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Lines of arrested growth and long bone histology in Pleistocene large mammals from Germany: What do they tell us about dinosaur physiology?

by

P. Martin Sander and Peter Andrássy, Bonn

With 9 text-figures and 1 table

Summary

Lines of arrested growth (LAGs) are a typical feature of the bone histology of ectothermic tetrapods but have received little study in mammals and birds. However, LAGs have figured prominently in the debate about dinosaur physiology. Here we describe the bone histology, including the occurrence of LAGs, in an extensive sample of herbivorous mammals from the Late Pleistocene of Germany, mainly from the Rhine-Herne ship channel. Taxa sampled include the cervids *Megaloceros giganteus*, *Cervus elaphus*, and *Rangifer tarandus*, the bovids *Bos primigenius* and *Bison priscus*, the equid *Equus* sp., the rhinocerotid *Coelodonta antiquitatis*, and the elephantid *Mammuthus primigenius*. Samples were preselected for macroscopic evidence of cyclical growth. Bones sampled were mainly metatarsals as well as tibiae and indeterminate long bone fragments. All samples show fibro-lamellar bone in the cortex that is replaced by secondary bone to varying degrees. Most samples show one or more regularly spaced LAGs, sometimes even preserved in secondary bone. Surprisingly, there are distinct differences in the histology of the various taxa in terms of the arrangement of the primary vascular network and the patterns of remodeling. The common development of LAGs in these endothermic Late Pleistocene mammals calls into question the argument that LAGs in dinosaur bone indicate an ectothermic physiology.

Key words: Mammalia – Pleistocene – bone histology – lines of arrested growth.

Zusammenfassung

Stillstandslinien (LAGs) sind typische Merkmale der Knochenhistologie ektothermer Tetrapoden, wurden aber bei Säugetieren und Vögeln bisher wenig untersucht. Allerdings spielen Stillstandslinien in der Debatte über die Physiologie der Dinosaurier eine wichtige Rolle. In dieser Arbeit wird die Histologie und insbesondere die Stillstandslinien im Knochen herbivorer Großsäuger aus dem Jungpleistozän von Deutschland beschrieben. Das Material wurde vor allem bei Bauarbeiten am Rhein-Herne-Kanal geborgen. Untersucht wurden die Cerviden *Megaloceros giganteus*, *Cervus elaphus* und *Rangifer tarandus*, die Boviden *Bos primigenius* und *Bison priscus* sowie *Equus* sp., *Coelodonta antiquitatis* und *Mammuthus primigenius*. Die Proben wurden nach makroskopischen Anzeichen für zyklisches Wachstum ausgewählt, wobei es sich vor allem um Metatarsi sowie Tibiae und unbestimmbare Knochenfragmente handelt. Alle Proben zeigen fibrolamellären Knochen in der Corticalis, der unterschiedlich stark durch sekundären Knochen ersetzt ist. Die meisten Proben weisen eine oder mehrere Stillstandslinien auf, die regelmäßige Abstände haben und sich manchmal sogar im sekundären Knochen durchpausen. Überraschender Weise gibt es deutliche Unterschiede in der Histologie der einzelnen Taxa, die sich in der Anordnung des primären vaskulären Netzwerkes und in den Mustern des Umbaues finden. Das häufige Auftreten von Stillstandslinien in diesen endothermen pleistozänen Säugetieren weckt Zweifel an der Sichtweise, daß Stillstandslinien im Dinosaurierknochen eine ektotherme Physiologie anzeigen.

Schlüsselwörter: Mammalia – Pleistozän – Knochenhistologie – Stillstandslinien.

Addresses of the authors: Dr. P. Martin Sander, Institute of Paleontology, University of Bonn, Nussallee 8, D-53115 Bonn, Germany, e-mail: martin.sander@uni-bonn.de
Dipl.-Geol. P. Andrássy, Institute of Paleontology, University of Bonn, Nussallee 8, D-53115 Bonn, Germany, e-mail: p.andrassy@uni-bonn.de

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1. Introduction

Compared to dinosaurs, the bone histology of large mammals has received very little attention by paleontologists. This is surprising, because a thorough understanding of mammalian bone histology is crucial to the paleobiological interpretation of the bone histology of extinct tetrapods such as dinosaurs and non-mammalian synapsids. Although birds and crocodiles are phylogenetically closer to dinosaurs than mammals, the bone histology of large modern mammals probably is most informative for the interpretation of dinosaurian bone histology. Crocodile bone histology differs from dinosaur bone histology too much because of the much lower growth rates of crocodiles. Except for ratites such as ostriches and moas, birds are poorly suited for comparison because of their much smaller body size and their adaptation to flight rather than to a terrestrial lifestyle, despite being surviving dinosaurs. Large herbivorous mammals over 1 t body mass ("megaherbivores") empirically offer the best basis of comparison for many aspects of dinosaurian biology despite the convergent acquisition of many dinosaurian characters such as large body size, high growth rates, and a fully upright stance.

Bone histology of dinosaurs is rapidly becoming a major source of information on the biology of dinosaurs, and has figured prominently in controversial discussions about metabolism and thermoregulation of dinosaurs, epitomized by the back-to-back review chapters on physiology (Chinsamy & Hillenius 2004, Padian & Horner 2004) in the authoritative volume on dinosaurs, "The Dinosauria" (Weishampel et al. 2004). An important role in the debate is played by the lines of arrested growth (LAGs) observed in the long bone cortex of many tetrapods. This includes most dinosaurs (e.g. Ricqlès 1983, Reid 1987, Padian 1997, Klein 2004, Werning 2005, Sander & Klein 2005, Bybee et al. 2006). The most notable exception is the sauropod dinosaurs. They have only in a minority of specimens well developed LAGs in their long bones (Sander 2000, Sander & Tückmantel 2003, pers. observ.). In living tetrapods, a LAG indicates an interruption in cortical bone apposition. In living reptiles and fishes, the interruption in growth is often due to unfavorable environmental conditions that the ectothermic reptiles and amphibians cannot compensate for metabolically. LAGs in living reptiles are regularly used for aging purposes, a method known as skeletochronology (Castanet et al. 1993, Castanet 1994).

While Chinsamy & Hillenius (2004 and references therein) argue that regularly spaced LAGs in dinosaur bones indicate a metabolic rate below that of modern mammals, which are believed to generally lack LAGs, Padian & Horner (2004 and references therein) take a phylogenetic perspective by pointing out that LAGs in dinosaurs may be a poor indicator of metabolic status because they are plesiomorphic. According to Padian & Horner (2004), LAGs thus simply may be inherited from some ectothermic ancestor of the dinosaurian lineage and have no particular meaning in terms of thermoregulation. In addition, Padian & Horner note that LAGs have been occasionally observed in large mammals such as elk (*Cervus elaphus*; Horner et al. 1999), seals (*Phocoena phocoena*; Buffrenil 1982), and polar bears (*Ursus maritimus*; Chinsamy et al. 1998). Elk in particular are of interest because unlike bears, they do not hibernate. Temperate zone small mammals also commonly show LAGs (e.g. Klevezal 1996), as do large birds such as moas (Turvey et al. 2005).

The purpose of this paper is to describe the histology of the long bones of large Pleistocene mammals with a particular emphasis on the occurrence of LAGs in the long bone cortex. We will show that LAGs are common in

essentially Recent mammals that ostensibly had the same metabolism as their living relatives, thus supporting the view of PADIAN & HORNER (2004) that LAGs per se are a plesiomorphic trait and cannot be cited in support of an ectothermic metabolism in dinosaurs. Our study grew out of the fortuitous observation of LAGs in fracture surfaces of Pleistocene fossil bones. We expanded on this observation by studying fossil bone because samples of Pleistocene large mammals are much easier to obtain in museum collections than those of Recent mammals. Fragmentary but identifiable material is usually not found in recent osteological collections, but only complete skeletons to which sampling access is more restricted.

In the past, fossil mammal bone was repeatedly studied histologically, but often as part of larger histological surveys, and it received only a minor treatment (e.g. GROSS 1934, WARREN 1963). The most comprehensive study of mammalian bone histology is still that of ENLOW & BROWN (1958) as part of their comparative work on tetrapod bone histology (ENLOW & BROWN 1956, 1957, 1958). While GROSS (1934) was almost purely descriptive in his approach, ENLOW & BROWN (1956, 1957, 1958) were already interested in physiological interpretation as well as description of histology. Also of relevance is the work of KLEVEZAL, summarized in KLEVEZAL (1996), who was specifically interested in the growth marks found in mammalian bone. Since these seminal papers, little has been published on fossil mammal bone histology.

