



General palaeontology, systematics and evolution (Vertebrate palaeontology)

The ontogeny of bone growth in two species of dormice: Reconstructing life history traits

Ontogénie de la croissance osseuse chez deux espèces de dormice

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ABSTRACT

Though bone histology has become a powerful tool to reconstruct life history strategies and physiology in living and extinct reptiles and amphibians, it is of limited use in mammals. Dormice (Myoxidae) are good candidates for assessing the relation between bone microstructure and life history due to their long life span, marked physiological cycles and negligible bone remodelling. We carried out the most comprehensive study so far analyzing 16 wild individuals of unknown age belonging to two different species of dormice, *Glis glis* and *Eliomys quercinus*. Our study shows a high degree of consistency in the number of resting lines present in bones of the same individual, with femora providing the most accurate age estimations. Moreover, the presence of a single LAG in some juveniles allows discerning between offspring from different reproductive events (early or late litters).

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R É S U M É

Bien que l'histologie des os soit devenue un outil précieux pour la reconstitution des stratégies de l'histoire de la vie et de la physiologie chez les reptiles et amphibiens vivants et éteints, elle est d'une utilité limitée chez les mammifères. Les Dormice (Myoxidae) sont de bons candidats pour évaluer la relation entre microstructure de l'os et histoire de la vie, en raison de leur longue durée de vie, de leurs cycles physiologiques marqués et des modifications négligeables de leurs os. Nous avons réalisé l'étude jusqu'à présent la plus complète, en analysant 16 individus sauvages d'âge inconnu, appartenant à deux différentes espèces de dormice, *Glis glis* et *Eliomys quercinus*. Notre étude montre un degré élevé de cohérence dans le nombre de lignes de repos présentes dans les os du même individu, avec les fémurs fournissant les estimations d'âge les plus précises. En outre, la présence d'un unique LAG chez certains juvéniles permet de faire la distinction entre progénitures, à partir de différents événements de la reproduction (portées récentes ou anciennes).

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1. Introduction

Life history is the schedule of events in the life of an organism from conception to death, with growth rate, age at first reproduction and length of reproductive span (generally equalling longevity) being of special importance because of their direct impact on fitness (Ricklefs, 2007). Life histories are strategies to enhance reproductive success by adjusting the developmental schedule to current environmental conditions. The study of life histories, hence, provides valuable insights into ecological conditions, biodiversity, demography, vulnerability and many other aspects of a species' biology and ecology (Ricklefs, 2007; Roff, 2002; Stearns, 1992). Therefore, an understanding of life history strategies is key to conservation management of endangered species. Even more, reconstruction of life histories from the past in their environmental context provides important cues to reconstruct past ecosystem dynamics or to predict the vulnerability and survival chance of extant populations (Köhler and Moyà-Solà, 2009; Raia and Meiri, 2006; Raia et al., 2003; Schwartz et al., 2002).

Bones and teeth of vertebrates record important events that took place during development (Klevezal, 1996), amongst them two important life history traits: the transition from young to adult (age at first reproduction, also called the age at sexual maturity) and age at death (which in some cases coincides with maximum longevity). One important goal of hard tissue histology is to estimate these life history components in extant and fossil vertebrates (Jordana and Köhler, 2011; Köhler, 2010; Köhler and Moyà-Solà, 2009).

The biological concept of "adult" is undeniably the attainment of sexual maturity, which is not to be confounded with somatic maturity. Sexual maturity can be attained before somatic maturity (in *Homo* for instance) or after (for instance in birds, Erickson et al., 2007). Bone tissue records age at sexual maturity in form of an important decrease in the rate of periosteal bone apposition (Chinsamy-Turan, 2005; Klevezal, 1996). This has a biological explanation based on the concept of trade-offs in life history theory. Because energy is limited, resources must be shared between different vital functions. As long as an organism needs to grow in order to attain a minimum size for successful reproduction, resources are channelled towards growth (and maintenance). As soon as this size is attained, resources are channelled away from growth towards reproduction (Ricklefs, 2007; Roff, 2002; Stearns, 1992). In mammalian bone, this is usually recorded as the transition from fast-growing to slow-growing tissue or even to a halt in bone apposition. It is a common belief that in mammals, contrary to ectotherm vertebrates, bone growth stops completely. This, however, is not the case. Many mammals such as our myoxids, but also primates (Castanet et al., 2004; Klevezal, 1996), ungulates (Klevezal, 1996; Köhler and Moyà-Solà, 2009), bears and kangaroos (Chinsamy-Turan, 2005), and probably many others deposit various annual resting lines periosteally that indicate an alternation between ceasing and resuming growth. Such growth rings, or lines of arrested growth (LAGs) are characteristic features in bone tissues of ectotherms

(reptiles and amphibians) (Guarino et al., 2003; Starck and Chinsamy, 2002; Tumarkin-Deratzian, 2007), but they have also been observed in endotherms (mammals and birds) (Castanet et al., 2004; Klevezal, 1996; Starck and Chinsamy, 2002). LAGs record cyclical cessation of bone growth and are deposited annually (Castanet et al., 2004; Chinsamy-Turan, 2005), except for certain amphibians, which form LAGs biannually in environments with a dry summer and a cold winter seasons (Chinsamy-Turan, 2005). The number of growth lines in histological cross sections, hence, provides the age of an individual, a fundamental trait for demographic studies such as reconstruction of growth curves (Stearns, 1992), determination of the age structure of populations (Guarino et al., 2003), and conservation management (Chinsamy and Valenzuela, 2008) among others. In mammals, where bone remodelling tends to increase with age, estimated ages must be considered as minimum ages (Castanet et al., 2004). The spatial organization of LAGs and the histology of tissue deposited during cycles of active osteogenesis result from both local and general rates and rhythms of bone growth (Castanet, 2006), and provide additional information about an organism's life history such as age at first reproduction, or environmental (especially resource) conditions.

