J. Zool., Lond. (2004) **263**, 31–39 © 2004 The Zoological Society of London Printed in the United Kingdom DOI:10.1017/S0952836904004844

Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*

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(Accepted 21 October 2003)

Abstract

In primates, age determination using lines of arrested growth (LAGs) from bones has rarely been attempted, and the reliability of these structures has never been experimentally validated. In order to test skeletochronology in primates, LAGs were studied mainly in the long bones of known age *Microcebus murinus*, a small primate, whose potential longevity may reach 12 years. LAGs were extensively studied in 43 males and 23 females ranging from juveniles to 11-year-old adults. All individuals were born and reared in captivity. Some young individuals were injected with fluorescent dyes to quantify bone growth rates. LAGs in the diaphysis of the tibia are well correlated with age and this skeletal element appears to be the best for assessing skeletochronology in *Microcebus murinus*. There is strong evidence that the seasonal cycle of photoperiodicity is more important than age alone in producing LAGs.

Key words: age determination, skeletochronology, primates, *Microcebus murinus*

INTRODUCTION

In many fields of biology, the assessment of individual and populational life-history traits is essential. Most of these traits, such as individual age and longevity, age at sexual maturity, or ontogenetic trajectory, include a time dimension. Nevertheless, few direct and reliable methods are available to get this 'time dimension' in the wild. Most of them deal with a series of continuous morphometric characters, but their extrapolation from reference data (e.g. size-frequency data) has been previously assessed in a similar population. Thus as Halliday & Verrel (1988) said, 'the method assumes what it sets out to demonstrate'. Moreover such methods do not work for extinct species, except those based on skeletal elements (Kerley, 1965; Bouvier & Ubelaker, 1977; Thompson, 1980; Ericksen, 1991).

Skeletochronology, the use of lines of arrested growth (LAGs) periodically laid down in skeletal tissues, is one of the best ways to obtain the data stated above. LAGs, which largely depend on environmental rhythms, can directly provide, at least in theory, a temporal informative dimension. In ectothermic vertebrates (actinopterygians, amphibians and most non-avian reptiles), several hundred studies have already used this method. In endotherms (birds and mammals) or supposedly endotherms (e.g. dinosaurs), LAGs are also present, even when growth is considered a regular and finite process.

In mammals, Scheffer (1950) and Laws (1952) were the first to use LAGs for age determination in the teeth of pinnipeds. Later, LAGs in mammals were mainly analysed in dental cementum and dentine in many terrestrial and aquatic species (for additional references and syntheses see: Klevezal & Kleinenberg, 1967; Bourlière & Spitz, 1975; Perrin & Myrick, 1980; Klevezal, 1996). LAGs have also been studied in bone tissue, especially in the jaw and the diaphysis of long bones (Morris, 1970, 1972; Ohtaishi, Nachiya & Shibata, 1976; Frylestam & Schantz, 1977; Fiala, 1978; Klevezal & Fedyk, 1978; Kovacs & Ocsényi, 1981; Watts & Gaskin, 1989; Puzachenko, 1991; Garlich-Miller *et al*., 1993) and the results occasionally compared with those obtained from tooth cementum (e.g. Petersen & Born, 1982; Quéré & Pascal, 1983). Nevertheless, chronological studies in mammals performed only from bones, are scarce in comparison to similar studies in amphibians, lepidosaurs, archosaurs and chelonians (Castanet, 2002).

One of the main goals of skeletochronology is to calibrate precisely the relationship between the number of LAGs and the individual age, throughout life in a reference population. Three main techniques have been used for this assessment: (1) the use of individuals of known age; (2) the comparison of growth rings in similar bones (phalanges), removed from captured–recaptured

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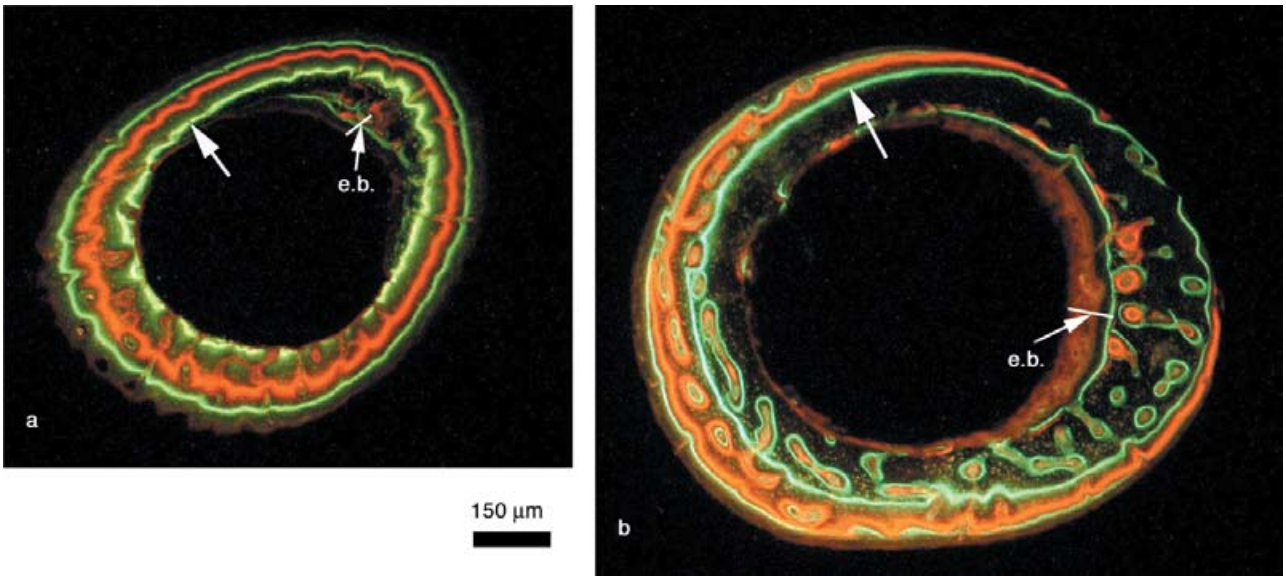