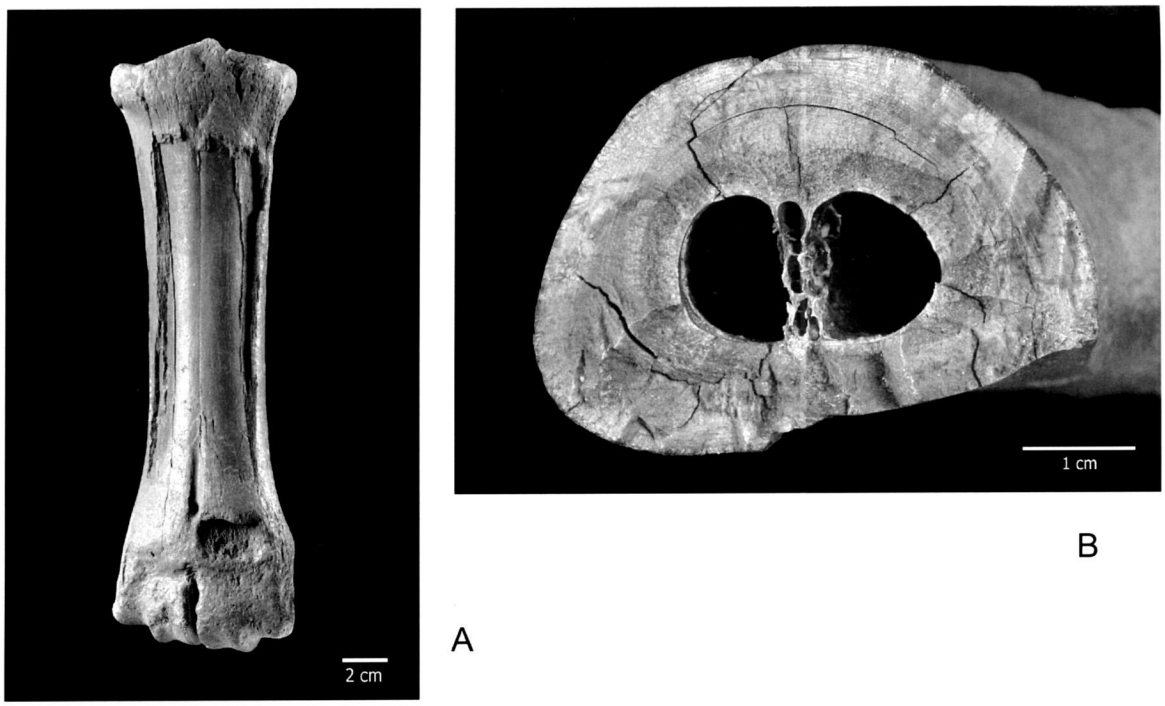
2. Material and methods

2.1. Material

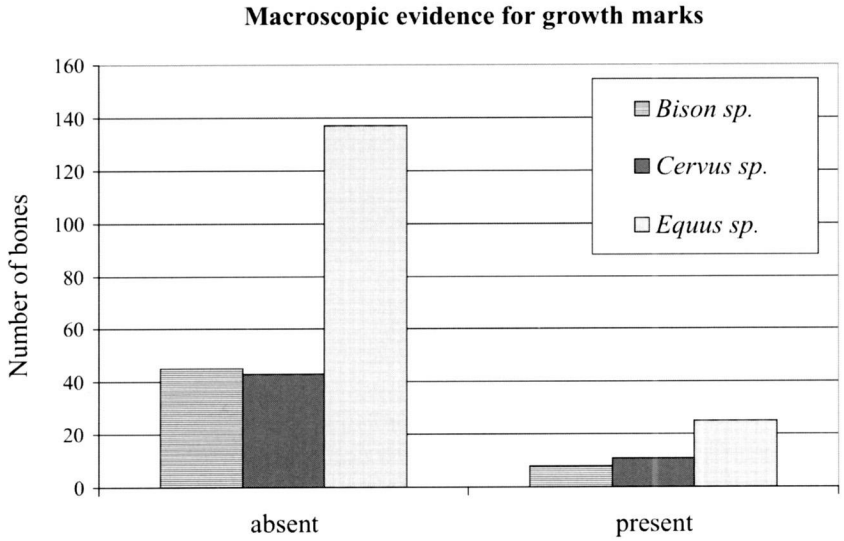
The study material (Table 1) consists of long bones and is primarily derived from the collections of the Museum für Ur- and Ortsgeschichte Quadrat, Bottrop (MQB), which holds a large collection of Late Pleistocene large mammal remains that were discovered during construction of the Rhine-Herne ship channel (*Rhein-Herne-Kanal*). The faunal composition indicates that the bones date from the early and middle Weichselian glaciation (KOENIGSWALD & WALDERS 1995). The long bones were screened for external and macroscopic indications of cyclical growth such as surface-parallel linear features visible in fractures and surface-parallel spalling of bone layers (Text-figs 1 A+B). Only a fraction of the screened bone showed such indications (Text-fig. 2), and only those were selected for histologic sampling.

Table 1. Taxa studied, locality, section ID number, bone type, and reference to the figures of the thin sections studied. RHK = Rhein-Herne ship channel, MQB = Museum Quadrat Bottrop, IPB = Institut für Paläontologie, Universität Bonn.

Taxon	Locality	Section ID	Bone	Text-fig.
Megaloceros giganteus	RHK	MQB 4.8.304	metatarsal III+IV	Text-fig. 4A
Megaloceros giganteus	RHK	MQB 4.8.308	metatarsal III+IV	
Megaloceros giganteus	RHK	MQB 4.8.309	metatarsal III+IV	
Megaloceros giganteus	RHK	MQB 4.8.315	metatarsal III+IV	Text-fig. 4B
Cervus elaphus	RHK	MQB 4.8.295	metatarsal III+IV	
Cervus elaphus	RHK	MQB 4.8.299	metatarsal III+IV	Text-fig. 5A
Rangifer tarandus	RHK	MQB 4.8.277	metatarsal III+IV	Text-fig. 5B
Bison priscus	RHK	MQB 4.8.349	metatarsal III+IV	Text-figs 6A, B
Bison priscus	RHK	MQB 4.8.351	metatarsal III+IV	
Bison priscus	RHK	MQB 4.8.372	metatarsal III+IV	Text-fig. 6C
Bos primigenius	Gross-Rohrheim	IPB M 26	tibia	Text-fig. 6D
Equus sp.	RHK	MQB 4.4.614	metatarsal III	
Equus sp.	RHK	MQB 4.4.634	metatarsal III	Text-fig. 7B
Equus sp.	RHK	MQB 4.4.657	metatarsal III	
Equus sp.	RHK	MQB 4.4.733	tibia	
Equus sp.	RHK	MQB 4.8.516	metatarsal III	Text-fig. 7C
Equus sp.	?	IPB M 2042	metatarsal III	Text-fig. 7A
Coelodonta antiqitatis	Brexbachtal	IPB Brex 01/1	tibia	
Mammuthus primigenius	RHK	MQB RHK 01.2	indet. long bone	Text-figs 8A, B
Mammuthus primigenius	RHK	MQB RHK 02.1	indet. long bone	
Mammuthus primigenius	RHK	MQB RHK 03.1	indet. long bone	



Text-fig. 1. **A** Fused metatarsals III+IV of *Bos*, MQB RHK uncatalogued, in anterior view. Note the spalling of bone layers on the shaft of the bone. **B** Fused metatarsals III+IV, *Bos* MQB RHK uncatalogued, fracture across the shaft with macroscopically visible growth cycles.



Text-fig. 2. Frequency of metatarsals and tibiae with macroscopic evidence of growth marks in material from the Rhine-Herne ship channel, collections of the MQB. Note that such evidence is rare.

The specimens from the Rhine-Herne ship channel are all very well preserved and easily processed into thin sections. This material was augmented by a screening of long bones in the collections of the Institute of Paleontology, University of Bonn (IPB). These specimens are from various German Late Pleistocene localities (Table 1); accordingly, they show greatly varying quality of preservation and were sometimes difficult to process. The specimen from Groß-Rohrheim (Table 1) is derived from Last Interglacial Rhine River sands, while the Brexbachtal locality (Table 1) is a Late Pleistocene loess deposit laid down during glacial conditions.

The focus of this study was exclusively on long bones, with the initial aim to sample primarily humeri and femora. However, screening the collections yielded primarily metacarpals and metatarsals pertaining to Cervidae, Bovidae, and Equidae. Other samples included tibiae and indeterminate long bone fragments. The sample from the IPB collections includes five samples from complete humeri and femora of *Mammuthus*, while in the MQB collections only fragmentary bones of this taxon were available.

There were several reasons for selecting the long bones mentioned above. One is that these long bones are very commonly preserved and thus easily available for sampling, albeit sometimes only as fragments. In addition, the size of the long bones suggests that they have a sufficiently thick cortex to preserve a record of their growth. The most important reason, however, is that long bones are least affected by remodeling during growth because of their simple shaft morphology. This results in simple appositional growth of the cortex, increasing the chance of preserving growth marks in the bone tissue.

Specific taxa sampled were giant elk (*Megaloceros giganteus*), red deer (*Cervus elaphus*), and reindeer (*Rangifer tarandus*) among the Cervidae, aurochs (*Bos primigenius*) and steppe bison (*Bison priscus*) among the Bovidae, horse (*Equus* sp.), woolly rhino (*Coelodonta antiquitatis*), and mammoth (*Mammuthus primigenius*). The taxa sampled are characteristic of full-glacial conditions (*Megaloceros giganteus*, *Rangifer tarandus*, *Coelodonta antiquitatis*, *Mammuthus primigenius*; KOENIGSWALD 2002) or are climatically insensitive (*Cervus elaphus*, *Bos primigenius*, *Bison priscus*, *Equus* sp.; KOENIGSWALD 2002); while strictly interglacial taxa were not sampled.