Usually, studies of bone histology are conducted in vertebrates that grow continuously throughout their lives or at least over several years and that show little if any remodeling of their bone tissue such as living and extinct amphibians and reptiles including certain dinosaurs (Chinsamy-Turan, 2005; Chinsamy and Valenzuela, 2008; Erickson, 2005; Margerie de et al., 2002; Tumarkin-Deratzian, 2007), early birds (Cambra-Moo et al., 2006; Castanet et al., 2000) and mammal-like reptiles (Botha and Chinsamy, 2005; Bromage et al., 2009). Small mammals, in contrast, grow fast and reach somatic/sexual maturity long before their first year of life when the first LAG is deposited (Castanet et al., 2004). Therefore, their bone tissue is considered to provide little information about growth rates, age at maturity, age at death or other life history traits.

The common histological pattern of mammals is: (i) a bone matrix of highly vascularised reticular or plexiform type indicative of a high and constant rate of tissue deposition in juvenile individuals; (ii) rather abruptly followed by dense periosteal bone at the transition from juvenile to adult, indicative of an important decrease in growth rate. Later in life, rest lines can form in this periosteal region (Chinsamy-Turan, 2005). Mammals with an extended juvenile period, generally large mammals such as certain ungulates (Köhler and Moyà-Solà, 2009), follow a slightly different pattern with one or occasionally two LAGs deposited within the fast-growing bone tissue before the beginning of dense periosteal bone, indicating that the animal ceased and resumed relatively fast growth over two or three years before maturity. Some studies, however, provide compelling evidence that mammalian bone growth does not necessarily follow this apparently uniform pattern (Castanet, 2006; Castanet et al., 2004; Chinsamy and Hurum, 2006; Klevezal, 1996; Ponton et al., 2004). A recent example is the finding of reptile-like zonal bone in a

Table 1

Describing different life history traits and hibernation periods of both species included in this study.

Tableau 1

Tableau décrivant différents traits de l'histoire de vie et les périodes d'hibernation des deux espèces étudiées.

Traits	<i>Eliomys quercinus</i>	<i>Glis glis</i>
Size (length in mm)	100–175	130–190
Body weight (g)	45–120	70–180
Sexual maturity (months)	3–6	3–6
Lifespan (years)	5,5 (captivity)	3–4 years (wild), 8 (captivity)
Hibernation periods	November to February	November–May
Reproductive strategy	March–May & August–October	June–August (after 1st hibernation)
Gestation (days)	22–28	20–30
Weaning (weeks)	4	4
Litter size (n° indiv.)	2–8	2–10
Litters/year	2	1–2

Data from Nowak, 1999.

fossil insular mammal (Köhler, 2010; Köhler and Moyà-Solà, 2009).

These studies illustrate that we are not only far from understanding aspects of mammalian bone tissue such as tissue formation and development, associated physiological traits, or the evolution of bone tissues, but that we do not even possess an acceptable database of mammalian bone microanatomy necessary for a correct interpretation of bone tissue from living or fossil mammals. Therefore, survey studies in the area of mammalian bone histology are urgently needed to increase the few data hitherto available.

Here, we provide the first description of the ontogeny of appositional growth in representatives of Myoxidae (dormice), *Glis glis* and *Eliomys quercinus*. We examined the nature of the bone tissues of various skeletal elements (long bones and mandibles). Dormice are especially suitable for our study because (Table 1): (i) they have a high mean longevity (Moreno, 2005; Pilastro et al., 2003); (ii) they have slow life histories for their size (Krystufek et al., 2005); (iii) they are hibernators with a pronounced biological rhythm that closely matches the seasonal changes in their environment (seasonal differences in growth rates are particularly evident in hibernators) (Moreno, 2005; Pilastro et al., 2003); (iv) bone remodeling is negligible so that their bone tissue is likely to present growth marks that reliably record their physiological cycles over years (Klevezal, 1996).

The conservation status of certain myoxid species in Spain is close to vulnerable. While *G. glis* is of least concern (Moreno, 2005), *E. quercinus* populations are particularly fragile due to destruction, degradation, pollution and fragmentation of their micro-habitats (Castián and Gosálbez, 2007). Recent data indicate a serious decline in the abundance of dormice (Blanco, 2007). This is especially concerning in the case of *E. quercinus* on which only a few (ecological and morphological) studies have been conducted (Gil-Delgado et al., 2006; Magalhaes de et al., 2009; Moreno, 2002). The knowledge of key life history traits is extremely important for dormice conservation. Life span is one such trait. Hitherto, however, life span has been determined for captive specimens of *E. quercinus* only (Magalhaes de et al., 2009) (Table 1), while data on natural populations are not available so far. Here, bone histology provides a valuable alternative to the classical methods of capture-recapture as it is a powerful tool to determine

life span and, ultimately, to assess population dynamics of threatened species.