Fig. 1. Fluorescent labelling for periostic bone growth rate calculation. Tibia of *Microcebus murinus*. Cross section of the diaphysis. Three labels (green, fluorescein–DCAF; red, alizarin). Ten days delay between each label and between the last label and death (bone periphery). (a) One month old individual at the first (arrow) DCAF label. Growth rate is about 7–8 µm day^{-1} during this second month of life. (b) A 2-month-old individual at the first (arrow) DCAF. Growth rate decrease to 3–4 µm day^{-1} during the third month of life. At this age the diameter of the diaphysis corresponds to that of the vascularized crown deposited before the first lines of arrested growth in older individual (see Fig. 2b,c,d at the same magnification). Note the differential growth rate and the deposition of endosteal bone (e.b.), with a DCAF label, according to the spatial drift of this diaphysis during ontogenesis.

amphibians (e.g. Hemelaar & Van Gelder, 1980); (3) The multiple bone labelling by fluorochroms *in vivo* (see below and Fig. 1). After death, position of LAGs is compared with position of fluorescent labels.

In ectotherms these techniques have largely confirmed the annual periodicity of the deposition of LAGs and their value as a reliable record of time (Castanet *et al*., 1993). Results in mammals, mainly from individuals of known age, reveal that the number of LAGs in bones generally appears positively correlated with age (Ohtaishi *et al*., 1976; Sullins, McKay & Verts, 1976; Frylestam & Schantz, 1977; Fiala, 1978; Pascal & Castanet, 1978; Broekhuizen & Marskamp, 1979; Bryan & Bowen, 1979; Pascal & Kovacs, 1983), although the results depend on the bone used (mandible, tibia, phalanx). Some studies show that age and number of LAGs have only a weak or even no relationship (Lemnell, 1974; Klevezal & Fedyck, 1978; Pascal & Delattre, 1981). These results, using bone tissue of individuals of known age, come from a few species belonging to only three taxa: rodents, lagomorphs and carnivores (details in Klevezal, 1996).

In primates, LAGs have not yet been extensively used as an age criterion. Some studies (Wada, Ohtaisha & Hchiya, 1978) use cementum and dentine, with relative success in non-human primates (Stott, Sis & Levy, 1980; Yoneda, 1982; Kay, Rasmussen & Beardk, 1984) and humans (Kawasaki, Tanbaka & Ishikawa, 1980; Stott, Sis & Levy, 1982; Charles *et al*., 1986; Condon *et al*., 1986; Lipsinic *et al*., 1986; Miller, Dove & Cottone, 1988; Groskopf, 1990; Dean, Thackeray & Macho, 1993). However, only Wada *et al*. (1978) and Kay *et al*. (1984) had the opportunity to observe individuals of known age, and showed that, in general, the number of LAGs in cementum is well correlated with age.

To our knowledge, only one study (Herrmann & Danielmeyer, 1994) has analysed the relationship between age and LAGs in long bones of young children from past human populations, but it did not test the reliability of this practice on living individuals of known age.

In order to test the validity of skeletochronology in primates, we report here the results of analysing LAGs from a sample of a large population of captive grey mouse lemurs *Microcebus murinus*, the age of which ranged from juveniles to 11-year-old adults. LAGs were mainly studied in the long bones of the limbs, and multiple fluorescent labels were applied to some juvenile individuals to measure the significance of LAGs in the context of bone growth dynamics.

