

Physiological and life history strategies of a fossil large mammal in a resource-limited environment

Meike Köhler^{1,2} and Salvador Moyà-Solà¹

Catalan Institute for Research and Advanced Studies, Catalan Institute of Paleontology, Autonomous University of Barcelona, 08193 Bellaterra, Spain

Edited by Paul E. Olsen, Columbia University, Palisades, NY, and approved October 2, 2009 (received for review January 2, 2009)

Because of their physiological and life history characteristics, mammals exploit adaptive zones unavailable to ectothermic reptiles. Yet, they perform best in energy-rich environments because their high and constant growth rates and their sustained levels of resting metabolism require continuous resource supply. In resource-limited ecosystems such as islands, therefore, reptiles frequently displace mammals because their slow and flexible growth rates and low metabolic rates permit them to operate effectively with low energy flow. An apparent contradiction of this general principle is the long-term persistence of certain fossil large mammals on energy-poor Mediterranean islands. The purpose of the present study is to uncover the developmental and physiological strategies that allowed fossil large mammals to cope with the low levels of resource supply that characterize insular ecosystems. Long-bone histology of *Myotragus*, a Plio-Pleistocene bovid from the Balearic Islands, reveals lamellar-zonal tissue throughout the cortex, a trait exclusive to ectothermic reptiles. The bone microstructure indicates that *Myotragus* grew unlike any other mammal but similar to crocodiles at slow and flexible rates, ceased growth periodically, and attained somatic maturity extremely late by ≈ 12 years. This developmental pattern denotes that *Myotragus*, much like extant reptiles, synchronized its metabolic requirements with fluctuating resource levels. Our results suggest that developmental and physiological plasticity was crucial to the survival of this and, perhaps, other large mammals on resource-limited Mediterranean Islands, yet it eventually led to their extinction through a major predator, *Homo sapiens*.

islands | artiodactyl | paleohistology | growth rate | metabolism

Energy availability is a key factor in the evolution of physiological and life history strategies of organisms. Therefore, much interest has recently been shown in the ecophysiological adaptations of vertebrates endemic to ecosystems with low energy flux (1). Ectotherms, although frequently thought of as primitive (2), are actually specialists in coping with low levels of available energy (3, 4). Ectotherm vertebrates have slow and flexible growth rates and a notable physiological plasticity, which allows a close matching of their energy requirements to prevailing resource conditions (3, 5, 6). Endotherms, instead, typically have high and steady growth rates and a constant thermometabolic regime, and they depend on high and continuous food intake to maintain their elevated metabolism (7). Therefore, in environments such as islands, where resource bases are narrow and resource availability is unpredictable (1), reptiles frequently replace mammals (8, 9).

Certain mammals, however, were dominant faunal elements on Mediterranean islands, where they persisted for long time periods, some of them over millions of years (10). This is particularly perplexing in the case of insular dwarf mammals such as elephants, deer, and hippos, which should be expected to have even higher resource requirements than small mammals because of the scaling of metabolic rate with body mass. Unsurprisingly, therefore, hypotheses aimed to explain the evolution of dwarfism and gigantism on islands (the Island Rule) (11) traditionally evoked resource availability as the driving force behind these, often dramatic, changes in body size (8, 9). More

recently, however, several studies drew attention to the tight correlation between body size and life history traits, suggesting that not body size itself but fitness-related life history traits were the chief goal of selection on islands (12, 13). Thus, it has been argued that dwarfing is a corollary of selection for an increase in production rate in low-mortality environments (12–15) through an increase in growth rate (14) and a decrease in age at maturity (14, 15). This contrasts with a model that predicts shifts in adult body size in function of the magnitude of adaptive changes in growth rate and age at maturity in response to resource availability and extrinsic mortality (16). For environments such as islands, where resources are scarce and extrinsic mortality is low, this model predicts a decrease in adult body size through a decrease in growth rate, associated to an increase in age at maturity (16). Data that might provide empirical support for any of these essentially theoretical approaches, however, are scarce and come from observations on small extant vertebrates only (see ref. 16 for a more comprehensive review), because almost all large insular mammals went extinct following human settlement (1). The only way to reconstruct the physiological and life history strategies of dwarfed insular mammals, hence, is the study of their fossil remains. *Myotragus*, a dwarf bovid from the Plio-Pleistocene of Majorca (Balearic Islands, Spain), is particularly suitable for this purpose because it evolved under known selective pressures (chronically low resource levels and lack of predators) (10, 17) in a completely isolated ecosystem, conditions that closely resemble experiments on natural populations but at a timescale that only the fossil record can provide.