Krystufek et al. (2005) found LAGs in a series of histological sections of mandibles of the less vulnerable taxon *G. glis*, which they utilized to reconstruct population structure, mean age and maximum life span for this wild population. However, they did not provide any histological description of the mandibular tissue and disregarded postcranial bones. Our survey, hence, is the first comprehensive study of the microstructure of long bones and mandibles of dormice. The aim of our study is to determine the histological patterns of the long bones (tibia, femora, humerus, radius, ulna) and mandibles in dormice, to describe the ontogenetic growth and to estimate age at death of individuals, thus providing new data on the life span of both species.

2. Material and methods

Our sample consists of 16 wild individuals of unknown age belonging to two different species of dormice, *G. glis* ($n=6$) and *E. quercinus* ($n=10$), which share some life history traits (Table 1). Age class was estimated according to:

- the degree of epiphyseal fusion:
 - immature individuals, with unfused epiphyses,
 - mature adults with fused epiphyses;
- the stage of tooth wear of the P₄ protoconid:
 - class I: protoconid unworn or slightly worn,
 - class II: protoconid worn up to two-thirds of its height,
 - class III: protoconid almost completely or completely worn (Fig. 1).

With the exception of one (*G. glis* from Sant Feliu del Racó, Barcelona), all individuals are from museum collections (Table 2). Only the specimens from the IPE (Instituto Pirenaico de Ecología) were ready to be sectioned, the remaining material required a laborious preparation process. The material from Museum de Ciències Naturals de Granollers (MCNG) (which consisted of only the right half of each individual preserved in alcohol) and the individual from Sant Feliu del Racó (which was found dead) were emaciated and then macerated in a KOH solution at 6% for 48 hours. The bones used for our analysis are tibia, femur, ulna, humerus, radius and mandible. Most individ-

Table 2

Information about the material.

Tableau 2

Informations à propos du matériel.

Specie	Sex	Origin	Bones	Museum Collection	ID
<i>G. glis</i>	f	Aigües tortes (Lleida, Spain)	J	IPE	3
<i>G. glis</i>	f	Vall d'Aran (Lleida, Spain)	H, R, U, F, T	MCNG	4
<i>G. glis</i>	m	Vall d'Aran (Lleida, Spain)	H, R, U, F, T	MCNG	5
<i>G. glis</i>	f	Vall d'Aran (Lleida, Spain)	H, R, U, F, T	MCNG	6
<i>G. glis</i>	m	St. Julià de Cerdanyola (Barcelona, Spain)	H, R, U, F, T	MCNG	7
<i>G. glis</i>	i	St. Feliudel Racó (Barcelona, Spain)	H, R, U, F, T	ICP	15
<i>E. quercinus</i>	i	Meranges (Girona, Spain)	J	IPE	1
<i>E. quercinus</i>	i	Ait Hhamed (Morocco)	J	IPE	2
<i>E. quercinus</i>	m	Py de Conflent (France)	J, H, R, U, F, T	MCNG	8
<i>E. quercinus</i>	i	Gredos (Ávila, Spain)	R, U, F, T	MCNG	9
<i>E. quercinus</i>	f	Py de Conflent (France)	J, H, F	MCNG	10
<i>E. quercinus</i>	f	Vall d'Aran (Lleida, Spain)	J, H, R, U, F, T	MCNG	11
<i>E. quercinus</i>	i	Vall d'Aran (Lleida, Spain)	J, H, R, U, F, T	MCNG	12
<i>E. quercinus</i>	m	Vall d'Aran (Lleida, Spain)	J, H, R, U, F, T	MCNG	13
<i>E. quercinus</i>	m	Gredos (Ávila, Spain)	J, R, U, F, T	MCNG	14
<i>E. quercinus</i>	m	Aisa (Huesca, Spain)	J	IPE	16

f: female; m: male; i: unknown; J: jaw; H: humerus; R: radius; U: ulna; F: femur; T: tibia; MCNG: Museum de Ciències Naturals de Granollers; IPE: Instituto Pirenaico de Ecología; ICP: Institut Català de Paleontologia.

f: femelle; m: mâle; i: inconnu; J: mâchoire; H: humérus; R: radius; U: ulna; F: fémur; T: tibia; MCNG: Musée de Sciences Naturelles de Granollers; IPE: Instituto Pirenaico d'Ecologie; ICP: Institut catalan de Paléontologie.

uals preserved all long bones. In some cases, however, only mandibles were available (Table 2). Quantitative data on femora are summarized in Table 3.

Before sectioning, each bone was embedded in epoxy resin (Araldite 2020). We prepared thin sections of the central part of the diaphysis of long bones and of the jaws at the level of the third molar (Fig. 2). The surface of interest is exposed using a Buehler Isomet low speed saw, and later polished on a glass sheet coated with carborundum powder, in decreasing particle size (e.g. 600, 800 and 1000 grit). The bone is fixed to a frosted glass slice using ultraviolet curing glue (Loctite 358). The ground section is prepared with a diamond saw (Buehler, PetroThin) to a final thickness of about 100–120 µm. The thin section is polished with a gradient of carborundum (800 and 1200). The slice is dehydrated through a graded series of alcohol baths, cleared in Histo-Clear II during five minutes and finally mounted in DPX mounting medium. The slices were observed under circularly polarized transmitted light (Leica DM 2500P).

Our descriptions of bone tissue of dormice are based on the typological classification established by de Ricqlès et al. (1991, see also Margerie et al., 2002). However, we follow Horner et al. (1999) in considering growth marks as lines of arrested growth (LAGs) and annuli only if they

can be traced around the whole circumference of the bone.

