAG spacing in a cross-section of a metatarsal, phalanx, fibula or rib may be another matter completely (e.g. figure 2b, see also [33]). The patterns within individual bones cannot always be easily extrapolated, or changes easily detected, in relation to overall somatic growth, and depending on the element sampled, the histological record may indicate that the bone itself is approaching maturity in the sampled area, but this may not be true of the organism as a whole.
5. Conclusion

Overall, LAG spacing (or zone thickness, change in radius) can be a useful indicator of relative ontogenetic patterns, but it should only be used in conjunction with (and potentially in subordination to) other proxies such as tissue organization and vascularity changes, as well as zonal area and growth mark circumference measurements. We recommend the following when attempting to use LAG spacing and related data to infer total body growth rates and relative maturity:

1. Care should be taken in interpretation, given that the scaling relationship between the radius and area of bone being grown, and consequently the body mass increase, is not linear, nor necessarily immediately intuitive from a direct reading of LAG space changes.

2. In particular, caution is recommended in using LAG spacing when working with isolated elements and/or material which preserves only a small segment of the overall growth pattern (particularly if lacking preservation of either end of the growth period or the area around the growth curve inflection point).

3. Multi-element sampling assists in alleviating some of these issues, most significantly those relating to potential misinterpretations of allometric growth patterns of a single bone for those of whole-organism growth, but even in multi-element analyses, these assessment criteria should be observed in order to avoid possible misinterpretations of the growth record. Data from skeletochronology, pathologies and other age-related traits can be referred to as additional support when evaluating growth stages.

4. Double/multiple LAGs (also known as split LAGs) should be traced around the full circumference of a section to confirm if they merge/split or remain completely distinct. If the former, the outermost growth mark of the set should be measured, representing the closest equivalent to a ‘complete’ annual cycle as possible for these multiple LAG structures.

Data accessibility. All data necessary for the replication of this study are found in the manuscript and associated electronic supplementary material, data files. The data are provided in the electronic supplementary material [77].

Authors’ contributions. T.M.C. conceived the project, collected the data, made histological sections, performed the analyses, wrote the manuscript, made the figures and supplementary tables, performed the revisions and wrote/editied the revised manuscript; C.M.B. conceived the project, collected the data, performed the analyses, assisted in writing the manuscript, made figures and supplementary tables, performed the revisions and wrote/editied the revised manuscript; K.C. assisted in development of the project, collected the data, made histological sections, assisted in writing the manuscript and assisted in editing the revised manuscript; K.S.B. assisted in development of the project, assisted in writing the manuscript and wrote/editied the revised manuscript; P.J.M. assisted in development of the project, assisted in histological sampling, assisted in writing the manuscript and wrote/editied the revised manuscript; D.C.E. conceived the project, assisted in data collection, assisted in histological sampling, assisted in writing the manuscript and assisted in editing the revised manuscript.

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References

1. Amprino R. 1947 La structure du tissu osseux envisagée comme expression de différences dans la vitesse de l’accroissement. Arch. Biol. 58, 317–330.

2. Francillon-Viellet H, de Buffrénil V, Castanet J, Géraudie J, Meunier FJ, Sire JF, Zylberberg L, de Ricqles A. 1990 Microstructure and mineralization of vertebrate skeletal tissues. In Skeletal Bioinorganicization: Patterns, Processes and Evolutionary Trends, vol. 5 (ed. JG Carter), pp. 175–234. Washington, DC: American Geophysical Union. (doi:10.1029/SC005p0175)

3. de Ricqles A, Meunier FJ, Castanet J, Francillon-Viellet H. 1991 Comparative microstructure of bone. In Bone volume 3: bone matrix and bone specific products (ed. BK Hall), pp. 1–78. Boca Raton, FL: CRC Press.

4. Castanet J, Francillon-Viellet H, Meunier E, De Ricqles A. 1993 Bone and individual aging. In Bone (ed. BK Hall), pp. 245–283. London, UK: CRC Press.