Physiological and life history strategies of fossil vertebrates are recorded in their hard tissues. Long-bone tissues of slow and flexibly growing ectotherms and fast and constantly growing endotherms differ substantially. Ectotherms are characterized by lamellar-zonal bone throughout the cortex. This bone is formed in a periodic manner whereby the deposition of lamellar (parallel-fibered) bone (18, 19) cyclically comes to a halt. These seasonal pauses in bone formation are recorded in the bone tissue as growth rings or lines of arrested growth (LAGs) (20–22). Endotherms are characterized by uninterrupted (azonal) fast growing fibrolamellar tissue throughout the cortex and a thin outer cortical layer (OCL) of slow growing lamellar bone deposited after attainment of somatic/sexual maturity (21, 23, 24). LAGs, if present, appear near the periosteum in the OCL (21, 23). An “intermediate” pattern, the fibrolamellar-zonal complex (25) composed of alternating zones of fibrolamellar tissue and LAGs, can be observed in extinct tetrapods only (dinosaurs and nonmammalian therapsids) (24, 25). Fossil evidence indicates that fast and uninterrupted growth has been acquired independently by birds and mammals (21). The capa-

Author contributions: M.K. designed research; M.K. performed research; M.K. and S.M.-S. analyzed data; and M.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

¹M.K. and S.M.-S. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: meike.kohler@icrea.es.