3. Results

3.1. Ontogenetic stages of bone tissue

3.1.1. Primary bone

Immature individuals: the tissue of immature individuals is formed exclusively of primary bone. The bone matrix may consist either of parallel-fibered bone (PFB) or woven bone, both types with rounded osteocytes (Fig. 3).

Vascularisation is scarce, and consists basically of primary osteons with an essentially longitudinal orientation, although some channels with radial orientation can be observed occasionally. There are open channels within the outer cortex; some of them are not filled with lamellar bone.

Individuals 13 (*E. quercinus*) and 15 (*G. glis*), though undoubtedly juveniles (unfused epiphyses) show a single LAG (Fig. 4), which is an unexpected result considering current literature (Chinsamy-Turan, 2005; Klevezal, 1996) and will be dealt with later.

Adults: the bone tissue formed during the adult stage is lamellar with flattened osteocytes (Fig. 5). In this tissue, LAGs appear as simple or double rest lines (Fig. 6).

Table 3Measurements of femora (in mm) of *G. glis* and *E. quercinus*.**Tableau 3**Mesures de fémur (en millimètres) de *G. glis* et *E. quercinus*.

ID	<i>Glis glis</i>					<i>Eliomys quercinus</i>						
	5	6	15	4	7	13	8	9	10	11	12	14
APD	1.16	1.77	1.94	2.04	2.01	1.64	1.79	1.65	1.88	1.66	1.62	1.97
TD	2.17	2.39	2.39	2.63	2.73	2.23	2.47	2.03	2.02	2.12	1.86	2.51
mL	17.71	16.75	21.87	25.64	26.10	21.76	23.75	-	-	-	20.68	25.14

APD: anteroposterior diameter; TD: transversal diameter; mL: maximal length; (-): data not available.

APD: diamètre antéro-postérieur; TD: diamètre transversal; ml: longueur maximum; (-): données non disponibles.

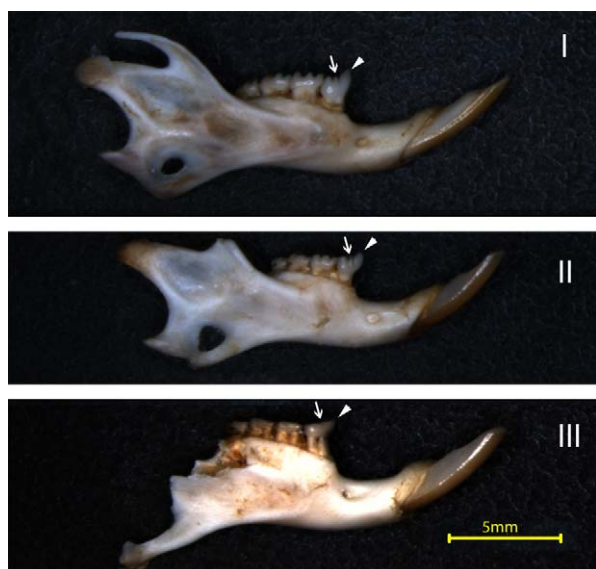


Fig. 1. Tooth wear classification based on the wear stage of the P₄ protoconid (arrow heads): Class I, protoconid unworn or slightly worn (the wear surface does not reach two-thirds of the protoconid height); Class II, wear has eliminated up to two-thirds of the height of the protoconid; Class III, the protoconid is completely or almost completely worn (the wear surface can reach the valley separating protoconid and mesoconid (arrows)).

Fig. 1. Classification de l'usure dentaire fondée sur le stade d'usure du protoconide de la P₄ (flèches). Classe I, protoconide non usé ou peu usé (la surface d'usure n'atteint pas les 2/3 de la hauteur du protoconide). Classe II, l'usure a éliminé jusqu'aux 2/3 de la hauteur du protoconide. Classe III, le protoconide est complètement ou presque complètement usé (la surface d'usure peut atteindre le sillon séparant le protoconide du mésoconide (flèches)).

Channels in adults are sparse, in some cases almost non-existent. Channels, if present in lamellar bone, are usually simple. All adult ulnae ($n = 6$, Table 2) show alternations of different types of bone tissue: in addition to lamellar bone, there may have formed either PFB or woven bone between LAGs indicating variable growth rates at different ontogenetic stages (Fig. 6). These faster-growing tissues may present a greater number of channels.

3.1.2. Bone remodeling

Ontogenetic, bone drift may erase part of the primary bone while lamellar bone is deposited endosteally, usually in adult individuals. In our studied material, bone drift is

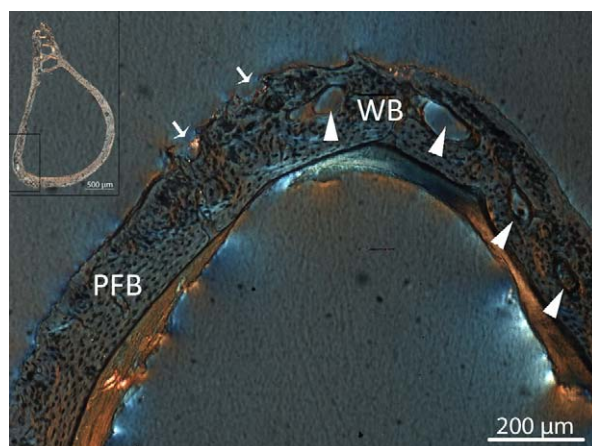


Fig. 3. Humerus of an immature individual. The cortex consists of parallel-fibered bone (PFB) and woven bone (WB). Open blood vessels on the outer cortex (arrows) and vessels not filled up with lamellar bone (arrow heads) indicate a period of fast growth.