2.2. Methods of study

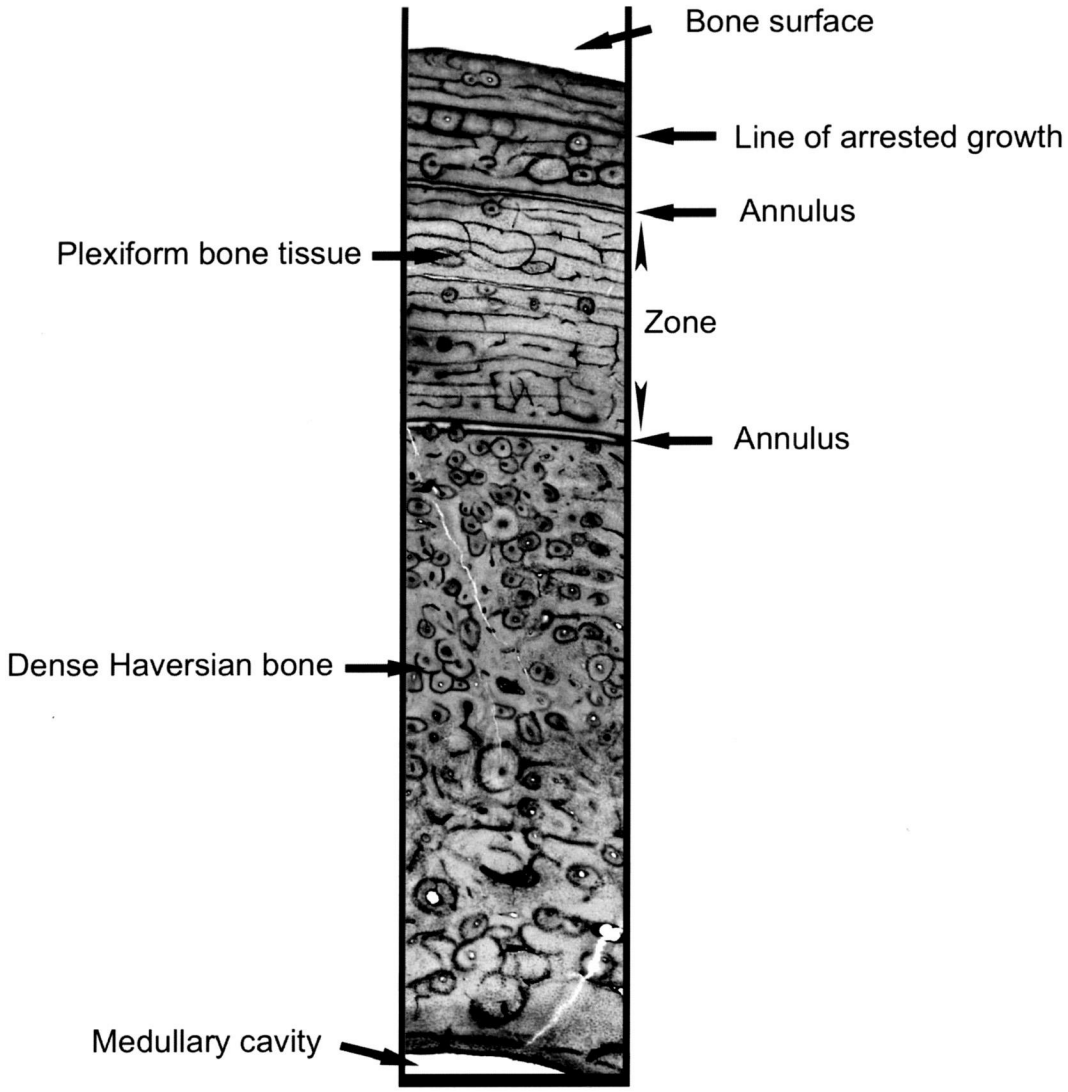
Where possible, the bones to be sampled were sectioned across the diaphysis with a rock saw. Some specimens were deemed too valuable to section completely, and a core sample from the region of the mid-diaphysis with the thickest cortex was taken using the method described by SANDER (2000). The cores have a diameter of 15 mm. This method was modified by using vegetable oil rather than water as a lubricant of the core drill. Many Pleistocene bones retain much collagen, which takes up water and makes the bone swell.

After sampling, the diaphysial cross sections and core sections were impregnated under vacuum with Araldite-2020® or Biresin-L84®. The cores were then sectioned longitudinally, perpendicular to the long axis. The section thus obtained represents a sector of the diaphysial cross section of the bone. Initially, each sample was processed into a thin section and a polished section because growth marks that cannot be detected in thin section may become visible in polished sections (e.g. the polish lines of SANDER 2000, SANDER & TÜCKMANTEL 2003). However, in this study, the polished sections did not show additional growth marks so that from most of the samples, only thin sections were produced. Because the samples were sensitive to water, in the final stages of grinding (below a section thickness of about 0.2 mm), vegetable oil was again used successfully as a lubricant. The final thickness of 50–60 µm of the sections was obtained by hand grinding.

The thin sections were studied using a Leitz Ortholux® polarizing microscope (magnifications 40 ×, 100 ×, 400 ×) and a Wild® binocular microscope (magnifications 6 ×, 12 ×, 25 ×, 50 ×), using a transmitted light stage as well as incident light. Photomicrographs were taken with a digital camera mounted on the microscopes. Photomicrographs were processed with Adobe Photoshop© and Corel Draw©. Descriptive terminology (Text-fig. 3) follows FRANCILLON-VIEILLOT et al. (1990) and CASTANET et al. (1993).

3. Histological descriptions: Cervidae

Among the Cervidae, bone material of *Megaloceros giganteus*, *Cervus elaphus*, and *Rangifer tarandus* was studied histologically. In general, bone tissue in the long bone cortex of the Cervidae is of the fibro-lamellar type,



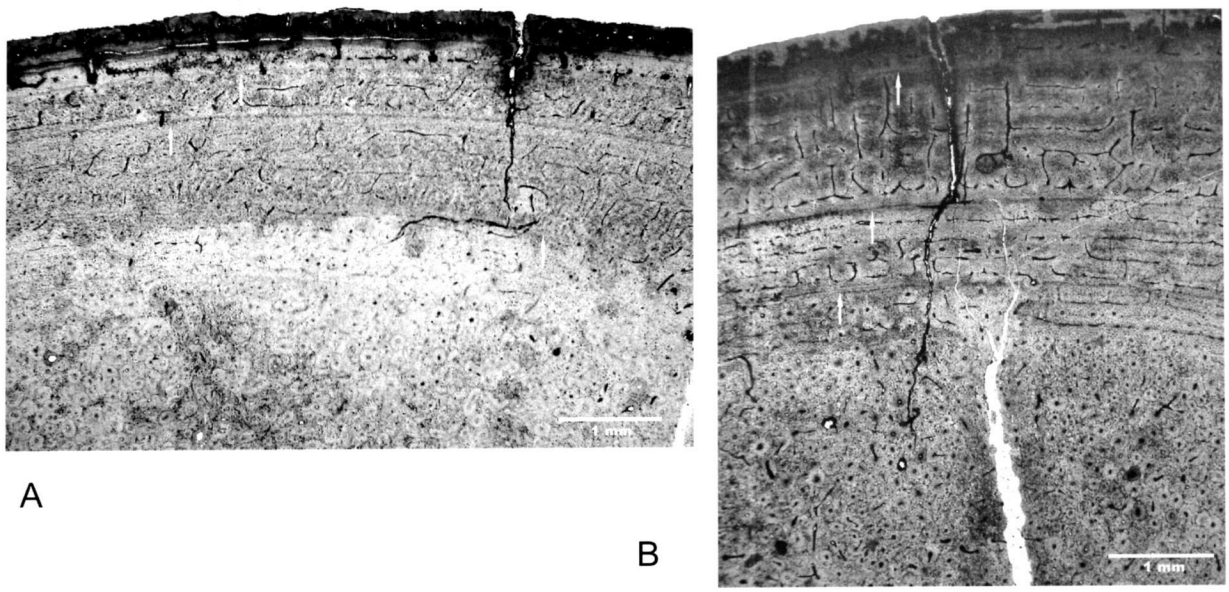
Text-fig. 3. Types and terminology of bone tissues and bone growth marks illustrated in a thin section across the cortex of a *Bison priscus* long bone. Unless noted otherwise, all thin sections illustrated in this and the following figures were photographed under normal light.

with a plexiform arrangement of the vascular canals. In this arrangement, the vascular canals run primarily circumferentially, and are connected by radial canals (canals of Volkmann). The plexiform tissue of cervids is characterized by a long, slightly wavy bone tissue which in turn is subdivided by a well developed vascular network. Secondary osteons are very common in specific regions of the diaphysial cross section.

