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Source: *Acta Ornithologica*, 39(2) : 137-140

Published By: Museum and Institute of Zoology, Polish Academy of Sciences

URL: <https://doi.org/10.3161/068.039.0210>

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Variation of the outer circumferential layer in the limb bones of birds

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Ponton F., Elżanowski A., Castanet J., Chinsamy A., de Margerie E., de Ricqlès A., Cubo J. 2004. Variation of the outer circumferential layer in the limb bones of birds. *Acta Ornithol.* 39: 137–140.

Abstract. The core of the limb bone cortex of mammals and birds is made of rapidly deposited, fibro-lamellar bone tissue (also present in non-avian theropods), which is usually surrounded by an avascular outer circumferential layer (OCL) of slowly deposited parallel-fibered bone. We present the first comparative allometric study of the relative OCL thickness (expressed as a fraction of the diaphyseal radius) in modern birds. Body size explains 79% of the OCL variation in thickness, which is inversely correlated with size, that is, shows negative allometry (slope -0.799). This may explain the apparent absence of OCL in the ratites. Since the OCL is deposited at the end of growth, we propose that its relative thickness probably correlates with the amount of slow, residual growth, which our results suggest to be on the average larger in small birds.

Key words: bone histology, postnatal growth, ossification, skeleton, periosteal bone, phylogenetically independent contrasts

Received — July 2004, accepted — Sept. 2004

INTRODUCTION

In mammals, birds and some non-avian archosaurs, the bulk of the limb bone cortex is made of highly vascularized, fibro-lamellar bone tissue, which is deposited at higher rate than parallel-fibered or lamellar bone. In mammals and birds, the fibro-lamellar core is often surrounded by avascular outer circumferential layer (OCL) of slowly deposited parallel-fibered bone, which has also been referred to as the outer circumferential lamellae (Ham 1953), outer layers of circumferential lamellae (Enlow & Brown 1957), periosteal zone (Klevezal et al. 1972, van Soest & van Utrecht 1978), periosteal layers (Lewis 1979, Nelson & Bookhout 1980), and external fundamental system (Cormack 1987). The presence of outer circumferential layer in birds and mammals is most probably another homoplasy of these two taxa since their respective lineages split in the Carboniferous soon after the beginning of amni-

ote evolution and the OCL is not differentiated in any other tetrapods.

The OCL has been found in the majority of hitherto studied neognathous birds (Amprino & Godina 1947, Klevezal et al. 1972, van Soest & van Utrecht 1978, Koubek & Hrabe 1984, Chinsamy et al. 1995, Castanet et al. 1996, Chinsamy et al. 1998, Zhang et al. 1998), although it seems poorly if at all developed in the ratites studied to date including *Struthio*, *Rhea*, and *Casuaris* (Amprino & Godina 1947, Chinsamy 1995, Chinsamy et al. 1995). The OCL is well developed in the basal bird *Confuciusornis* (de Ricqlès et al. 2003), but absent in the studied specimens of odontognathous birds, *Ichthyornis* and *Hesperornis*, which was taken as evidence of their immature age (Chinsamy et al. 1998), as well as in the Mesozoic flightless bird *Patagopteryx* (Chinsamy et al. 1995). In the Enantiornithes, which branched off after *Confuciusornis* but before the odontognaths, the entire cortex is made of poorly vascularized par-

allel-fibered tissue (Chinsamy et al. 1995) which must have completely replaced the fibrolamellar bone found in the enantiornithine perinatal skeletons (Chinsamy & Elzanowski 2001).

The slowly formed OCL is deposited in grown-up individuals and smooths off the rough periosteal surface of juvenile bones, hence their presence is commonly taken as an indication of the termination of growth. Accordingly, the only detailed study of timing of the OCL deposition showed that parallel-fibered bone appears in the major limb bones and coracoid of immature but already adult-sized Mallards *Anas platyrhynchos* at the age of about 5 months (Castanet et al. 1996). However, the termination of growth seems to vary in its temporal extent between avian taxa, e.g. the fast growth phase ends early on and growth continues at a slow rate in Crowned Cranes *Balearica pavonina*, whereas Sandhill Cranes *Grus canadensis* maintain their initial growth rate until almost fully grown (Pomeroy 1980). Variation in the terminal growth phases may in fact be reflected in bone histology. In most of the studied neognathous species, the OCL is sharply set off from the remaining cortex by a "subperiosteal resorption line" (de Ricqlès et al. 2001), but in some of them, including large passerines such as *Corvus* (Enlow & Brown 1957: fig. 27/5) and large accipitrids such as *Aquila* and *Gyps* (Amprino & Godina 1947) and *Buteo* (Enlow & Brown 1957), the outer avascular tissue is poorly defined from the remaining fibro-lamellar cortex. The OCL of birds is commonly layered itself, showing what appears to be growth rings separated by lines of arrested growth (LAGs), although their