Fig. 3. Humérus d'un individu immature. Le cortex consiste en os à fibres parallèles (PFB) et en os à fibres enchevêtrées (WB). Les vaisseaux sanguins ouverts dans la partie externe du cortex (flèches) et les vaisseaux non remplis d'os lamellaire (têtes de flèches) indiquent une période de croissance rapide.

more extensive than expected. Tibia, humerus, ulna and jaws showed an important drift, with endosteal bone making up most of the cortex in tibia, humerus and ulna. In the femur, the drift is less pronounced. This important remodeling leads to a loss of the innermost (ontogenetically earliest formed) tissue, so that some LAGs might have disappeared and age estimation in certain bones may be skewed.

The incidence of Haversian systems is very low. They are usually found in primary bone deposited during the juvenile stage.

3.2. Consistency of growth patterns and number of LAGs among bones of single individuals

Bone tissue types provide precise information on the rate of bone deposition and, hence, on overall growth rate of an organism (Margerie de et al., 2002). This, together with skeletochronology, is a powerful tool in reconstructing life history traits in vertebrates. The consistency of these signals throughout the bones of an individual is therefore of extraordinary importance. We



Fig. 2. Cutting plane of the bones. At midshaft in long bones (left). At third lower molar in jaws (right).

Fig. 2. Plan de coupe des os au milieu de la hampe pour les os longs (à gauche). Au niveau de la 3^e molaire inférieure pour les mâchoires (à droite).

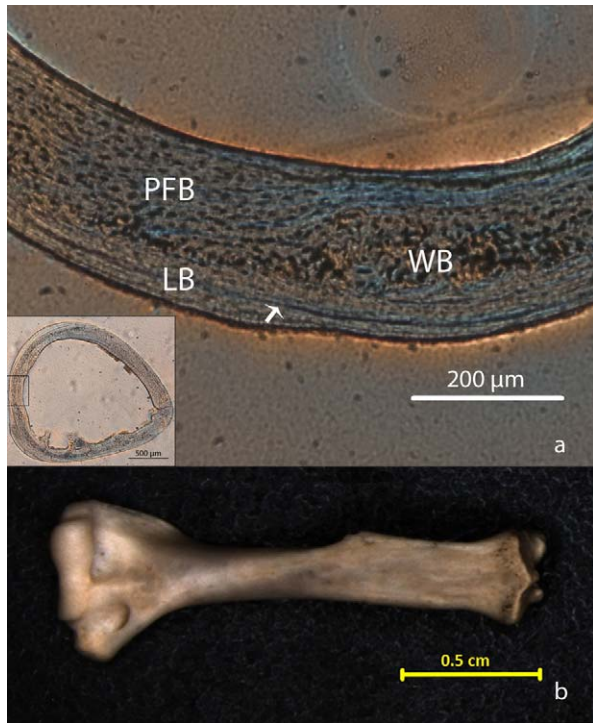


Fig. 4. a: humerus of an immature individual showing a single LAG (arrow) located in an area of slower growing bone (LB); b: the entire bone of Fig. 4a before cutting. Observe the still unfused proximal epiphyses.
Fig. 4. a: humérus d'un individu immature montrant un unique LAG (flèche) localisé dans une zone à croissance plus lente de l'os (LB); b: os entier de la Fig. 2a avant coupe. Observer les épiphyses proximales encore non fondues.

found cases of intra-individual variation that span from full consistency in the number of LAGs between bones of the same individual (*G. glis*; jaws were not included because of the limited availability in this species), to considerable variability, with a maximum difference of ± 3 LAGs (*E. quercinus*) (Table 4). The uniformity, however, observed in *G. glis* may be biased by the low representativeness of this sample (because of both the number

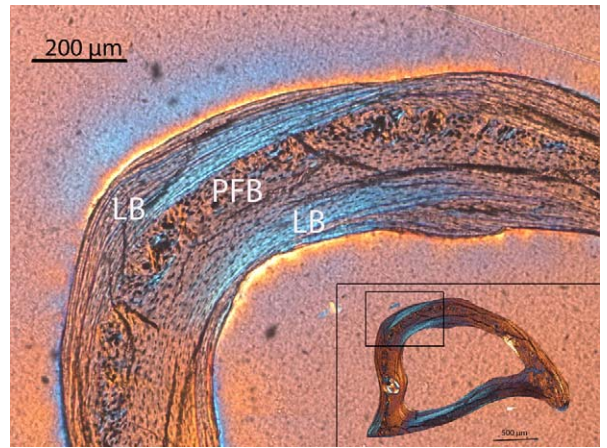


Fig. 5. Adult tibia. Observe tissue of faster growth (PFB) in the center of the cortex and slow-growing tissue (LB) deposited peripherally and endosteally.
Fig. 5. Tibia d'adulte. Observer le tissu à croissance rapide au centre du cortex (PFB) et le tissu à croissance lente (LB) déposé en zone périphérique et endostique.

of individuals and the available bones for each individual).

In all specimens except for the immature/young adult individuals of *E. quercinus* (ID 11, 12 and 13 that we will discuss later), we found the maximum number of LAGs in the femora (Table 4). This suggests that this bone is more reliable for age estimation than the other bones. We contrasted the number of LAGs in femora with the stage of tooth wear (Table 4, see methods). We found that the stage of advanced tooth wear (class III, ID 8, 14, Table 4) correlates with the highest number of LAGs in femora (6 and 5 respectively), while other long bones and the jaws of the individual 14 show a number of LAGs comparable to individuals with an intermediate tooth wear.