3.1. *Megaloceros giganteus*

MQB 4.8.304, *Megaloceros giganteus*, fused metatarsals III+IV (Text-fig. 4A)

Four fragmentary metatarsals of *Megaloceros giganteus* were sampled. MBQ 4.8.304 is the largest of these metatarsals, and is 25.4 cm long as preserved, with a diameter of 4.7 cm. The cortex is more than 1 cm thick. Generally, the cortex of *Megaloceros* is relatively thicker than in *Cervus* but thinner than in *Bos* and *Bison*. The specimen was sampled at about the middle of the shaft. The medullary cavity is free of spongy bone. The thin section represents the lateral part of the rather square cross section of the bone. In this region, only the inner



Text-fig. 4. **A** MQB 4.8.304, *Megaloceros giganteus*, fused metatarsals III+IV. The white arrows mark lines of arrested growth (LAGs) in the primary bone. The lower half of the section consists of dense Haversian bone. **B** 4.8.315 MQB *Megaloceros giganteus*, fused metatarsals III+IV. Three LAGs (white arrows) are visible.

cortex is remodelled, and two LAGs are preserved, with the possible presence of a third (Text-fig. 4A). The strongest remodeling occurs in the areas of greatest curvature, possibly because the highest stresses occur along the edges of the shaft.

MQB 4.8.309/4.8.315, *Megaloceros giganteus*, fused metatarsals III+IV (Text-fig. 4B)

These two metatarsals are equivalent to MQB 4.8.304 but somewhat smaller (length 20.2 cm and 20.9 cm, diameter of 4.2 cm and 4.3 cm). The cortex of both is between 7 mm and 10 mm thick. Both bones have a longitudinal split through the shaft, so only half of the cross section is represented in the thin sections. Both bones also have a large medullary cavity devoid of spongy bone. LAGs are preserved in the outer third of the cortex (Text-fig. 4B).

MQB 4.8.308, *Megaloceros giganteus*, fused metatarsals III+IV

The fourth specimen is the smallest (length 26.6 cm and diameter 3.5 cm). The cortical thickness is only 7–8 mm. Again, the medullary cavity is completely free of spongy bone up to the epiphyses. No LAGs or any other growth marks were detected. All other histologic features of this sample are the same as in the other *Megaloceros* samples.

3.2. *Cervus elaphus*

Two metatarsal fragments of *Cervus elaphus* were sampled. Both of these preserve only the distal ends. The *C. elaphus* samples are characterized by more remodeling than those of *Megaloceras* because the cross section of the bone is not quadrangular as in *Megaloceras* but more rounded. The regions of greatest remodeling are therefore not concentrated in the corners but are evenly distributed over the entire cross section, so the primary cortex is preserved only as isolated remains. Apart from this, there are no histologic differences between *Megaloceras* and *Cervus.*

MQB 4.8.295, *Cervus elaphus*, fused metatarsals III+IV

The preserved length of this bone is 19.8 cm, the shaft diameter is 2.9 cm, and cortical thickness is 5 mm. The thin section covers the lateral half of an oval cross section, and two LAGs are visible.

MQB 4.8.299, *Cervus elaphus*, fused metatarsals III+IV (Text-fig. 5A)

The thin section covers the entire cross section of the shaft, which has a diameter of 2.4 cm and a cortical thickness of 7-8 mm. The preserved length is 14.1 cm. There is no spongy bone in the medullary cavity. Again, two LAGs are preserved (Text-fig. 5A).

3.3. *Rangifer tarandus*

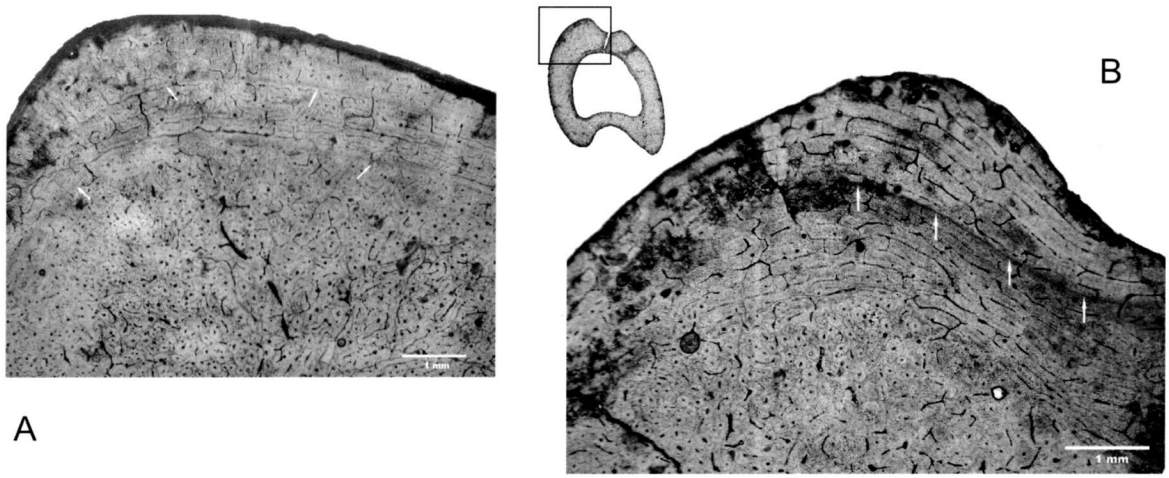
In its histology, *Rangifer* is a typical cervid and does not differ from *Cervus* and *Megaloceros*. As in the other cervids, the primary bone of the cortex is of the plexiform fibro-lamellar type. Remodeling is also very characteristic.

MQB 4.8.277, *Rangifer tarandus*, fused metatarsals III+IV (Text-fig. 5B)

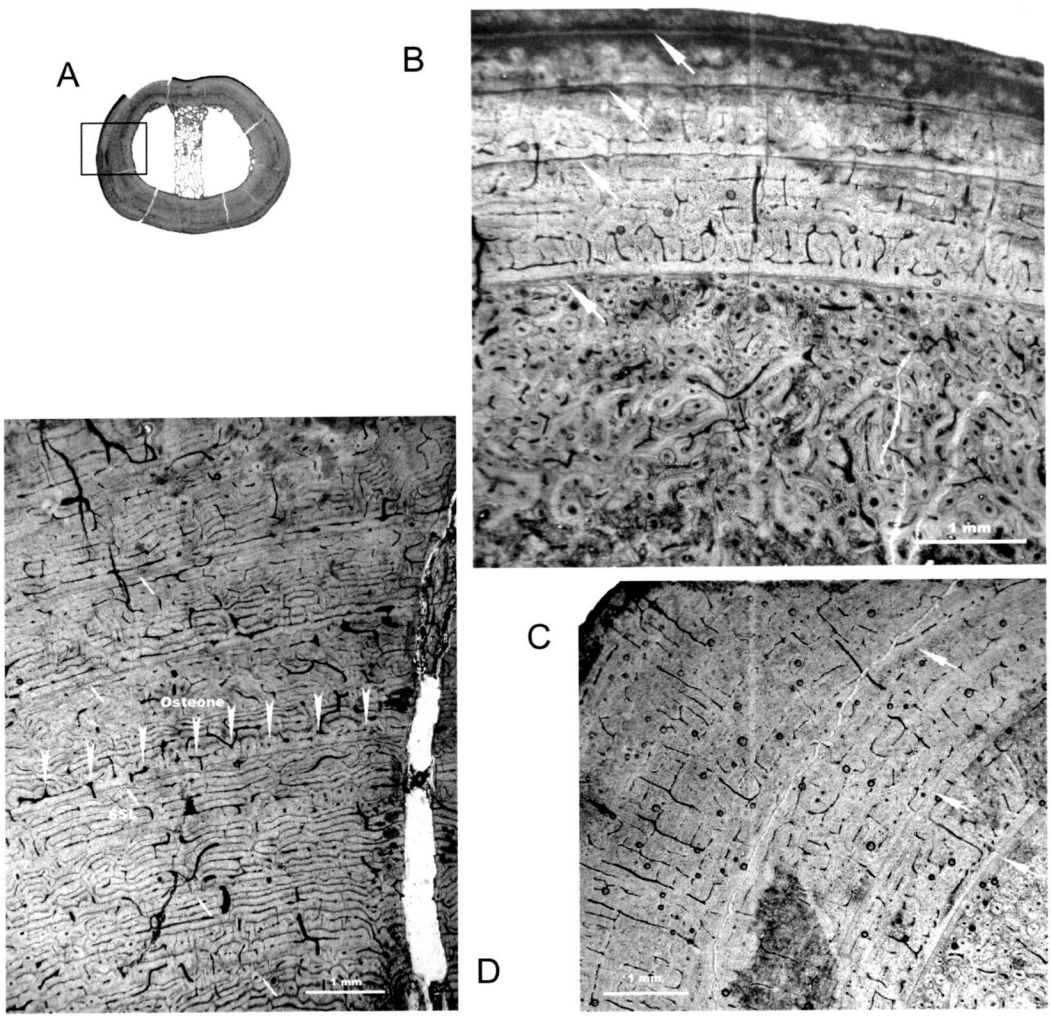
The specimen is a distal metatarsal of 11.9 cm preserved length and a diaphysial diameter of 1.7 cm. The cortical thickness is 4–5 mm. The thin section covers the entire diaphysial cross section with a free medullary cavity devoid of any spongy bone. The bone tissue is largely remodelled; only in the region of the edges of the dorsal groove are remains of the primary tissue. Along one of the edges, a LAG is visible (Text-fig. 5B).