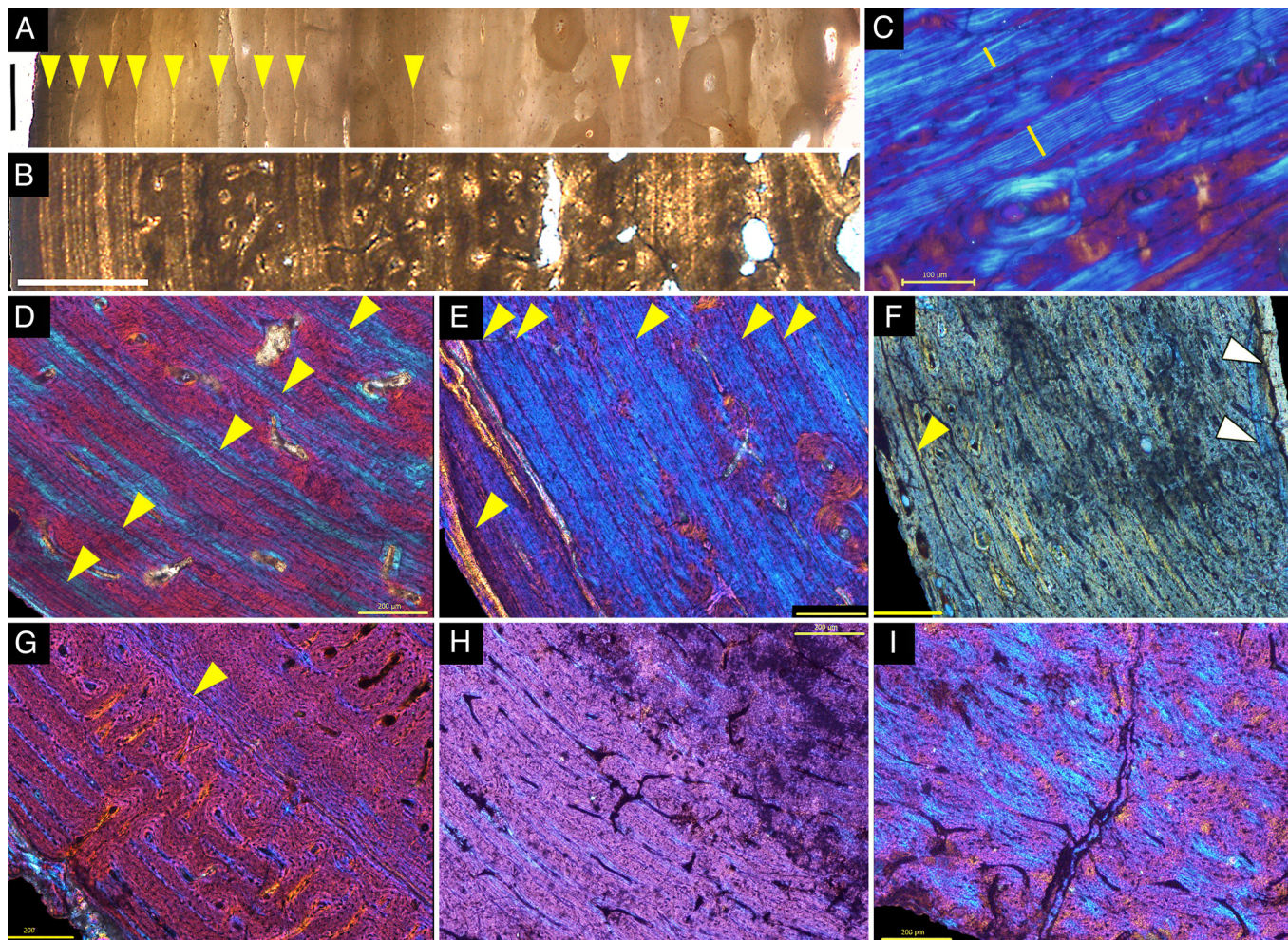


Fig. 1. Micrographs of long bone tissues. (A) *Myotragus balearicus* (IPS 44929; entire section through cortical wall), adult distal tibia with completely fused epiphysis, 11 lines of arrested growth (LAGs), some Haversian systems. (B) Crocodile (IPS 4913, Eocene, Spain; entire section through cortical wall), adult femur. Observe the similarities with *Myotragus* (A) in the spacing of growth lines. (C) *M. balearicus* (IPS 44923c), subadult tibia, annuli (bars) interrupting FLC and LPO bone. (D) Crocodile gen. et sp. indet. (IPS 4930-h, Eocene, Spain), proximal femur with alternating lamellar annuli and fibrolamellar zones. (E) *M. balearicus* (IPS 44929), complete tibia with alternating LAGs, lamellar annuli, and fibrolamellar zones (elongated vascular channels, red). Note the resemblances with crocodile (D). (F) *M. balearicus* (IPS 26158-1), very tiny humerus of ≈ 4 -cm length, FLC bone, one LAG (yellow arrowhead), two generations of endosteal bone (white arrowheads). (G) Cervid gen. et sp. indet. (IPS 3811-f, Pleistocene, Spain), adult distal tibia with completely fused epiphysis. Densely vascularized FLC tissue is shown with alternating formation of radial, concentric, and irregular oriented channels and one isolated LAG (arrowhead). Compare with the almost nonvascular zonal bone of *Myotragus* (E). (H) *Gazella borbonica* (IPS 26760-c, Pliocene, Spain), adult proximal femur with uninterrupted FLC bone. Compare with the zonal bone of adult *Myotragus* (E). (I) *G. borbonica* (IPS 26780, Pliocene, Spain) juvenile distal femur without epiphysis. Loosely formed azonal tissue of FLC type is shown with rounded osteocytes. Compare with the more compact and organized bone with flattened osteocytes and advanced remodeling of juvenile *Myotragus* (F). Periosteal surface is shown in all micrographs at lower left (left in A and B). (A and B) Transmitted light; (C–E and G–I) polarized light with 1λ filter; (F) circularly polarized light. (A and B) are composed of various micrographs. (Scale bars: A and D–I, 200 μm ; B, 1,000 μm ; C, 100 μm .)

bility to stop growth periodically is therefore considered to be a plesiomorphic trait reflecting an intermediate physiological condition (20) that has been lost in modern vertebrates (20, 24) or is simply a phylogenetic legacy (26).