MATERIALS AND METHODS

Animals

The grey mouse lemur *Microcebus murinus* is a small nocturnal Malagasy primate (60–80 g) considered as a good representative of the ancestral primate stock (this species apparently retained the ancestral growth pattern of primates, as recently shown by Cubo *et al*., 2002*a*). This species has complex behavioural and physiological adaptations to cope with both seasonal changes in climatic conditions and resource availability in its natural habitat. During the cold and dry winter, both sexes are

sexually quiescent and enter an inactive phase. The short breeding season is restricted to the hot and rainy summer months and is associated with sustained behavioural and physiological activities. These biological rhythms are photoperiod-dependent and can be reproduced in captivity by cyclic variations of the day length (Perret, Aujard & Vannier, 1998). Exposure to long days (>12 h light per day) induces the breeding season. In contrast, exposure to short days (<12 h light per day) leads to increased fat deposition, reduced activity, torpor and complete sexual rest in both sexes (Perret & Aujard, 2001*a*,*b*).

In captivity, grey mouse lemurs were kept in controlled conditions with constant ambient temperature (24–26 °C), constant relative humidity (55%) and food available *ad libitum*. Females reproduce during long days and give birth to 2–3 babies (6–7 g). Weaning is achieved after 2 months and animals present all the characteristics of the adult state when 3 months old. The potential longevity of grey mouse lemurs is considered to be 12–14 years but in captivity the mean life span is about 5 years and maximum survival reaches 9–11 years (Perret, 1997).

Skeletochronological analysis

All grey mouse lemurs used in this study (43 males and 23 females; Table 1) were born at the laboratory breeding colony (Brunoy, MNHN, France, European Institutions Agreement n° 962773) from a stock originally caught in southern Madagascar 30 years ago. From birth to death, animals were exposed to 2 different photoperiodic regimens. Most of the studied animals ($n = 56$) were exposed to an accelerated photoperiodic regimen consisting of a 20-week period of Malagasy winter-like short day length (light/dark 10:14) and a 20-week period of Malagasy summer-like long day length (light/dark 14:10), a situation ensuring 1 seasonal cycle over a 40-week period instead of the yearly 52 weeks. The other animals ($n = 10$) were maintained under natural photoperiod in Paris, corresponding to 1 seasonal cycle per year. The acceleration from an annual cycle to an accelerated cycle led to a reduction of about 30% of the life span (Perret, 1997). This reduction is independent of sex and is not accompanied by a desynchronization of biological rhythms. It suggests that, in mouse lemurs, longevity may depend on the expression of the number of seasonal cycles, rather than the true age (Perret, 1997). These data provide the opportunity to test the potential relationship between LAGs and both the number of accelerated cycles and the true age.

The ages of the animals at death were expressed both in years and in number of seasonal cycles the animals experienced. To provide a more accurate definition of the number of seasonal cycles at an animal's death, each seasonal cycle was reported as a multiple of 365 or 280 days for annual or accelerated photoperiodic conditions, respectively. After death, animals were autopsied and tissue samples stored at − 20 °C for further analysis.

Because no previous skeletochronological study was available for *M. murinus*, it was necessary to choose the

Table 1. Skeletochronological data for *Microcebus murinus*. LAGs, lines of arrested growth. *Individuals under natural photoperiod

	Number of LAGs	Age in year	Age in cycle
Males			
M1	**0**	0.2	0.0
M2	**0**	0.2	0.3
M3	**0**	0.2	0.3
M4	**2**	0.9	1.0
M5	**2**	1.1	1.2
M6	**1**	1.1	1.3
M7	**2**	1.1	1.3
M8	**2**	1.3	1.4
M9	**2**	1.3	1.6
M10	**2**	1.5	1.7
*M11	**2**	1.5	1.2
M12	**2**	1.8	2.1
M13	**3**	1.9	2.2
M14	**3**	2.0	2.5
M15	**2**	2.2	2.9
M16	**2**	2.3	2.7
M17	**3**	2.5	3.0
M18	**3**	2.5	3.2
M19	**3**	2.8	3.5
*M20	**3**	2.8	2.5
M21	**3**	3.2	3.8
*M22	**4**	3.4	3.4
*M23	**2**	3.6	3.3
M24	**4**	4.3	5.4
M25	**5**	4.3	5.4
M26	**4**	4.4	5.4
*M27	**3**	4.6	4.0
*M28	**5**	4.6	4.3
M29	**3**	4.9	6.2
M30	**5**	5.2	6.6
*M31	**4**	5.6	5.0
M32	**4**	5.7	7.3
M33	**4**	6.2	7.8
M34	**5**	6.2	8.0
M35	**4**	6.4	8.2
M36	**7**	6.6	8.4
M37	**6**	7.1	9.0
*M38	**5**	8.2	7.5
M39	**6**	8.2	10.5
M40	**5**	8.2	10.5
M41	**7**	8.8	11.0
M42	**6**	9.1	11.4
*M43	**7**	11.1	10.8
Females			
F1	**0**	0.2	0.0
*F2	**1**	0.4	0.1
F3	**1**	0.6	0.4
F4	**2**	0.7	0.6
F5	**2**	0.7	0.7
F6	**2**	0.8	0.8
F7	**2**	1.1	1.3
F8	**2**	1.3	1.7
F9	**2**	1.5	1.8
F10	**3**	1.5	1.8
F11	**3**	2.3	2.9
F12	**3**	2.4	2.9
F13	**2**	3.0	3.5
F14	**4**	3.7	4.6
F15	**4**	3.8	4.7
F16	**4**	3.9	5.0
F17	**6**	3.9	5.1
F18	**5**	5.3	6.9
F19	**5**	5.9	7.1
F20	**6**	6.2	7.9
F21	**7**	7.3	9.3
F22	**4**	7.4	8.9
F23	**5**	7.5	9.7