4. Histological descriptions: Bovidae

The bovids also have plexiform fibro-lamellar bone. However, unlike the situation in the cervids, the circumferential vascular canals do not appear laminar but wavy (Text-fig. 6). In both, the primary bone is fibro-lamellar. The well developed network of longitudinal canals is the same as that in cervids. Also comparable are the general distribution and the common occurrence of secondary osteons that are concentrated in specific regions, presumably those experiencing the highest stresses.



Text-fig. 5. **A** MQB 8.299, *Cervus elaphus*, fused metatarsals III+IV. The inner three-fourths of this section consist of Haversian bone that replaced the primary bone. In the outer third of the cortex, two LAGs are seen (white arrows). **B** 4.8.277 MQB *Rangifer tarandus*, metatarsal III+IV. One LAG is visible, the section is from the anterior margin of the shaft (see inset).



Text-fig. 6. **A, B** MQB 4.8.349, *Bison priscus*, fused metatarsals III+IV. **A** Entire thin section with the septum of trabecular bone dividing the medullary cavity. Box marks area of close-up. **B** Close-up of the cortex with four zones marked by three LAGs (white arrows). **C** MQB 4.8.372 *Bison priscus*, fused metatarsals III+IV. Thin section with three zones and LAGs. The radial vascular canals (canals of Volkmann) are especially well developed in this section. **D** IPB M26 *Bos primigenius*, fused metatarsals III+IV, with very well developed laminar to plexiform fibro-lamellar bone tissue. The large white arrows indicate secondary osteons lined up along a LAG. Small arrows mark LAGs.

4.1. *Bison priscus*

MQB 4.8.349, *Bison priscus*, fused metatarsals III+IV (Text-figs 6A+B)

This bone fragment is 17.8 cm long and was sampled in the middle of the shaft. The shaft is 4.3 cm in diameter, and its cross section is nearly circular. Cortical thickness is 1–1.3 cm. The medullary cavity is nearly devoid of spongy bone apart from a central bridge toward the proximal end of the bone. This bridge is oriented anteroposteriorly and probably represents the plane of ontogenetic fusion of the metatarsals (Fig. 6A).

The medullary cavity of this specimen is lined with fine layers of endosteal bone that also covers the struts of the spongiosa. In the perimedullary region, erosion cavities are filled by incomplete secondary osteons, and scattered erosion cavities are found throughout the innermost third of the cortex. This is followed peripherally by a region of secondary Haversian bone. Only the outer third of the cortex (sometimes up to the outer half) consists of fibro-lamellar bone, although this region contains scattered secondary osteons as well. While the secondary osteons are usually distributed randomly, there are also instances in which they trace the original vascular network and thus the periosteal surface. A first LAG is observed just exterior to the zone of strong remodeling (Text-fig.

6B). Another three follow peripherally, separated by wide zones (Text-fig. 6B). Assuming that two to three growth cycles may have been resorbed by the expansion of the medullary cavity, this individual was around seven years old. LAG spacing indicates that it grew relatively rapidly in the first three to four years, followed by a decrease in growth rate.

MQB 4.8.351, *Bison priscus*, fused metatarsals III+IV

The sampled bone fragment is 16.2 cm long. The cross section of this metatarsal is rounded rather than oval, its diameter being 3.8 cm. The cortex is up to 1 cm thick. The medullary cavity resembles that of MQB 4.8.349. However, as a special feature, MQB 4.8.351 shows a pathology (hyperplasy?) that could not be identified further. The pathology did not affect the entire bone but remained localized. Possibly the pathology was a reaction to a more proximally located fracture. The pathology consists of laminar bone that is largely remodelled into Haversian bone. In the center of the pathology, there are some erosion cavities that indicate local resorption.

In the remaining cortical bone, there are several well developed zones delimited by LAGs, five of which can be detected with certainty.

MQB 4.8.372, *Bison priscus*, fused metatarsals III+IV (Text-fig. 6C)

This bone has a preserved length of 12.3 cm. The thin section covers a nearly complete cross section that was cut somewhat distal to the middle of the shaft. The cross section is oval and measures 3.2 cm by 5.2 cm. Cortical thickness is 1–1.3 cm. There is scant spongy bone in the medullary cavity. The spacing of LAGs and zones is similar to MQB 4.8.349, and three zones ending in LAGs can be recognized (Text-fig. 6C).

4.2. *Bos primigenius*

IPB M 26, *Bos primigenius*, fragment of a probable tibia (Text-fig. 6D)

The thin section covers two-thirds of the original cross section of the bone. The preserved length of the bone is 28.8 cm. The maximum cortical thickness is 2.2 cm, and the diameter at this location is 5.7 cm. The histology of *Bos* is basically similar to that of *Bison*, at least based on the single specimen sampled. However, there are differences in the degree of remodeling. In IPB M 26, the cortex is much less remodelled than that of the *Bison* samples. The details of the zones and LAGs are the same. In the outer third of the cortex, four zones separated by LAGs are seen. A characteristic feature is that the secondary osteons are lined up just exterior to the LAGs, thus following a pre-existing vascular network as described above for *Bison* sample MQB 4.8.349 (Text-fig. 6D).

5. Histological descriptions: Equidae

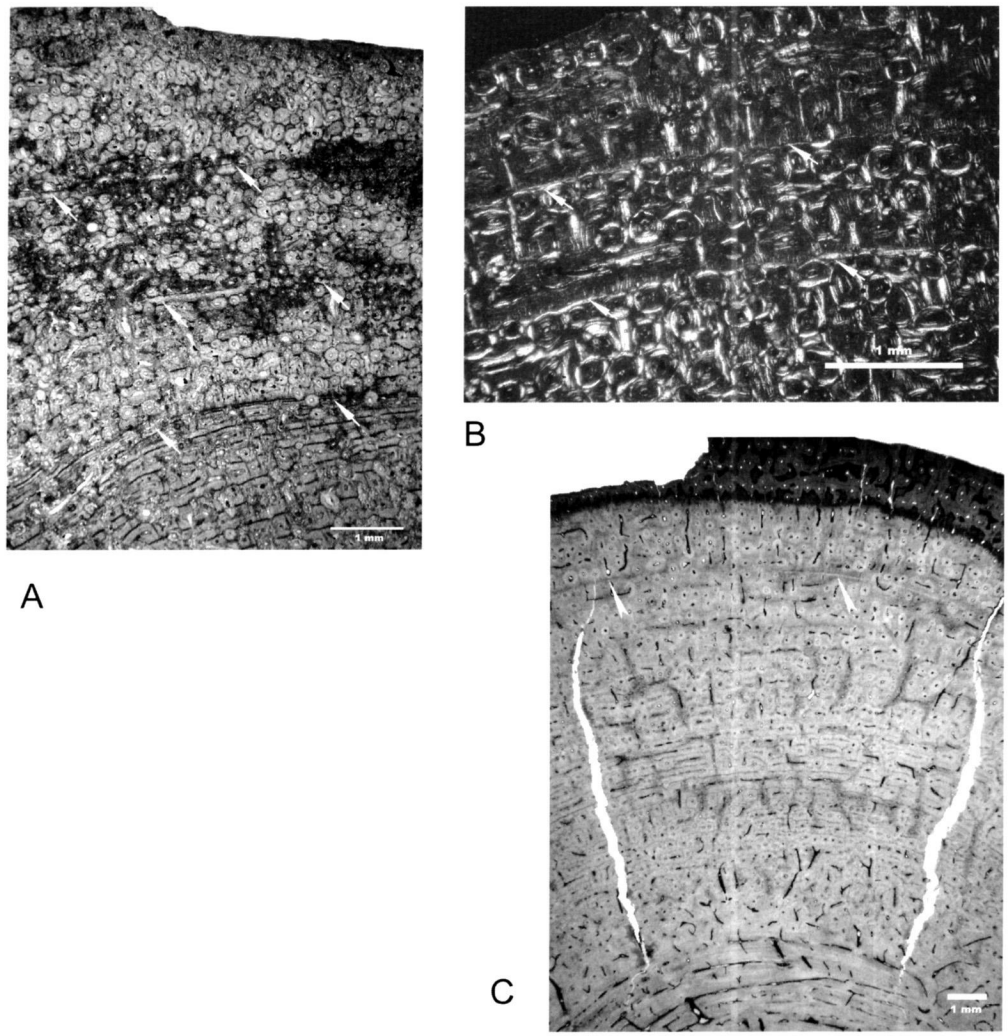
Because it is difficult to distinguish among the species of European Late Pleistocene horses (*Equus germanicus*, *Equus ferus*, *Equus hydruntinus*) based on long bone morphology, all material was subsumed under *Equus* sp. This also follows the labelling in the MBQ collections.