Results

Ontogenetic Stages of Bone Tissue. Our descriptions of bone tissues of *Myotragus* are based on the typological classification established by de Ricqlès (ref. 18; see also ref. 19). Thin sections from an ontogenetic series of 57 long bones of *Myotragus* reveal that the primary bone tissue consists of zonal bone throughout (Fig. 1A, C, E, and F; Fig. 2B–E), comparable to that of crocodiles (compare Fig. 1B and D). LAGs appear as simple (Fig. 1A, E, and F), double, or even triple rest lines (Fig. 2B–E). They are spaced fairly homogeneously throughout the cortex (Fig. 2C). In older individuals, LAGs are closer spaced the more they ap-

proach the periosteal surface (Fig. 1A), indicating that growth rate decreased with age. At an early ontogenetic stage (Figs. 1F and 2B), fibrolamellar-zonal (23) tissue or lamellar bone with primary osteons (LPO) prevails, alternating with annuli of lamellar nonvascular bone (LNV) with flattened osteocytes and LAGs (Fig. 2B and D). Vascularization is moderate with an essentially circumferential orientation of the channels. Early remodeling becomes manifest at the inner medullary surface (erosion of innermost primary tissue and deposition of inner circumferential layers, first Haversian systems) (Figs. 1F and 2B). The primary bone pattern of this early ontogenetic stage indicates a moderately rapid rate of bone deposition interrupted by low rates of bone deposition and growth arrest. It sharply contrasts with the early ontogenetic stage of other bovids (here *Gazella borbonica*, Fig. 1I), which is characterized by an azonal fibro-lamellar complex (FLC) throughout the cortex deposited

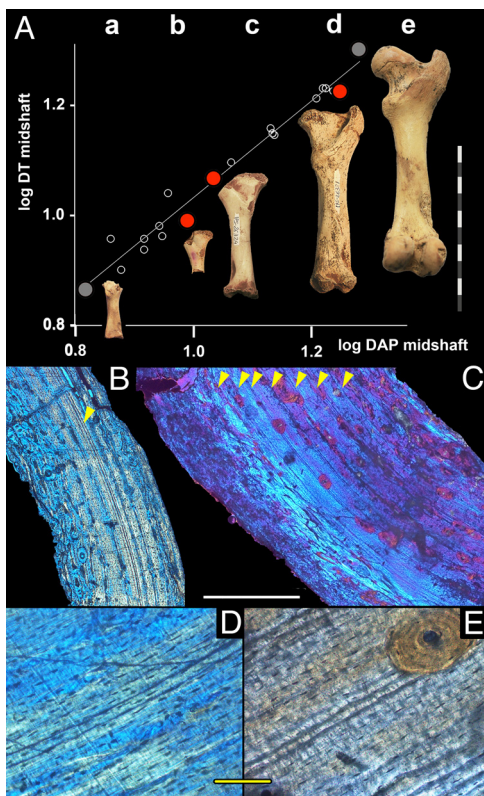


Fig. 2. Growth series for three aged femora and micrographs of their sections. (A) Growth series of femora from the smallest juvenile (a, gray dot; mbcn7160) to a large adult individual (e, gray dot; mbcn7260) [logarithmic regression of anterior–posterior diameter (DAP) against transversal diameter (DT) at midshaft]. Sectioned specimens (red dots) provided ages of 2 (b, IPS 26444e), 3 (c, IPS 26324), and 8 (d, IPS 26321) years. Note the surprisingly small size at 2 and 3 years. (Scale bar, 10 cm.) (B) Section through cortical wall of IPS 26444e (femur in Ab) with a double LAG (arrowhead) in the central cortical wall, embedded in LNV annuli. Bone tissue is of FLC type close to the medullary cavity and of LPO type before and after the annuli. (C) Section through cortical wall of IPS 26321 (femur in Ad) with seven LAGs (arrowheads). Their regular distances and their presence only on the central cortex suggest that there might have been more LAGs that have been deleted by microbial attack (dark clouds) and remodeling; Haversian systems (red) scattered throughout inner cortex; erosion and endosteal bone surrounding medullary cavity. (D) Higher magnification of IPS 26444e-1 (femur in Ab) showing the double LAG embedded in LNV tissue with flattened osteocytes. (E) Higher magnification of IPS 26321 (femur in Ad) showing multiple LAGs embedded in LNV tissue with flattened osteocytes. (Scale bars: B and C, 500 μm ; D and E, 100 μm .) C is composed of multiple micrographs.