most suitable long bones for our study (Castanet *et al*., 1993). In a first sample of 9 individuals (2 females, 7 males) we studied LAGs in the bones of the upper limb (femur/humerus) and lower limb (radius, ulna/tibia, fibula) bones and in the proximal phalanx of the third pedal digit. LAGs in the tooth cementum and in transverse sections of the jaw were analysed in 8 other individuals.

All the studied bones were cleaned by hand. After demineralization by nitric acid (12 h in 5% NO_3H) and washing for at least 1 h in tap water, 15-μm-thick frozen sections were prepared from the diaphyses of each bone. They were stained by Ehrlich's hematoxylin and analysed under the light microscope independently by 2 authors (S. Croci & J. Castanet) without knowledge of individual size and age ('blind reading', Castanet *et al*., 1993). In most individuals, LAGs were easily counted by the 2 observers. For each individual of the sample ($n = 66$) the number of LAGs was the same for the 2 observers or differed by only 1 LAG (overall mean difference between the 2 observers: 0.8 ± 0.06 LAGs, $n = 66$). In these last cases, data were compared for a consensual result of the number of LAGs after a thorough re-analysis of the slide.

Fluorescent labelling

This experiment was conducted on 8 juveniles divided into 2 groups, 1 and 2 months old respectively. Each mouse lemur received, without anaesthesia, 3 intra abdominal injections of vital fluorescent dies: 40 mg/kg fluorescein (DCAF – yellow) and 80 mg/kg alizarin (Alz – red), in the following order: DCAF–Alz–DCAF with a delay of 10 days between each injection. The individuals were killed by overanaesthesia with pentobarbital sodium 10 days after the last injection. Because fluorochromes are linked to the mineralized border of newly deposited bone, undecalcified sections were needed. After embedding in polyester resin, 80-μm-thick sections were prepared and analysed under the fluorescent microscope (Axiovert, Zeiss). Rates of osteogenesis correspond to the distance between 2 fluorescent labels/time elapsed, calculated at several points in the bone section.

Statistics

All values are expressed as mean ± SEM. The correlation between the number of LAGs and individual ages expressed in years or in number of seasonal cycles, was tested using Spearman or Pearson methods according to the distribution of the values (statistic package for personal computer).

RESULTS

Rate of osteogenesis

The calculation of radial growth rates from the successive fluorescent labels of the diaphyses of the tibiae of the eight young mouse lemurs gives moderate growth rate values: a range of 7–8 μm day^{-1} during the first month (Fig. 1). This rate decreases to 3–4 μm day^{-1} during the second and third months. Finally in the tibia of adults, 80 to 90% of the thickness of the vascularized diaphyseal cortex was already deposited at 2 to 3 months.

LAGs in different structures

Tooth cement and jaw bone

In the root of the molar 2 or molar 3 of the eight individuals analysed, the tooth cement is narrow, even in adults. Although LAGs can be detected inside, they are very difficult to count and thus cannot be a good indicator of individual age. In the lower part of the jaw bone, the density of blood vessels is generally low and weakly remodeled as Haversian bone. LAGs are present but they frequently split as an outcome of jaw morphogenesis. Consequently, the number of LAGs changes locally, both in a single section and between sections at different levels of the jaw. The number of LAGs is an inaccurate indicator of age.

Long bones

The periosteal cortices at the diaphyseal level of various long bones (humerus, radius, ulna, femur, tibia and first phalanx of the third pedal digit) are largely made of primary bone in both sexes. Except in individuals less than 1 year old, these cortices show two parts. (1) the inner part, the broader one, is made of parallel-fibred bone matrix; it contains primary blood vessels and scarce primary osteons, running mainly longitudinally but rarely anastomosing. In some individuals a local discontinuity inside this vascularized crown (generally in its middle part) can be observed (Fig. 2a). This discordance cannot be a LAG (see discussion). Secondary osteons are scarce in this inner part. They appear only in some individuals older than 5 years. (2) the outer part of the cortex, separated by a first LAG, is never vascularized. Its thickness slightly increases with age, but it always remains narrow compared to the inner part, even in older individuals (Fig. 2). LAGs are present only in this outer part, giving it a typical layered pattern. Generally these LAGs are well expressed, although they can divide locally, as the result of bone morphogenesis and drift.