5. Erickson GM, Tumanova TA. 2000 Growth curve of Psittacosaurus mongoliensis Osborn (Ceratopia: Psittacosauridae) inferred from long bone histology. Zool. J. Linn. Soc. 130, 551–566. (doi:10.1111/j.1096-3642.2000.tb02210.x)

6. Erickson GM, Rogers KC, Yerby SA. 2001 Dinosaurian growth patterns and rapid avian growth rates. Nature 412, 429–433. (doi:10.1038/35086558)

7. Erickson GM, Makovicky PJ, Currie PJ, Norell MA, Yerby SA, Brochu CA. 2004 Gigantism and comparative life-history parameters of tanygnosaurid dinosaurs. Nature 430, 772–775. (doi:10.1038/nature02699)

8. Chinsamy-Turan A. 2005 The microstructure of dinosaur bone: deciphering biology with fine scale techniques. Baltimore, MA: Johns Hopkins University Press.

9. Lee AH, Werning S. 2008 Sexual maturity in growing dinosaurs does not fit reptilian growth models. Proc. Natl Acad. Sci. USA 105, 582–587. (doi:10.1073/pnas.0708903105)

10. Cooper LN, Lee AH, Taper ML, Horner JR. 2008 Relative growth rates of predator and prey dinosaurs reflect effects of predation. Proc. R. Soc. B 275, 2609–2615. (doi:10.1098/rspb.2008.0912)

11. Köhler M, Marin-Moratalla N, Jordana X, Aanes R. 2012 Seasonal bone growth and physiology in endotherms shed light on dinosaur physiology. Nature 487, 358–361. (doi:10.1038/nature11264)

12. Griebeler EM, Klein N, Sander PM. 2013 Aging, maturation and growth of sauroporpod dinosaurs as deduced from growth curves using long bone histological data: an assessment of methodological constraints and solutions. PLoS ONE 8, e67012. (doi:10.1371/journal.pone.0067012)

13. Lee AH, O’Connor PM. 2013 Bone histology confirms determinate growth and small body size in the neoaosaur theropod Masiakasaurus knopfleri. J. Verteb. Paleontol. 33, 865–876. (doi:10.1080/02724634.2013.743898)

14. Horner JR, de Ricqles A, Padian K. 1999 Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. Paleobiology 25, 295–304. (doi:10.1017/S0094837300021308)
15. Horner JR, De Ricqlès A, Padian K. 2000 Long bone histology of the hadrosaurid dinosaur Maiasaura peeblesorum: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *J. Vertebr. Paleontol.* 20, 115–129. (doi:10.1671/0272-4634(2000)020[0115:LBPEET]2.0.CO;2)

16. Woodward WN, Horner JR, Farlow JO. 2011 Osteohistological evidence for determinate growth in the American alligator. *J. Herpetol.* 45, 339–342. (doi:10.1670/07-274.1)

17. Erickson G. 2005 Assessing dinosaur growth patterns: a microscopic revolution. *Trends Ecol. Evol.* 20, 677–684. (doi:10.1016/j.tree.2005.08.012)

18. Lee AH, Hutterlenkock AK, Padian K, Woodward WH. 2013 8. Analysis of growth rates. In *Bone histology of fossil tetrapods*, pp. 217–252. Berkeley, CA: University of California Press.

19. Sereno PC, Tan L, Brusatte SL, Kriegstein HJ, Zhao X. 2009 Cloward K: tyrannosaurid skeletal design first evolved at small body size. *Science* 326, 418–422. (doi:10.1126/science.1177428)

20. Watanabe A, Erickson GM. 2013 Druckenmiller PS: paleohistology of the hadrosaurid dinosaur *Sauropoda*. *J. Vertebr. Paleontol.* 33, 1169–1175. (doi:10.1080/02724634.2013.770750)

21. MyhreNPD. 2013 Revisiting the estimation of dinosaur growth rates. *PLoS ONE* 8, e81917. (doi:10.1371/journal.pone.0081917)