during uninterrupted fast growth. At a later juvenile stage (Fig. 2 C and E), alternating LNV and LPO bone becomes predominant and vascularization decreases. Equidistant LAGs embedded in LNV tissue with flattened osteocytes (annuli) denote that growth slowed down and ceased periodically. Haversian systems become increasingly abundant throughout the inner half of the cortex. Older individuals (Fig. 1E) show very slow growing lamellar-zonal bone in which nonvascular annuli (LNV) and/or LAGs alternate with poorly vascularized zones (LNV/LPO) throughout cortex. This pattern of bone microstructure is frequent among wild alligators (27) (compare Fig. 1 B and D), but contrasts with the presence of fast growing fibrolamellar bone (FLC) throughout the cortex in other artiodactyls (here adult *G. borbonica*, Fig. 1H, and adult *Cervus* indet., Fig. 1G). None of the *Myotragus* specimens available for sectioning shows a distinct OCL that might indicate a rather abrupt onset of somatic maturity and/or sexual maturity as in other mammals. Instead,

some of the specimens simply show an increasingly closer spacing of LAGs toward the outer cortex, a trait that characterizes crocodiles but not mammals (compare Fig. 1A with Eocene crocodile, Fig. 1B). Table 1 summarizes the main histological traits of *Myotragus* in comparison with the bone microstructure of crocodiles and large mammals.

Skeletochronology. Skeletochronology is consistent with the slow and variable-rate growth pattern deduced from the long-bone tissue. The earliest ontogenetic stage available for sectioning is a very tiny and immature humerus without epiphyses (IPS 26158–1, length ≈ 4 cm; Fig. 1F). The tissue consists largely of FCL with longitudinal and circular osteons, although at the middle of the bone wall the tissue is more compact and of LPO type. Two clearly distinguishable generations of endosteal bone are deposited along the medullary cavity. At the periosteum, one LAG is observable followed by a thin annulus and, most peripherally, by FLC tissue, indicating that the individual resumed growth after the unfavorable season but died shortly after at the age of somewhat more than 1 year. A small proximal femur without epiphysis (IPS 26444e; Fig. 2 Ab, B, and D) shows FLC bone around the medullary cavity followed by LPO and LNV bone. Erosion is observable along the medullary cavity and some Haversian systems are scattered over the inner bone wall. At the middle of the bone wall there is a double LAG embedded in lamellar (LNV) annuli with flattened osteocytes, followed by alternating LPO/LNV bone. This tissue indicates that the individual recovered a faster growth rate after a period of slow growth and growth arrest. Age at death, hence, was at ≈ 2 years. A slightly larger immature femur (IPS 26324, Fig. 2Ac) that still lacks epiphyses, trochanter major, and trochanter minor shows little vascularized primary tissue of LPO type throughout. It presents two LAGs, large erosion cavities on the inner cortical (medullary) surface, endosteal bone, and more extensive Haversian remodeling, providing a minimum age of almost 3 years. We found a similar tissue pattern with two LAGs in a similar-sized humerus without epiphyses (IPS 26430). A minimum of six LAGs has been observed in a tibia of only two-thirds the size of a fully grown tibia in which the proximal epiphysis is not completely fused (IPS 44923-c), providing a minimum age of 7 years. Seven LAGs and, hence, a minimum age of close to 8 years, correspond to a juvenile femur of nearly adult size that still lacks both proximal and distal epiphyses and that shows an initial fusion of the trochanter major (IPS 26321; Fig. 2 Ad, C, and E). The primary bone largely consists of LNV tissue type; LAGs are mostly double or triple. Haversian systems invaded the inner cortical wall, and erosion and formation of endosteal bone along the medullary cavity are advanced. The presence of a minimum of 11 LAGs in fully grown individuals with epiphyses completely or almost completely fused (Fig. 1A, IPS 44929) denotes that *Myotragus* grew for at least 12 years before it attained skeletal/sexual maturity, more than sixfold the time of bovids of similar body mass (28) and even longer than large males of highly dimorphic *Bison*, which stop somatic growth at 7 years (29).