Among the various long bones chosen for analysis in nine individuals, LAGs are best expressed in the tibia. Moreover, in that bone, endosteal resorption and remodeling never entirely destroys the inner primary bone (and consequently the first LAG), as they do in phalanges, for instance. For these nine individuals, the maximum number of LAGs counted in the tibia shows the best fit to the individual age. Thus, the tibia was the only bone used for the analysis of the whole sample.

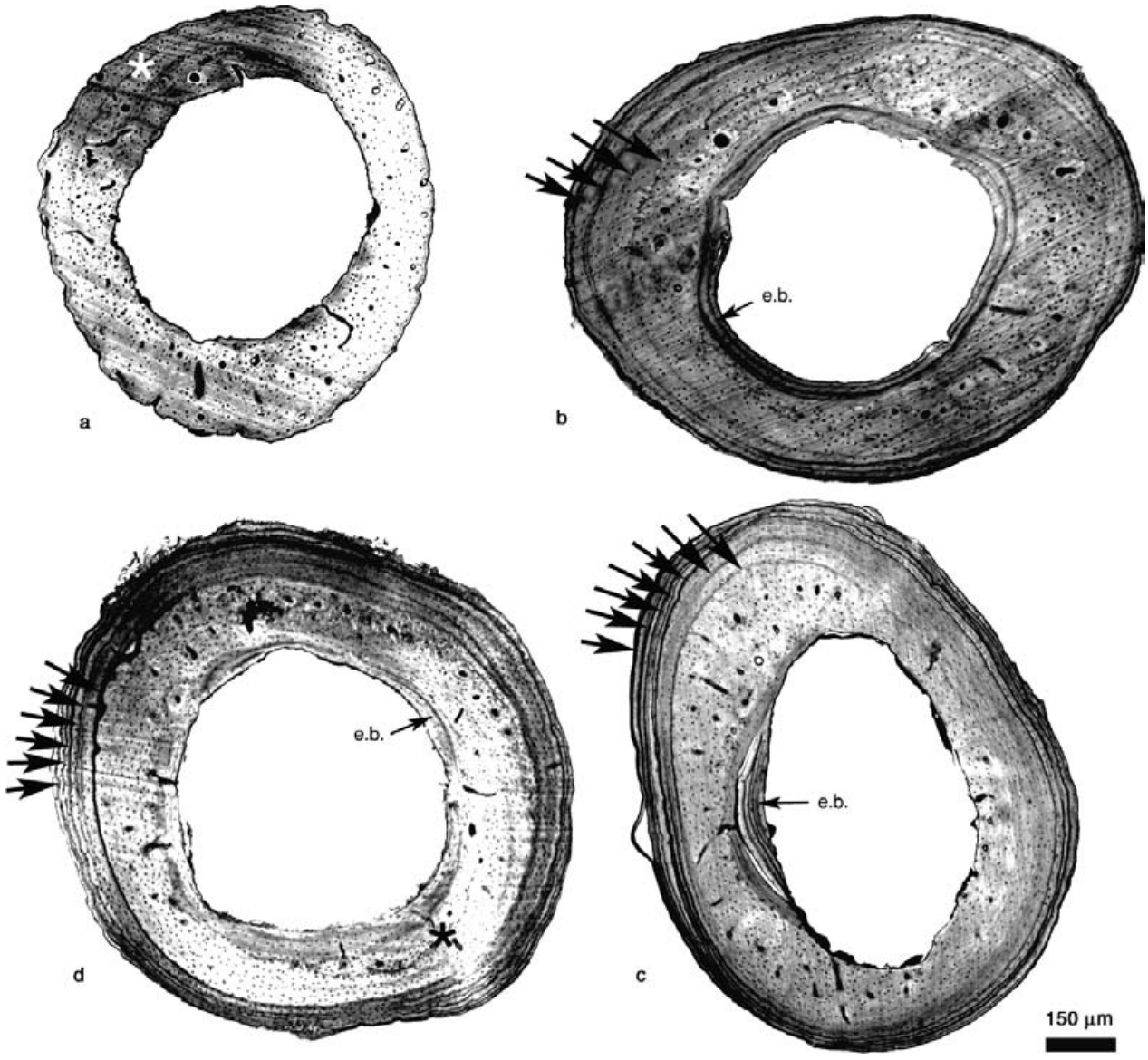

Fig. 2. *Microcebus murinus*. Cross sections in the tibial diaphysis of individuals at different ages. (a) Male 70 days old. Bone crown similar to this of Fig. 1a. Only a local discordance (star) which is not a line of arrested growth (LAG), can be observed inside this scarcely vascularized bone. (b) Female 4 years old and five artificial cycles. Four LAGs (arrows). Note LAGs in endosteal bone (e.b.). (c) Male 6.5 years old and 10 artificial cycles. Seven LAGs (arrows). (d) Male 8 years old and 11 artificial cycles. Six LAGs (arrows). The discordance (star) shown in (a) is present in the vascularized crown.