22. de Margerie E. 2004 Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin (Aptenodytes patagonica). *J. Exp. Biol.* 207, 869–879. (doi:10.1242/jeb.08041)

23. de Buffrénil V, Quilhac A, Castanet J. 2021 Chp 31: Skeletal histology and paleohistology. *Vertebrate skeletal histology and paleohistology: implications for diversity of growth strategies and osteohistological features within Theropoda and Sauropoda*. *Vertebr. Anatomy Morphol. Palaeontol. CVP* 2021 Abstr. 9, 22.

24. Brusatte SL, Carr TD, Erickson GM, Bever GS. 2009 Deyo-Murray MA: a long-snouted, multithorned tyrannosaurid from the Late Cretaceous of Mongolia. *Proc. Natl. Acad. Sci. USA* 106, 17 261–17 266. (doi:10.1073/pnas.0906911106)

25. Padian K, de Boef Miara M, Larsson HC, Wilson L, Venier S, Schlingemann MA, Rössner GE, Monaghan NT, Sánchez-Villagra MR. 2018 Palaeohistology and life history evolution of early sauropods. *Curr. Biol.* 28, 3143–3151. (doi:10.1016/cub.2018.07.063)

26. Woodward WH, Horner JR, Farlow JO. 20111 Osteohistological evidence for determinate growth in the American alligator. *J. Herpetol.* 45, 339–342. (doi:10.1670/07-274.1)

27. Erickson G. 2005 Assessing dinosaur growth patterns: a microscopic revolution. *Trends Ecol. Evol.* 20, 677–684. (doi:10.1016/j.tree.2005.08.012)

28. Lee AH, Hutterlenkock AK, Padian K, Woodward WH. 2013 8. Analysis of growth rates. In *Bone histology of fossil tetrapods*, pp. 217–252. Berkeley, CA: University of California Press.

29. Sereno PC, Tan L, Brusatte SL, Kriegstein HJ, Zhao X. 2009 Cloward K: tyrannosaurid skeletal design first evolved at small body size. *Science* 326, 418–422. (doi:10.1126/science.1177428)

30. Watanabe A, Erickson GM. 2013 Druckenmiller PS: paleohistology of the hadrosaurid dinosaur *Sauropoda*. *J. Vertebr. Paleontol.* 33, 1169–1175. (doi:10.1080/02724634.2013.770750)

31. MyhreNPD. 2013 Revisiting the estimation of dinosaur growth rates. *PLoS ONE* 8, e81917. (doi:10.1371/journal.pone.0081917)

32. de Margerie E. 2004 Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin (Aptenodytes patagonica). *J. Exp. Biol.* 207, 869–879. (doi:10.1242/jeb.08041)

33. de Buffrénil V, Quilhac A, Castanet J. 2021 Chp 31: Skeletal growth and skeletochronology. In *Vertebrate skeletal histology and paleohistology*, pp. 626–644. Boca Raton, FL: CRC Press.

34. Harlow S, Benson RB, Botha-Brink J, Bordy EM, Choiniere JR. 2018 A new specimen of the sauropod dinosaur *Diplodocus sp.* from the earliest Jurassic of South Africa and the transition to quadrupedality in early sauropodomorphs. *Curr. Biol.* 28, 3143–3151. (doi:10.1016/cub.2018.07.063)

35. Padian K, de Boef Miara M, Larsson HC, Wilson L, Venier S, Schlingemann MA, Rössner GE, Monaghan NT, Sánchez-Villagra MR. 2018 Palaeohistology and life history evolution of early sauropods. *Curr. Biol.* 28, 3143–3151. (doi:10.1016/cub.2018.07.063)

36. Woodward WH, Horner JR, Farlow JO. 2011 Osteohistological evidence for determinate growth in the American alligator. *J. Herpetol.* 45, 339–342. (doi:10.1670/07-274.1)