Discussion

The peculiar bone histology of *Myotragus* provides direct evidence of the developmental and growth strategy and indirect evidence regarding the physiology of this insular dwarf mammal. The presence of lamellar-zonal bone throughout the cortex indicates that *Myotragus* grew at slow and variable rates and ceased growth cyclically, which was associated with an important delay in the attainment of skeletal (sexual) maturity. Consistent with life history theory (30), the extended juvenile development of *Myotragus* was associated with an extended life span as indicated by the elevated number of very old individuals in the fossil assemblages (10). Our empirical finding, hence, does not support the prediction that life history traits of insular dwarfs

(Huesca, Spain); *G. borbonica*, Middle Pliocene, Layna (Spain); cervid (genus and species indet.), Upper Pliocene, Vilarroya (Spain). Specimens labeled IPS are housed at the Institut Català de Paleontologia, Universitat Autònoma de Barcelona, Bellaterra, Spain. Specimens labeled mbcn are housed at the Museu Balear de Ciències Naturals, Sóller, Majorca (Spain).

To avoid irreversible damaging of valuable material, we preferred fragmented specimens for sectioning (except for IPS 44923-c, IPS 26158-1, IPS 26324, and IPS 26321). Slices were made at midshaft following standard procedures (21, 23) and examined under transmitted light and under polarized and circularly polarized light with a 1 λ filter. Micrographs (Figs. 1 C–I and 2 B, D, and E) were taken on slices previously moistened with a drop of alcohol (98%) on their uncovered surface (a common procedure in petrography and crystallography). This procedure emphasizes the original tissue structure where this is affected by microbial attack or diagenetic processes, without damaging the fossil. Micrographs were taken with a polarization microscope (Leica DM 2500 P).