Relationship between age and the number LAGs

For each male and female grey mouse lemur sample studied, the number of LAGs is indicated in Table 1, according to the age in years or to the age in number of accelerated cycles at the time of death. A highly significant correlation appears between the number of LAGs and the age in years in both males ($r = 0.92$, $n = 43$, $P < 0.001$) and females ($r = 0.92$, $n = 23$, $P < 0.001$). Likewise, the number of LAGs is correlated with age expressed in cycles ($r = 0.92$, $n = 43$, $P < 0.001$, and $r = 0.93$, $n = 23$, $P < 0.001$ for males and females respectively). However, the maximum number of LAGs observed is seven, even in animals that are older than 7 years (mean age $= 8.3 \pm 0.4$ *versus* mean number of LAGs $= 5.8 \pm 0.3$, $n = 10$) or that have experienced more than seven accelerated cycles (mean accelerated cycles $= 9.3 \pm 0.3$ *vs* mean number of LAGs $= 5.6 \pm 0.3$, $n = 15$). For these animals, no correlation between the number of LAGs and age was evident (Fig. 3a,b). This suggests that radial bone growth likely stops after 6–7 years or seasonal cycles. Very young animals ($n = 4$, < 3 months old) have no visible LAGs.

Accordingly, a partial analysis was conducted on accelerated adult animals ranging from 6 months to 7 years or seven accelerated cycles. The regression slopes obtained from accelerated cycles as the criteria for age are different from those obtained from true age (0.51 *vs*

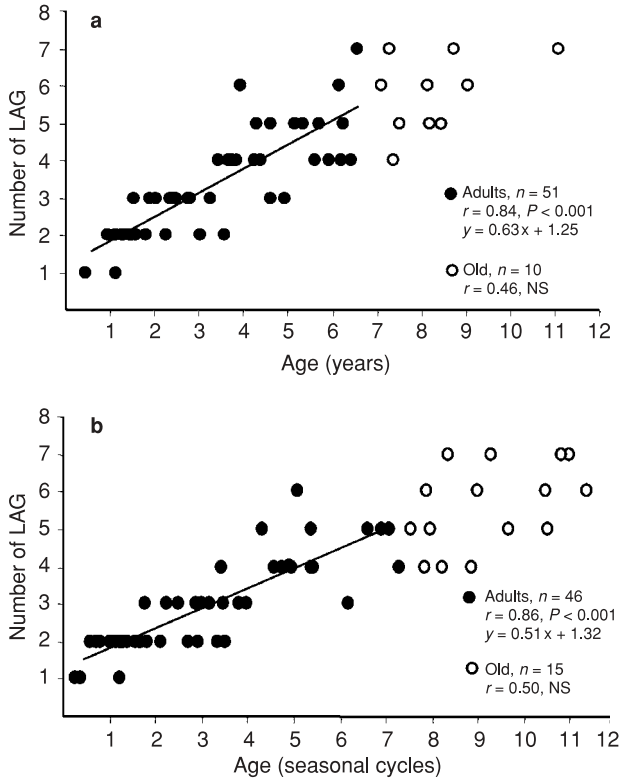

Fig. 3. *Microcebus murinus*. (a), (b) Relationship between age and the number of lines of arrested growth (LAG).

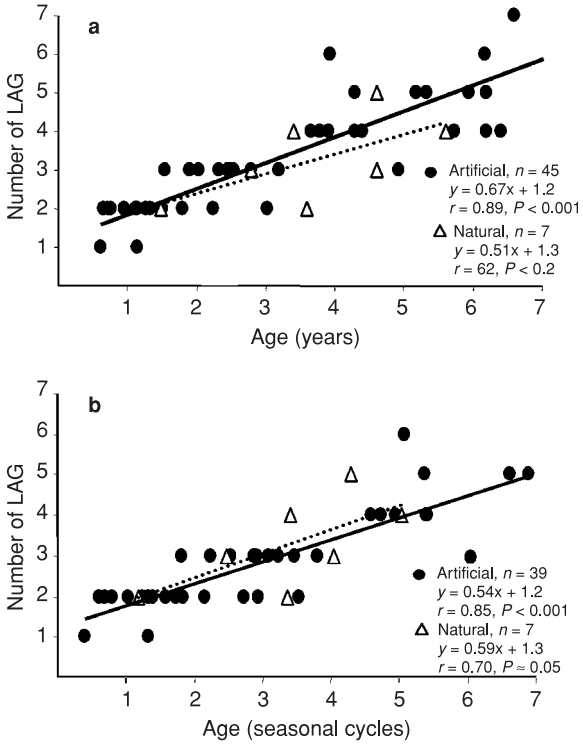

Fig. 4. *Microcebus murinus*. (a), (b) Effect of photoperiodic regimen on bone growth rate. LAG, line of arrested growth.

0.63, Fig. 3a,b) although not significant ($P = 0.1$). This suggests an effect of accelerated photoperiod regimen on the periodicity of bone growth (see below).