Because bone matrix tissue depends on the ontogenetic stage, within a single individual, all bones consistently show the same tissue type. Bone cortices of immature individuals consist of both PFB and woven bone. These fast-growing tissues are deposited only within the first months of life of the animal. The adult stage is characterized by

Table 4

Number of LAGs listed for bones and specimens. Data for degree of tooth wear and epiphyseal fusion is showed.

Tableau 4

Nombre de LAGs listés par os et par échantillon. Données relatives au degré d'usure et à la fusion de l'épiphysse.

ID	Number of LAGs <i>Glis glis</i>						Number of LAGs <i>Eliomys quercinus</i>									
	5	6	15	3	4	7	13	1	2	8	9	10	11	12	14	16
Jaw	-	-	-	3	-	-	1	2	3	4	-	2	1	1	3	1
Humerus	0	0	1	-	2	3	1	-	-	4	2	2	1	1	2	-
Radius	0	0	1	-	2	3	1	-	-	4	-	-	1	0	3	-
Ulna	0	0	1	-	2	3	1	-	-	4	2	-	-	1	3	-
Femur	0	0	1	-	2	3	0	-	-	6	3	2	0	0	5	-
Tibia	0	0	1	-	2	3	1	-	-	4	3	-	1	1	2	-
Epiphyses	U	U	U	-	F	F	U	-	-	F	F	F	F	F	F	-
Toothwear	-	-	-	II	-	-	I	II	II	III	-	II	I	I	III	I

-: bones not available for sectioning.

-: os non disponibles pour la coupe.

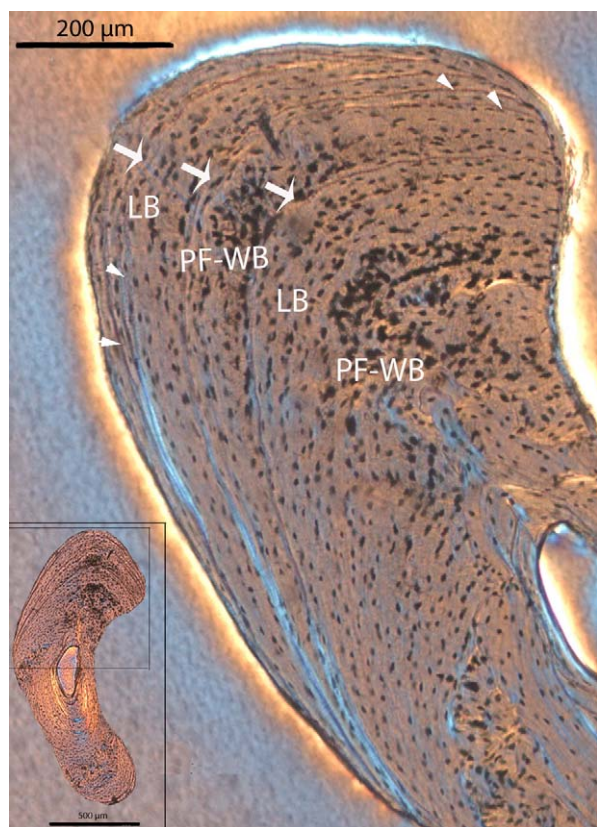


Fig. 6. Adult ulna showing three LAGs (arrow), two of them double (arrow heads), and the alternation of faster-growing, parallel-fibered woven bone (PF-WB) and slower growing, lamellar bone (LB) with LAGs.

Fig. 6. Ulna d'adulte montrant trois LAGs (flèches), deux d'entre eux étant doubles (têtes de flèches) et une alternance d'os à croissance rapide à fibres parallèles et enchevêtrées (PF-WB) et d'os lamellaire à croissance plus lente (LB) avec LAGs.

a residual apposition of periosteal, slow-growing lamellar bone. The only observable differences in tissue type within bones of the same individual are found in the ulnae where we observed alternation of faster tissue deposition (PFB or woven bone) and slower tissue deposition (lamellar bone).

3.3. Skeletochronology

LAGs appear as simple or double rest lines, which are counted here as single events.

Because the observed variability in the number of rest lines suggests the possibility that LAGs were lost during bone remodelling (Castanet et al., 2004), we chose the maximum number of LAGs for our age estimations. Following this procedure, the oldest individual of *G. glis* died at the age of 3 years, while the oldest individual of *E. quercinus* attained an age of 6 years (Fig. 7) (Table 4).

4. Discussion

The main goal of our study was to assess the ontogeny of hard tissue deposition in long bones and mandibles of dormice. All individuals of both species showed a fairly

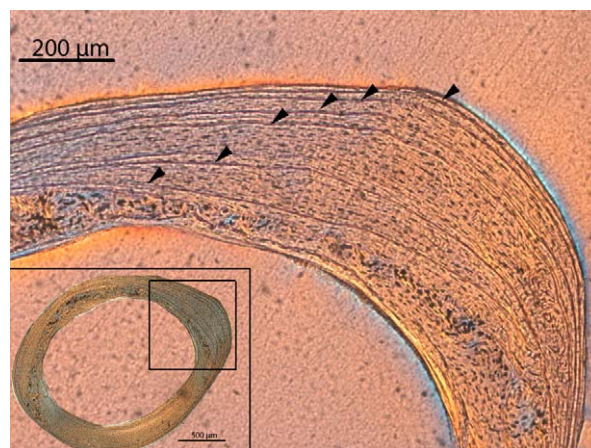


Fig. 7. Adult femur showing the greatest number of LAGs (ID 8); six LAGs are observed.