- McNab B (2002) *The Physiological Ecology of Vertebrates: A View from Energetics* (Cornell Univ Press, Ithaca, NY).
- Cox CB (1966) *Looking at Animals Again*, ed Arthur DR (Freeman, New York; San Francisco), pp 97–118.
- Shine R (2005) Life-history evolution in reptiles. *Annu Rev Ecol Syst* 36:23–46.
- Pough FH (1980) in *Behavioral Energetics*, eds Aspey WP, Lustick SI (Ohio State Univ Press, Columbus, OH), pp 141–189.
- Lance VA (2003) Alligator physiology and life history: The importance of temperature. *Exp Gerontol* 38:801–805.
- Rootes WL, Wright VL, Brown RW, Hess TJ (1991) Growth rates of American alligators in estuarine and palustrine wetlands in Louisiana. *Estuaries* 14:489–494.
- Bennett AF, Ruben JA (1979) Endothermy and activity in vertebrates. *Science* 206:649–654.
- McNab B (1994) Resource use and the survival of land and freshwater vertebrates on oceanic islands. *Am Nat* 144:643–660.
- McNab BK (2002) Minimizing energy expenditure facilitates vertebrate persistence on oceanic islands. *Ecol Lett* 5:693–704.
- Alcover JA, Moyà-Solà S, Pons-Moyà J (1981) *Chimeras of the Past* (Translated from Catalan). (Editorial Moll, Palma de Mallorca, Spain).
- Van Valen L (1973) Pattern and the balance of nature. *Evol Theory* 1:31–49.
- Brown JH, Marquet PA, Taper ML (1993) Evolution of body size: Consequences of an energetic definition of fitness. *Am Nat* 142:573–584.
- Brown JH, Sibly RM (2006) Life-history evolution under production constraint. *Proc Natl Acad Sci USA* 103:17595–17599.
- Raia P, Barbera C, Conte M (2003) The fast life of a dwarfed giant. *Evol Ecol* 17:293–312.
- Raia P, Meiri S (2006) The island rule in large mammals: Paleontology meets ecology. *Evolution* 60:1731–1742.
- Palkovacs E (2003) Explaining adaptive shifts in body size on islands: A life history approach. *Oikos* 103:37–44.
- Sondaar PY (1977) *Major Patterns of Vertebrate Evolution*, eds Hecht MK, Goody PC, Hecht BM (Plenum Press), pp 671–707.
- Ricqlès A de, Meunier FJ, Castanet J, Francillon-Vieillot H (1991) *Bone Matrix and Bone Specific Products*, ed Hall BK (CRC Press, Boca Raton, FL), Vol 3, pp 1–78.
- de Margerie E, Cubo J, Castanet J (2002) Bone typology and growth rate: Testing and quantifying ‘Amprino’s rule’ in the mallard (*Anas platyrhynchos*). *CR Biol* 325:221–230.
- Chinsamy A, Chiappe LM, Dodson P (1995) Mesozoic avian bone microstructure: Physiological implications. *Paleobiology* 21:561–574.
- Chinsamy A (2005) *The Microstructure of Dinosaur Bone: Deciphering Biology with Fine Scale Techniques* (Johns Hopkins Univ Press, Baltimore).
- Erickson GM (2005) Assessing dinosaur growth patterns: A microscopic revolution. *Trends Ecol Evol* 20:677–684.
- Klevezal GA (1996) *Recording Structures of Mammals: Determination of Age and Reconstruction of Life History* (AA Balkema, Rotterdam).
- Ray S, Botha J, Chinsamy A (2004) Bone histology and growth patterns of some nonmammalian therapsids. *J Vertebr Paleontol* 24:634–648.
- Castanet J (2006) Time recording in bone microstructures of endothermic animals; functional relationships. *C R Palevol* 5:629–636.
- Horner JR, de Ricqlès A, Padian K (2000) Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: Growth dynamics and physiology based on an ontogenetic series of skeletal elements. *J Vertebr Paleontol* 20:115–129.
- Tumarkin-Deratzian AR (2007) Fibrolamellar bone in wild adult alligator mississippiensis. *J Herpetol* 41:341–345.
- Magalhaes JP, Costa J, Toussaint O (2005) HAGR: The Human Ageing Genomic Resources. *Nucleic Acids Res* 33(Database Issue): D537–D543.
- Krasinska M, Krasinski ZA (2002) Body mass and measurements of the European bison during postnatal development. *Acta Theriol* 47:85–106.
- Stearns SC (1992) *The Evolution of Life Histories* (Oxford Univ Press, New York).
- Starck JM, Chinsamy A (2002) Microstructure and developmental plasticity in birds and other dinosaurs. *J Morphol* 254:232–246.
- Arnold W, et al. (2004) Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). *Am J Physiol* 286:R174–R181.
- Arnold W, Ruf T, Kuntz R (2006) Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) II. Energy expenditure. *J Exp Biol* 209:4566–4573.
- Ringberg T (1979) The Spitzbergen reindeer—A winter-dormant ungulate? *Acta Physiol Scand* 105:268–273.
- Ostrowski S, Williams JB, Ismael K (2003) Heterothermy and the water economy of free-living Arabian oryx (*Oryx leucoryx*). *J Exp Biol* 206:1471–1478.
- Ostrowski S, Williams JB (2006) Heterothermy of free-living Arabian sand gazelles (*Gazella subgutturosa marica*) in a desert environment. *J Exp Biol* 209:1421–1429.
- Lomolino M (2005) Body size evolution in insular vertebrates: Generality of the island rule. *J Biogeogr* 32:1683–1699.
- McNab B (2006) The energetics of reproduction in endotherms and its implication for their conservation. *Int Comp Biol* 46(6):1159–1168.
- McNab B, Bonaccorso FJ (2001) The metabolism of New Guinean pteropodid bats. *J Comp Physiol B* 171:201–214.
- Köhler M, Moyà-Solà S (2001) Phalangeal adaptations in the insular fossil goat *Myotragus*. *J Vertebr Paleontol* 21(3):621–624.
- Köhler M, Moyà-Solà S (2004) Reduction of brain size and sense organs in the fossil insular bovid *Myotragus*. *Brain Behav Evol* 63:125–140.
- Van Dam JA, et al. (2006) Long-period astronomical forcing of mammal turnover. *Nature* 443:687–691.