The histology of the Equidae is less diagnostic than that of the Bovidae and the Cervidae. In general, the samples show similarities with both of the other groups. However, equids, like cervids and bovids, are easily distinguished histologically from the woolly rhino and mammoth samples. The primary tissue of the equids is of the plexiform fibro-lamellar type. The vascular system is circumferential in all samples, but longitudinal canals are also present. Primary bone is restricted to the outer third of the cortex, the inner two-thirds being dominated by remodeling.

5.1. *Equus* sp.

IPB M 2042, *Equus* sp., tibia (Text-fig. 7A)

The bone sampled was a fragment of a tibia with a preserved length of 29.7 cm and a diameter of 4.1 cm. Preservation was poor at the histological level. The cortex ranges in thickness from 9 mm to 12.5 mm. The thin section covers the entire cross section of the bone. The medullary cavity is entirely devoid of spongy bone except



Text-fig. 7. **A** IPB M 2042, *Equus* sp., tibia. **B** MQB 4.4.634, *Equus* sp., metatarsal III. Fibro-lamellar bone and LAGs in polarized light. **C** MQB 4.8.516, *Equus* sp., metatarsal III. White arrows mark LAGs in all three sections.

for a few remnants. The histology is dominated by strong remodeling, with secondary osteons increasingly replacing the primary bone of the cortex until it was entirely replaced by dense Haversian bone. Only in a few places less affected by remodeling was primary tissue preserved. Despite this heavy remodeling, however, three LAGs can be easily discerned in the outer third of the cortex (Text-fig. 7A).

MQB 4.4.733, *Equus* sp., tibia

This is another tibia fragment, which has a cortical thickness of 1–1.25 cm. Its medullary cavity lacks spongy bone at the sampled cross section and is lined with endosteal bone. The entire cortex is heavily remodelled with a few small erosion rooms in the inner cortex. As in the previous specimen, a single LAG is preserved in the outer third, where remodeling is less severe.

MQB 4.4.614, *Equus* sp., metatarsal III

The sampled bone fragment is 14.8 cm long and has a diameter of 3.6 cm. Cortical thickness is 9–12.5 mm. The medullary cavity is relatively small but lacks any spongy bone. Histologically, sample MQB 4.4.614 is very similar to sample MQB 4.4.733. Here, too, there are two to three well developed LAGs. However, the

distribution of the regions of remodeling is different from the tibiae. The foci are not lateral but anterior and posterior. In this section, there are also secondary osteons aligned in a row exterior to the LAGs. Towards the internal cortex, the secondary osteons become denser and less organized.

MQB 4.4.634, *Equus* sp., metatarsal III (Text-fig. 7B)

This metatarsal has a preserved length of 20.3 cm and a diameter of 3.7 cm. Cortical thickness is 9–10 mm. In contrast to specimens MQB 4.4.614, 4.4.657, and 4.8.516, only a small part of the proximal part of the shaft remained. Thus, the sample was taken close to the metaphyseal region. For this reason, the medullary cavity is entirely filled with trabecular bone. Primary bone is only preserved in the outer third of the cortex in interstitial spaces between remodelled areas. As in the two tibiae, the bone is already in an advanced state of remodeling, but nevertheless two LAGs remain visible (Text-fig. 7B).

MQB 4.4.657 and MQB 4.8.516, *Equus* sp., metatarsal III (Text-fig. 7C)

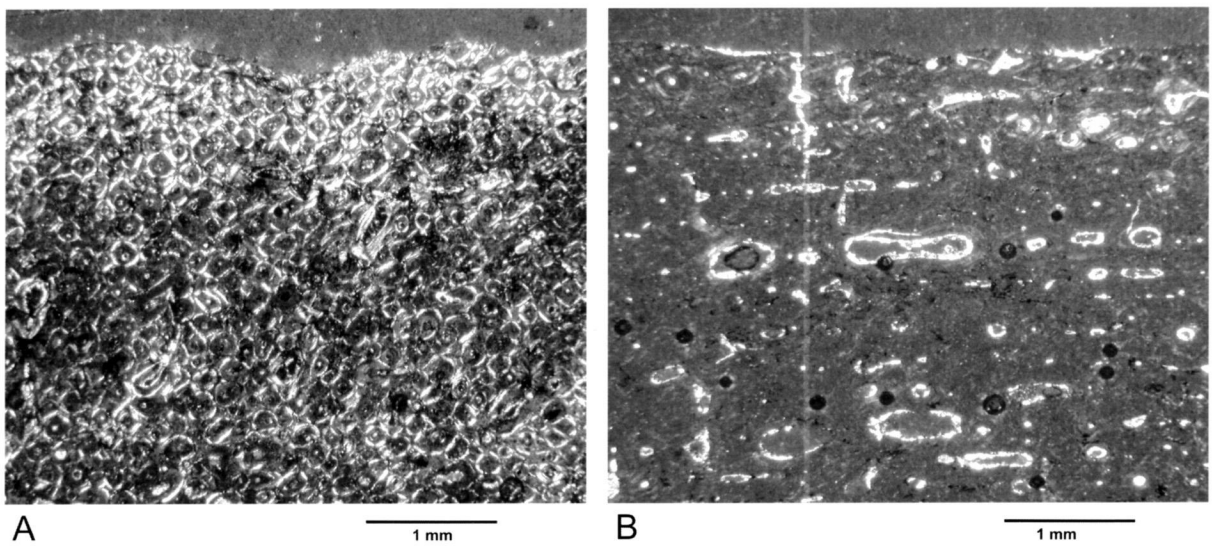
These two specimens have a diameter of 3.2 cm each and a preserved length of 15.3 cm and 16.1 cm. The cortical thickness of both is 1-1.1 cm. Preservation of the histology is very good, and vascularization is well developed. In sample MQB 4.8.516, the inner quarter of the cortex consists of endosteal bone lining the medullary cavity. Otherwise the histology of this sample is the same as that of metatarsus MQB 4.4.614. These two samples also agree with the other *Equus* metatarsals with respect to the distribution of the regions of remodeling. More than one LAG cannot be discerned with certainty in sample MQB 4.4.657 or in MQB 4.8.516 (Text-fig. 7C).

6. Histological descriptions: Rhinocerotoidea

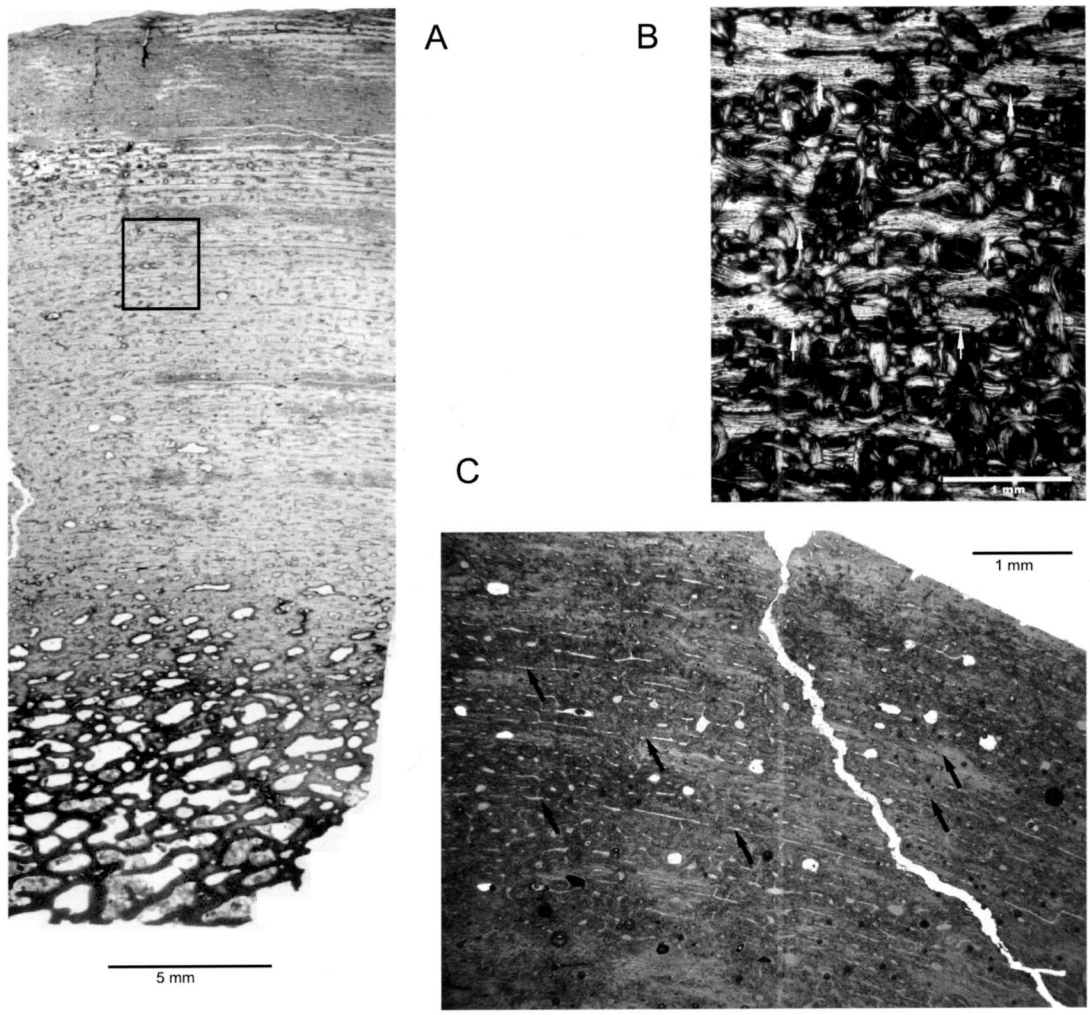
We sampled four tibiae of woolly rhinos. All have a similar histology. Three of the tibiae were sampled by coring; only from tibia IPB Ca BREX 01/1 was a complete cross section obtained. This will be described here in detail as representative for the other samples as well.