Fig. 7. Fémur d'adulte montrant le plus grand nombre de LAGs (ID 8); six LAGs sont observés.

similar bone tissue pattern at comparable ages. In immature individuals, the primary bone essentially consists of either parallel-fibered or woven bone with primary osteons. In adults, the bone tissue is lamellar in all long bones, except for the ulna. All available ulnae show alternating PFB-woven bone and lamellar bone tissue. This faster-growing tissue is likely to result from the important change in shape the ulna experiences during ontogeny, which triggers increased bone apposition in direction of the mechanical stresses that act on this bone during stance and, especially, during locomotion. The differences in bone tissue pattern between juveniles and adults denote a difference in the rate of bone deposition, with a faster growth in immature individuals compared to the residual bone deposition in adults (Ponton et al., 2004).

All bones were equally reliable for assessing the primary periosteal bone matrix. The uniformity in bone tissue makes all bones here analysed useful for estimating the rate of bone deposition and, hence, the individual growth rate for both species.

The number of growth lines was not always consistent throughout the bones of a single individual.

Classically, femur and tibia have been assumed to provide more reliable age estimations than other bones given their low degree of bone remodelling (Horner et al., 1999). Our results suggest that remodelling is indeed an important factor to take into account when skeletochronology is applied. We found different degrees of remodeling in bones of dormice, with femur presenting a less pronounced bone drift during ontogeny than other bones. Intriguingly, femora of the youngest individuals of *E. quercinus* (ID: 11, 12 and 13) do not show any resting line while humerus, ulna and tibia record a first LAG. At present, we cannot find a convincing explanation for this phenomenon. In adult individuals with high intra-individual variability in the number of LAGs, however, the femur exhibits the greatest number of growth marks. It is clearly the most conservative bone as it is less affected by remodelling. Altogether, this strongly suggests that the femur records more reliably the age at

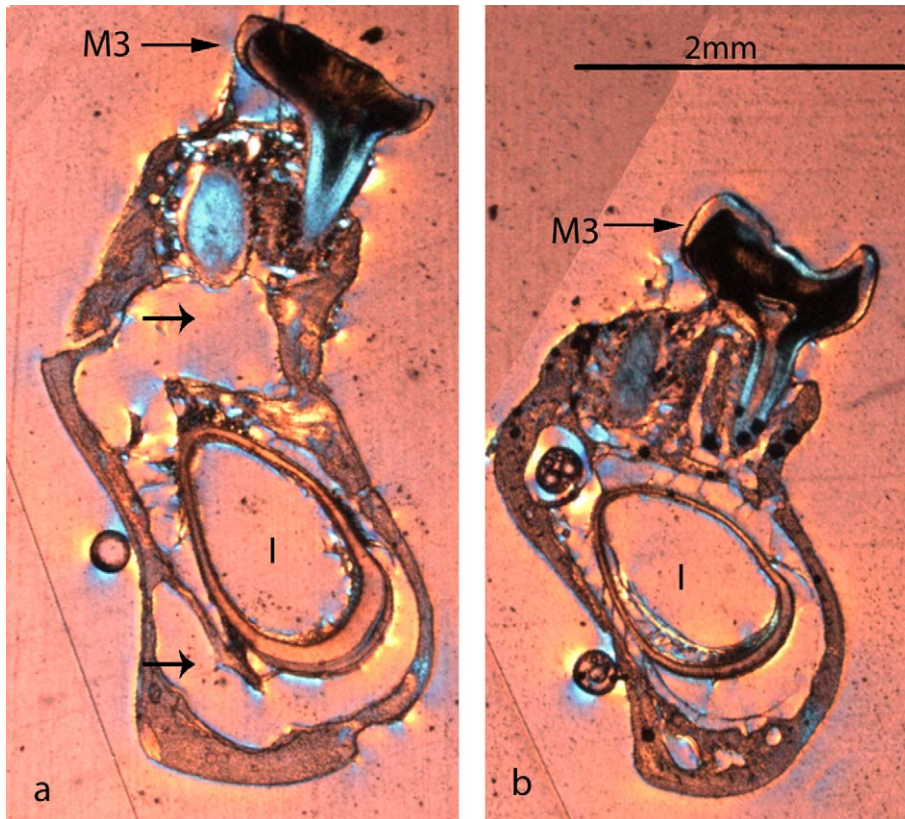


Fig. 8. Jaw of an adult (a) and immature (b) individual. Areas of bone reabsorption (arrows).

Fig. 8. Mâchoire d'un individu adulte (a) et d'un individu immature (b). Zones de réabsorption de l'os (flèches).

death than other long bones. This is supported by the tooth wear stages (class III, see methods) of the associated dental material.

In cases where jaw and femur were available for the same individual, we observed that the femur tends to show a greater number of LAGs. The smaller number of LAGs in the mandible suggests that during development the innermost (ontogenetically youngest) bone tissues were removed to provide space for the growing teeth (Fig. 8). Our results are in disagreement with previous studies in which the age estimates are based on bone histology of jaws (Krystufek et al., 2005). Altogether, our results indicate that the femur is more reliable for age estimations than the jaw or any of the long bones.