Effect of photoperiodic regimen

To assess a possible effect of accelerated photoperiodic regimen on bone growth, a comparison of the number of LAGs was done by separating animals kept under natural photoperiod (one season per year) from animals exposed to accelerated photoperiod (1.3 season per year). For the same true age, animals exposed to accelerated photoperiod generally demonstrate a higher number of LAGs than animals maintained under natural photoperiods (Fig. 4a). From linear regressions, the ratio of slopes, for LAGs by years, between accelerated and natural photoperiod, is 1.31 (0.67/0.51). This value corresponds to the 1.3-fold acceleration of seasonal cycle by year, and suggests that photoperiodicity is involved in bone growth. However, the difference in slopes is not significant because so few individuals were kept under natural photoperiodicity. When the number of seasonal cycles is chosen as the criterion for age for naturally exposed and photo-accelerated animals (Fig. 4b), the slopes for LAGs by seasonal cycles are quite similar (0.55/0.59) with no difference in the number of LAGs. This provides evidence that LAGs are deposited at each seasonal cycle, whatever the duration of the photoperiodic cycle.

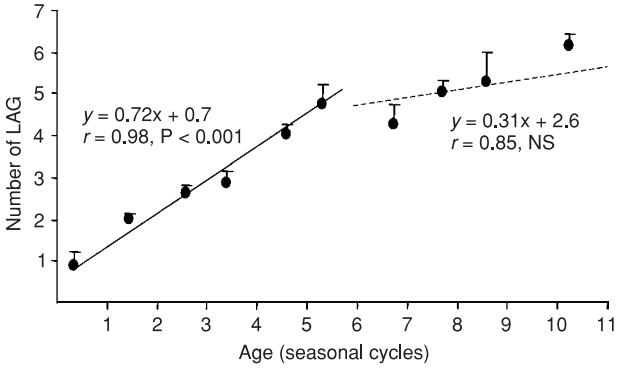

Fig. 5. *Microcebus murinus*. Estimation of age through the number of lines of arrested growth (LAG).

To estimate age by the number of LAGs, the average number of LAGs per seasonal cycle, regardless of the photoperiodic regimen, was calculated (Fig. 5). The number of LAGs deposited was high during the first two seasons: two LAGs were already observed in four to five animals after 1 year and were present in 13/14 animals after two seasonal cycles. Thereafter this number remained constant, with about one LAG/season until five seasons were completed ($r = 0.98$, $n = 6$, $P < 0.001$, Fig. 5). After five seasons, because bone growth rate was either very slow or stopped, few or no additional LAGs were deposited ($r = 0.85$, $n = 5$, NS).

DISCUSSION

Bone microstructures and bone growth dynamics

In vertebrates, primary periosteal bone with low or without vascularization and remodelling processes is a good recorder of cyclical growth rhythms, in ectotherms (Castanet *et al*., 1993) as well as in endotherms (Klevezal, 1996). This simple organization is clear in the long bones of the grey mouse, especially in the diaphyses where blood vessels are scarce, rarely remodeled and only localized in the inner part of the cortex. In this inner region, the lack of LAGs can be easily explained: the rate of apposition (3–8 μm day^{-1}) is close to that observed in other vertebrates with a similar tissue pattern (Ricqlès *et al*., 1991; Castanet *et al*., 1993; Castanet *et al*., 1996). It indicates that this entire vascularized part is deposited in a few (2–3) months before the end of the first growing season. This is confirmed by comparisons of bone diameter. Thus the first LAG can only appear at the periphery of this inner vascularized bone. It corresponds to the first 'winter' season that animals experience after their first summer growth period. The discontinuity observed locally in this inner vascularized crown is more problematic and we can only speculate on its origin. Two hypotheses appear possible. First, the discordant mark could be the result of an asymmetrical bone morphogenesis (cortical drift) without annual significance. Second, this mark, weakly expressed, represents weaning. This event happens about a month and a half after birth, a date that approximately corresponds to the position of the discordant mark in the middle part of the vascularized cortex. However, whatever its origin, this mark should not be taken into account for age estimation.

The outer bone cortex deposited after the first LAG, though narrow compared to the inner vascularized part, represents several years of growth. This outer cortex, which is avascular and made of a parallel fibred matrix, typically reflects a low rate of osteogenesis (Castanet *et al*., 1993). All the LAGs are concentrated inside this cortex, giving it its layered pattern. If we accept *a priori* that LAGs are annually deposited according to seasonal cycles, we can estimate the rate of deposition of this external cortex. The average values obtained between the first and the fifth LAG are about 0.1 μm day^{-1} (these values decrease after the third LAG). It is noteworthy that such values are similar to those calculated for the radial rate of osteogenesis in long bones of lizards and amphibians having a similar bone structure (avascular and parallel fibred matrix) (Ricqlès *et al*., 1991). Thus, this result implicitly is in agreement with an annual periodicity of the LAGs.