6.1. *Coelodonta antiquitatis*

IPB Ca BREX 01/1, *Coelodonta antiquitatis*, tibia (Text-fig. 8)



Text-fig. 8. **A** IPB CA BREX01/1, *Coelodonta antiquitatis*, tibia. **A** Outer cortex showing strong remodeling with several generations of secondary osteons. **B** Restricted region in outer cortex with poorly preserved primary laminar bone. The inner vascular canals are already enlarged by erosional processes. Polarized light, bone surface is visible at the top of both images.



Text-fig. 9. **A** MQB MP RHK 01.2, *Mammuthus primigenius*, indet. long bone. **A** Overview of thin section cut from core. Box marks area of close-up. **B** Close-up of an area with primary bone in polarized light showing two LAGs (white arrows). **C** IPB M 5962, *Mammuthus primigenius*, indet. long bone. Black arrows indicate growth marks representing possible LAGs.

This sample was not obtained from the middle of the diaphysis but close to the epiphysis. Therefore no distinctive medullary cavity is present but only one large and many small erosion rooms. The entire histology of the bone is characterized by strong remodeling (Text-fig. 8A). Except for a few islands of primary bone, the tissue is entirely replaced by secondary osteons, sometimes in several generations. What is preserved of the primary bone is more laminar than plexiform in its vascular system (Text-fig. 8B). The vascular density in the primary bone is the same as in the other large mammal taxa studied. Probably due to the strong remodeling, it was not possible to detect any growth marks although the sampled tibia showed macroscopic indications of LAGs which consist of spalling of bone layers and linear features on fracture surfaces.

7. Histological descriptions: Elephantidae

At a first glance, the histology of the elephants appeared similar to that of the rhinos because they are also strongly dominated by Haversian bone. However, as opposed to the rhinos, the thin sections often show the remains of primary fibro-lamellar bone with a laminar vascular network.

7.1. *Mammuthus primigenius*

MQB MP RHK 01.2, *Mammuthus primigenius*, indet. long bone fragment (Text-figs 9A+B)

In this specimen, cortical thickness ranges from 2.8 to 3.3 cm. The medullary cavity is filled with spongy bone. Primary bone is only preserved in the outer third of the cortex, which shows loosely spaced secondary osteons. These increase in density towards the center of the bone, and the inner third of the cortex is completely remodelled.

Sample MQB MP RHK 01.2 (Text-figs 9A+B) shows a distinct zonation in the laminar tissue. If the sample is observed under polarized light and rotated by 45 degrees, the limits of the zones are marked by bright lines. Using a gypsum plate makes these lines appear dark blue against the lighter background. However, this zonation could not be linked unequivocally to LAGs, and only more and better preserved *Mammuthus* long bone samples will resolve the issue of the presence of LAGs.

IPB M 5962, *Mammuthus primigenius*, indet. long bone fragment (Text-fig. 9C)

The cortical thickness of this specimen is 2.75 cm. The medullary cavity has little spongy bone. This sample also showed macroscopic indications of cyclical growth. However, in normal and polarized light under the microscope, only two zones could be distinguished in laminar fibro-lamellar bone (Text-fig. 9C).

MQB MP RHK 02.1, *Mammuthus primigenius*, indet. long bone fragment

This sample has a cortical thickness of 4.15 cm. There is a little spongy bone in the medullary cavity. The histology of the sample is similar to the previous one, but possible LAGs can only be detected under polarized light.

MQB MP RHK 03.1, *Mammuthus primigenius*, indet. long bone fragment

This bone fragment has the greatest cortical thickness of all specimens studied, amounting to 4.85 cm. The medullary cavity shows much spongy bone. The sample is more affected by remodeling than the other mammoth samples; otherwise the histology is similar to that of MQB MP RHK 01.2. There are no indications of growth marks, either under normal light or under polarized light.

8. Discussion

8.1. Taxonomic differences in histology

A surprising result of this study is that the different large mammal taxa can be discerned based on their long bone histology, i. e. that there are taxonomic differences in histology at the family level and above. In the third part of their study of tetrapod bone histology, Enlow & Brown (1958) described the long bone histology of some Recent artiodactyls, including *Bos*, *Capra*, *Equus*, and *Sus*, but did not mention specific differences among taxa of the kind discovered during our study. For example, they describe the histology of bovids as well organized plexiform tissue differing from reticular tissue, but do not differentiate further (Enlow & Brown 1958).

We found, on the other hand, that bovids, as represented by two species, are clearly distinguished from all other taxa studied by the wavy appearance of their circumferential vascular canals (Text-fig. 6). The cervids, represented by three species in our sample, also have a well defined histology, with straight appearance of the circumferential vascular canals and their limited amount of remodeling (Text-figs 4+5). This is in agreement with data from the literature, e. g. the Recent North American *Cervus elaphus* sample described by Horner et al. (1999), which was a sample from the cortex of the femur. However, a histologic distinction among *Megaloceros*, *Cervus*, and *Rangifer* was not unequivocally possible. Although *Equus* does not have clearly distinguishing characters in its primary tissue, it differs from the other taxa in the distribution of the regions of strongest remodeling in the bones studied (tibiae, metatarsals) (Text-fig. 7). The primary tissue of *Mammuthus* is more of the laminar fibro-lamellar type than the reticular type. It is also distinguished from the other taxa by its greater cortical thickness (Text-fig. 9). The sample (quantity and quality) of *Coelodonta* is too limited to allow generalizations about differences in histology, but heavy remodeling seems prevalent.

Although indications of growth marks were discovered in all taxa, the ungulates (cervids, bovids, and *Equus*) differ from the mammoth and the rhino in that their cycles and LAGs are more clearly expressed. The peculiar growth marks of *Mammuthus* may also turn out to be taxon-specific upon further study (Text-fig. 9B). As mentioned, in both *Bos* and *Equus*, LAGs may be followed by a parallel line of secondary osteons, making the detection of growth marks in almost completely remodelled bone possible (Text-figs 6B+D).

The result of this study, that large mammal taxa can be distinguished based on their bone histology, is in agreement with a recent study on sauropod long bones (SANDER 2000), in which individual taxa also could be distinguished based on bone histology.

8.2. The frequency and causes of growth marks in the sample

Growth marks, specifically lines of arrested growth, were detected in the great majority of all specimens studied in thin section. This leads to the question whether LAGs are a general but previously overlooked feature of all large Pleistocene mammals, or possibly of all large mammals in general. We do not have the data to provide the answer because we preselected the material for macroscopic evidence of cyclical growth (Text-fig. 1). We did not sample those bones that lacked this evidence (but may have LAGs), and these were the great majority (Text-fig. 2). On the other hand, not all samples that showed macroscopic evidence for LAGs did, in fact, show them in their histology. Especially in *Mammuthus*, the presence of typical LAGs has not been established, and additional work is necessary. The regular spacing of the LAGs in the ungulates observed in this study suggests a potential use in individual aging of Late Pleistocene mammals (skeletochronology).

However, we would like to make a few general points about the frequency of LAG occurrence. It is clear that LAGs are much more common than previously assumed, in frequency of occurrence in a specific taxon as well as in frequency across taxa. It is very likely that the presence and prominence of LAG vary among bones of the skeleton, as is well known from recent vertebrates (e. g. CASTANET et al. 1993) and from dinosaurs (e. g. HORNER et al. 1999, ERICKSON et al. 2004).

Because most of our sample derives from animals living during glacial conditions, this could also have affected the frequency of growth marks. The comparison of Pleistocene mammals with extant representatives of the same or very similar species offers great potential to address the issue of climate dependence of LAG occurrence in large mammals. It is tempting to link the LAGs to particular climatic conditions in Central Europe during the glacial periods. This included strong seasonality which possibly induced long seasonal migrations in the herbivores studied here. However, we refrain from speculating further in the virtual absence of modern comparative data on LAGs and their origin in large mammals.