In order to explain the presence of a LAG in long bones of immature individuals (defined by unfused epiphyses), life history traits that might affect the individuals energy budget such as weaning and first reproduction, as well as hibernation periods must be considered.

E. quercinus has two litters/year (the first event corresponds to the period from March to May and the second event to that from August to October). Sexual maturity is reached at 5 months (at average), thus, the first litter attains sexual maturity between August and October, and the second one between March and May after their first winter. Therefore, individuals of this second litter are expected

to interrupt growth during hibernation (from November to February) and before reaching sexual maturity (Fig. 9).

G. glis has a single reproductive period (June–August), producing one litter/year and it attains sexual maturity at 5 months (at average). The hibernation period is longer than in *E. quercinus* (from November to April or May). As a result, earliest born individuals have enough time to complete their growth before hibernation, while later born offspring are expected to cease growth during hibernation (Fig. 9), the same as the second litter of *E. quercinus*. Therefore, hibernation periods experienced before sexual maturity may explain the presence of one single LAG in immature individuals.

Appreciation of the conservation status of vulnerable species depends on our understanding population structure and dynamics, with age at death and other life history components being an essential tool in reconstructing demographic traits. Our results show that certain life history traits (period of reproduction, birth, life span and, possibly, weaning) can be reconstructed through histological studies in dormice. These parameters are extremely important for the conservation of both genera, especially for *E. quercinus*. Hitherto, all efforts have focused on the edible dormouse *G. glis*: individuals have been aged on the basis of colour (Bieber, 1998) or capture-recapture analyses

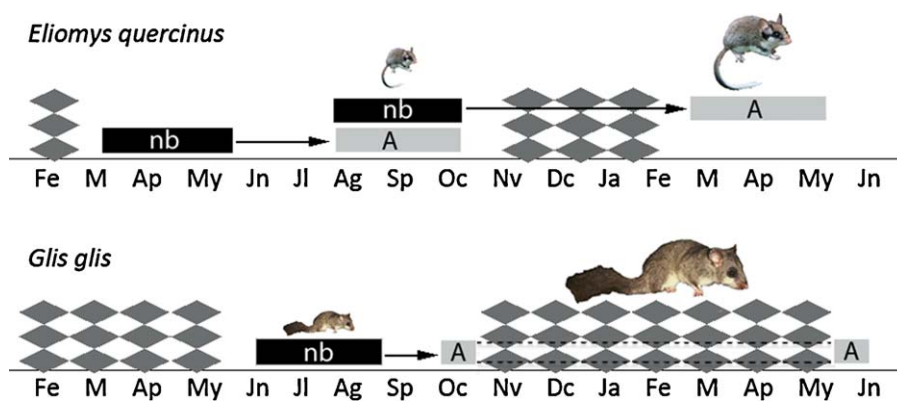


Fig. 9. The graphic shows the differences in life histories for both species of dormice (nb: new born, A: adults, diamonds: hibernation period). *E. quercinus*: the first litter (born between March and May) reaches sexual maturity before winter, the second litter ceases growth during hibernation and/or attains sexual maturity after winter (between March to May). *G. glis*: offspring is born between June and September, consequently the last-born individuals may have interrupted growth during hibernation. Both ways can explain the presence of a single LAG in immature individuals.

Fig. 9. Graphique montrant les différences dans les histoires de vie de deux espèces de dormice (nb: nouveau-né, A: adultes, losanges: période d'hibernation). *E. quercinus*: la première portée (née entre mars et mai) atteint la maturité sexuelle avant l'hiver, la seconde portée voit cesser sa croissance durant l'hibernation et/mais atteint sa maturité sexuelle après l'hiver (entre mars et mai). *G. glis*: la progéniture est née entre juin et septembre; en conséquence, les individus derniers-nés peuvent avoir interrompu leur croissance durant l'hibernation. Les deux modes peuvent expliquer la présence d'un unique LAG chez les individus immatures.

(Pilastro et al., 2003; Ruf et al., 2006). Krystufek et al. (2005), based on previous work by Morris (1972), estimated age at death by counts of resting lines in 109 mandibles of *G. glis*. He came to the conclusion that the number of LAGs varied between one and seven providing an average age at death of 3.3 years for females and 2.2 years for males. Though our study does not attempt to provide an account of age structure or similar demographic traits but instead is aimed at describing tissue patterns and growth rings in different bones throughout ontogeny, it provides some new data that add to previous demographic studies. The specimens studied in our small sample yield a maximum age of three years for individuals of *G. glis*, which is close to the mean age of females in the population studied by Krystufek et al. (2005) and the mean age for populations studied by Ruf et al. (2006) and Lebl et al. (2011), but much lower than the maximum age (7 years) of *G. glis* reported in the study of Pilastro et al. (2003). Our study of *E. quercinus* is the first to provide age estimations based on counts of rest line for individuals of this genus. We found a maximum age of 6 years, which slightly surpasses the maximum life span in captivity (5.5 years) hitherto reported (Magalhaes de et al., 2009), adding new data on wild individuals of this species.

5. Conclusions

The results obtained in this study permit the following conclusions: (i) seasonal physiological cycles (hibernation) of dormice are reflected in bone histology, and yield a valuable tool to assessing age and other life history parameters; (ii) there is a general histological pattern in all long bones of dormice, which is partially modified in the ulna; (iii) the femur seems to be the most suitable bone for studying microstructure and specifically, to perform skeletochronology.

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