More generally it must be noted that in *M. murinus*, the growth in the diameter of the long bones, even if very low after reaching maturity and adult size (6 months), is not completely finished at this stage and can be sustained until 5/6 years (see below). Nevertheless this does not contradict an earlier cessation of growth in bone length – and overall growth in body size – at about 6 months (Fig. 6a,b). Such a result, previously reported in the duck *Anas platyrhynchos* (Cubo *et al*., 2002*b*), is probably also true in other primates and mammals.

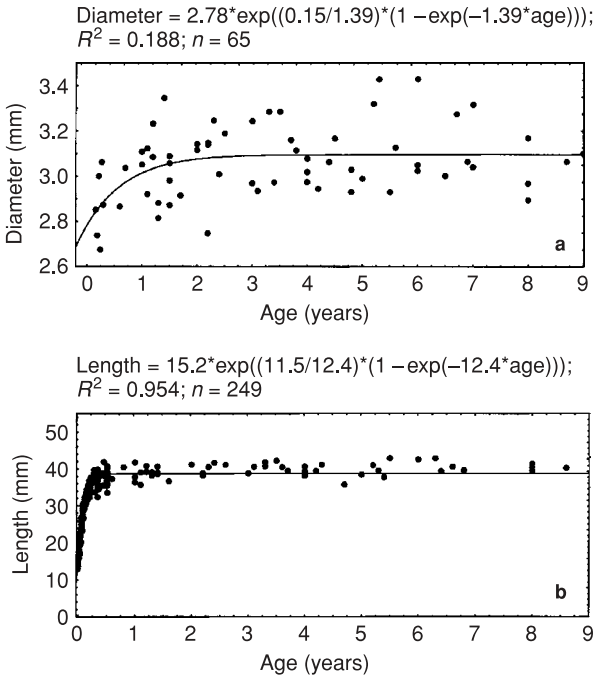

Fig. 6. *Microcebus murinus*. Regression between: (a) tibia diameter and (b) tibia length to age, through Gompertz function.

Skeletochronology and age estimation in *M. murinus*

As reported in the introduction, with the exception of Herrmann & Danielmeyer (1994), this study is the first attempt to age primates using LAGs from bones, and to validate the results using a sizeable sample of individuals of known age.

In *M. murinus*, the best results come from LAGs of the long bones. These lines are clear in the outer avascular cortex. Nevertheless some LAGs divide locally and the accuracy of their counted number will be ± 1 LAG. A careful analysis of the results in males and females shows a maximum of 7 LAGs and a strong relationship between age/LAG number for all individuals less than 6 or 7 years, regardless of sex. For older individuals, the number of LAGs can sometimes indicate the true age but more often underestimates it. In other words, an individual with six to seven LAGs is at least 6–7 years old but can be older. Because the longevity of *M. murinus* in captivity is about 9–11 years, the estimation of the age of individuals with six to seven LAGs can be underestimated by 3–4 years. Thus, the number of LAGs only gives the minimum age for older individuals. This result, not surprisingly, comes from the fact that growth in long bone thickness definitely stops at about 5–6 seasonal cycles in *M. murinus*, well before the upper limit of life expectancy, whatever the rhythmic conditions of life experienced by the animals. This cessation of radial bone growth seems normal for endotherms which clearly have a finite species-specific

body growth, but it was also observed in many ectotherms that are generally – but mistakenly – viewed to have indefinite growth throughout life. It has even been reported several times that within the same individual, either endo- or ectotherm, not all the bones grow at the same rate and for the same amount of time (e.g. Castanet, Newman & Saint-Girons, 1988). This process was called 'allochronic bone growth' (Castanet *et al*., 1996). This failure to record LAGs after 6 years is a limitation of skeletochronology (see Castanet *et al*., 1993). Nevertheless this problem will be really sensitive in organisms raised in captivity where individuals of our study can actually reach 11 years of age. In the wild, survival of *M. murinus* is greatly shortened by high predation pressure (Goodman, O'Connor & Langrand, 1993). From capture–recapture data in the wild, Kappeler (2000) showed that the oldest animal found was 6 years old. Thus in the wild, where most individuals are young adults (< 4 years old), the method will provide a reliable age estimation for most individuals of the population (with an accuracy of ± 1 year).

The number of LAGs is correlated to seasonal cycles. In *M. murinus* all behavioural and physiological parameters studied to date demonstrate a strong seasonal rhythm that has been shown to depend on photoperiodic variations. When exposed to short winter-like day lengths, animals exhibit pronounced increase in fat deposition, reduced activity, deep torpor and complete loss of sexual activity in both sexes (Perret *et al*., 1998; Perret & Aujard, 2001*a*,*b*). It can be strongly suspected that bone growth slows during winter when the organism's metabolism decreases, leading to the deposition of one LAG. This is corroborated by the fact that animals exposed to an accelerated photoperiodic regimen show a higher number of LAGs than animals of the same true age in which a yearly photoperiod is maintained.

Acknowledgements

The authors are grateful to M. M. Loth for the technical help in the preparation of the slides, and to A. de Ricqlès and to K. Padian for comments on this manuscript and correcting the English.

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