8.3. Significance for deductions about physiology

The occurrence of LAGs in the long bone cortex of Late Pleistocene large mammals described in this study serves as a caveat to the hypothesis (e. g., REID 1987, 1996, 1997, CHINSAMY 1994, CHINSAMY & HILLENIUS 2004) that the common occurrence of LAGs in dinosaur bones indicates that these animals had a lower metabolic rate than modern mammals. Our data, together with the observation of LAGs in moas (TURVEY et al. 2005), lend support to the view of PADIAN & HORNER (2004) that LAGs in dinosaurs are plesiomorphies and that they are of little use for physiology reconstruction. It is noteworthy that the largest mammals studied, the mammoth (this study), as well as the largest dinosaurs, the sauropods (CURRY 1999, SANDER 2000, SANDER & TÜCKMANTEL 2003, pers. observations) show the least development of lines of arrested growth.

Future work on mammals will need to include a more controlled approach to sampling to detect the frequency of LAG occurrence in cold-climate vs. warm-climate living and Pleistocene mammals. The reliability of macroscopic indicators for LAGs also needs to be tested by comprehensive sampling. A possible explanation for the apparent rarity of such indicators in the fossil population from the Rhine-Herne ship channel is that most long bones have undergone Haversian replacement in their cortex which would have obliterated LAGs (e. g. Text-fig. 8A). In other words, the absence of macroscopic indicators for LAGs may simply indicate the prevalence of Haversian bone in mammals compared to dinosaurs and not their rarity in primary bone. Long bones of large mammals differ in their histology from those of dinosaurs in generally being much more remodelled (ENLOW & BROWN 1958, pers. observ.).

This difference may not necessarily be an indicator of a different thermal physiology of mammals and dinosaurs but of different life history patterns. At least the largest dinosaurs, the sauropods, appear to differ from almost all large living mammals in their continued fast growth after sexual maturity (SANDER 2000). The continued fast apposition of bone throughout most of the life of the animal thus may have outpaced the front of Haversian remodeling moving peripherally. Only after growth had stopped, could Haversian remodeling entirely replace the primary bone, as seen in the largest sauropod long bones (SANDER 2000). In mammals, growth stops relatively early in the life of the animal, but remodeling continues, resulting in complete replacement of primary bone by secondary bone even in middle-aged individuals.

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References

BUFFRENIL, V. DE (1992): Donees preliminaires sur la presence des lignes d'arret de croissance periostiques dans la mandibule du marsouin commun *Phocoena phocoena* (L.), et leur utilisation comme indicateur de l'âge. – Can. J. Zool., **60** (11): 2557–2567.

BYBEE, P.J., LEE, A.H. & LAMM, E.-T. (2006): Sizing the Jurassic theropod dinosaur *Allosaurus*: Assessing growth strategy and evolution of ontogenetic scaling of limbs. – J. Morph., **267**: 347–359.

CASTANET, J. (1994): Age estimation and longevity in reptiles. – Gerontology **40:** 174–192.

CASTANET, J., FRANCILLON-VIEILLOT, H., MEUNIER, F.J. & RICQLÈS, A. DE (1993): Bone and individual aging. – In: HALL, B.K. (ed.): Bone. Volume **7**: Bone Growth – B. – (CRC Press) Boca Raton, pp. 245–283.

CHINSAMY, A. (1994): Dinosaur bone histology: Implications and inferences. – In: ROSENBERG, G.D. & WOLBERG, D.L. (eds.): Dino Fest. – Paleont. Soc. Spec. Publ. **7:** 213–227.

CHINSAMY, A., RICH, T. & VICKERS-RICH, P. (1998): Polar dinosaur bone histology. – J. Vert. Paleont., **18** (2): 385–390.

CHINSAMY-TURAN, A. & HILLENIUS, W. (2004): Physiology of non-avian dinosaurs. – In: WEISHAMPEL, D.B., DODSON, P. & OSMOLSKA, H. (eds.): The Dinosauria. – 2nd Ed., Berkeley (University of California Press), pp. 643–659.

CURRY, K.A. (1999): Ontogenetic histology of *Apatosaurus* (Dinosauria: Sauropoda): New insights on growth rates and longevity. – J. Vert. Paleont., **19** (4): 654–665.

ENLOW, D.H. & BROWN, S.O. (1956): A comparative histological study of fossil and recent bone tissues. Part I. – Texas J. Sci., **8** (4): 405–439.

–,– (1957): A comparative histological study of fossil and recent bone tissues. Part II. – Texas J. Sci., **9** (2): 186–214.

–,– (1958): A comparative histological study of fossil and recent bone tissues. Part III. – Texas J. Sci., **10** (2): 187–230.

ERICKSON, G., MACKOVICKY, P.J., CURRIE, P.J., NORELL, M.A., YERBY, S.A. & BROCHU, C.A. (2004): Gigantism and comparative life-history parameters of tyrannosaurid dinosaurs. – Nature, **430** (12 August 2004): 772–775.

FRANCILLON-VIEILLOT, H., BUFFRÉNIL, V.D., CASTANET, J., GÉRAUDIE, J., MEUNIER, F.J., SIRE, J.Y., ZYLBERBERG, L. & RICQLÈS, A. DE (1990): Microstructure and mineralization of vertebrate skeletal tissues. – In: CARTER, J.G. (ed.): Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. – Vol. **1:** 471–530, (Van Nostrand Reinhold) New York.

GROSS, W. (1934): Die Typen des mikroskopischen Knochenbaues bei fossilen Stegocepahlen und Reptilien. – Z. Anat. Entwicklungsgesch., **203** (6): 731–764.

HORNER, J.R., PADIAN, K. & RICQLÈS, A. DE (1999): Variation in dinosaur skeletochronology indicators: implications for age assessment. – Paleobiol., **25** (3): 49–78.

KLEIN, N.F. (2004): Bone Histology and Growth of the Prosauropod *Plateosaurus engelhardti* MEYER, 1837 from the Norian Bonebeds of Trossingen (Germany) and Frick (Switzerland). – Bonn (University of Bonn), 128 p.

KLEVEZAL, G.A. (1996): Recording Structures of Mammals. Determination of Age and Reconstruction of Life History. – Rotterdam (A.A. Balkema), 274 p.

KOENIGSWALD, W. v. (2002): Lebendige Eiszeit. Klima und Tierwelt im Wandel. – Stuttgart (Konrad Theiss Verlag), 190 p.

KOENIGSWALD, W. v. & WALDERS, M. (1995): Zur Biostratigraphie der Säugetierreste aus der Niederterasse der Emscher und der Fährtenplatte von Bottrop. – Münch. geowiss. Abh., **27:** 51–62.

PADIAN, K. (1997): Growth lines. – In: CURRIE, P.J. & PADIAN, K. (eds.): Encyclopedia of Dinosaurs. – San Diego (Academic Press), pp. 288–291.

PADIAN, K. & HORNER, J.R. (2004): Dinosaur physiology. – In: WEISHAMPEL, D., DODSON, B.P. & OSMOLSKA, H. (eds.): The Dinosauria. – 2nd ed., (University of California Press) Berkeley, pp. 660–671.

REID, R.E.H. (1987): Bone and dinosaurian "endothermy". – Mod. Geol., **11** (2): 133–154.

REID, R.E.H. (1996): Bone histology of the Cleveland-Lloyd dinosaurs and of dinosaurs in general. Part I: Introduction: Introduction to bone tissues. – Brigham Young Univ. Geol. Stud., **41:** 25–71.

–,– (1997): Dinosaurian physiology: the case for "intermediate" dinosaurs. – In: FARLOW, J.O. &. BRETT-SURMAN, M.K. (eds.): The Complete Dinosaur. – (Indiana University Press) Bloomington and Indianapolis, pp. 449–473.

RICQLÈS, A. DE (1983): Cyclical growth in the long limb bones of a sauropod dinosaur. – Acta Palaeont. Pol., **28** (1-2): 225–232.

SANDER, P.M. (2000): Long bone histology of the Tendaguru sauropods: Implications for growth and biology. – Paleobiol., **26** (3): 466–488.

SANDER, P.M. & KLEIN, N. (2005): Developmental plasticity in the life history of a prosauropod dinosaur. – Science, **310** (December 16, 2005): 1800–1802.

SANDER, P.M. & TÜCKMANTEL, C. (2003): Bone lamina thickness, bone apposition rates, and age estimates in sauropod humeri and femora. – Paläont. Z., **76** (1): 161–172.

TURVEY, S.T., GREEN, O.R. & HOLDAWAY, R. (2005): Cortical growth marls reveal extended juvenile development in New Zealand moa. – Nature, **435** (16 June 2005): 940–943.

WARREN, J.W. (1963): Growth Zones in the Skeleton of Recent and Fossil Vertebrates. – Unpubl. Ph.D., University of California, Los Angeles.

WEISHAMPEL, D.B., DODSON, P. & OSMOLSKA, H. (2004): The Dinosauria. – 2nd Ed., (University of California Press) Berkeley, 861 p.

WERNING, S. (2005): Ontogenetic osteohistology of the ornithopod dinosaurs *Tenontosaurus tilletti* (Cretaceous, North America). – J. Vert. Paleont., **25** (3): 128A–129A.