deposition does not seem to occur in an annual cycle (Lewis 1979, Nelson & Bookhout 1980).

We present the first comparative and allometric study of the relative OCL thickness in birds.

MATERIAL AND METHODS

We measured the cross-sectional parameters of the femoral shafts in 17 neognathous species from 14 families representing the entire spectrum of developmental modes (Table 1). All specimens were adults. Thin sections (14 to 16 μm thick) at the mid-shaft of decalcified femora were prepared using a freezing microtome and stained with Ehrlich's haematoxylin.

Using a reticule, we measured (in μm^2) the OCL area and the total cross-sectional area (including the medullary cavity). The relative thickness of the femoral OCL at the diaphysis of each section was determined as the ratio of the OCL area to the total cross-sectional area. The mean diaphyseal radius was calculated from total cross-sectional area and used as a proxy of body size. The cross-sectional measurements (diameter or circumference) of the femur can be treated as approximate measures of body size in birds (e.g. Campbell & Marcus 1992). The obtained figures were multiplied by 100 to avoid negative values after a log transformation.

In order to determine a size dependence of the relative OCL thickness, we used both a standard regression on raw data and the method of Phylogenetically Independent Contrasts (PIC), which addresses the non-independence of data

Table 1. The studied specimens with the calculated values of femoral radius (in mm) and taxon-specific developmental modes.

Family	Species	Femoral radius	Developmental mode
Megapodiidae	<i>Alectura lathamii</i>	5.207	precocial
	<i>Megapodius nicobariensis</i>	2.934	precocial
Phasianidae	<i>Phasianus colchicus</i>	3.342	precocial
	<i>Perdix perdix</i>	1.715	precocial
Anatidae	<i>Anas platyrhynchos</i>	2.306	precocial
Scolopacidae	<i>Scolopax rusticola</i>	2.050	precocial
Laridae	<i>Larus ridibundus</i>	2.424	semiprecocial
Ardeidae	<i>Bubulcus ibis</i>	2.429	semialtricial
Accipitridae	<i>Accipiter nisus</i>	2.209	semialtricial
	<i>Buteo buteo</i>	3.373	semialtricial
Falconidae	<i>Falco tinnunculus</i>	1.915	semialtricial
Columbidae	<i>Streptopelia decaocto</i>	1.547	semialtricial
Strigidae	<i>Asio flammeus</i>	2.126	semialtricial
Procellariidae	<i>Macronectes giganteus</i>	4.122	altricial
Corvidae	<i>Corvus corone</i>	2.089	altricial
Turdidae	<i>Erithacus rubecula</i>	0.604	altricial
Sturnidae	<i>Sturnus vulgaris</i>	1.291	altricial

points linked to phylogeny (Felsenstein 1985). For the latter purpose we used a recent avian phylogeny based on morphological characters (Mayr & Clarke 2003) and assumed a gradual model in which branch lengths were computed by using geological divergence times: Galloanserae-Neoaves: 104.2 MYA, Galliformes-Anseriformes: 89.8 MYA and basal Neoaves 89.3 MYA (van Tuinen & Hedges 2001). We used CAIC computer package (Purvis & Rambaut 1995) to calculate PICs: values for both the log relative thickness of the femoral OCL and the log femoral radius at all nodes in the phylogeny were estimated and sister-taxon contrasts for both variables computed.

We checked the validity of statistical and evolutionary assumptions of the PIC analysis for our data. Then, we constructed a scaling equation by using a phylogenetically independent slope (obtained from a regression through the origin of contrasts) and an intercept calculated from the estimate of each variable for the root node (Garland et al. 1993, Weathers & Siegel 1995).

RESULTS AND DISCUSSION

The result obtained from the raw data regression ($y = 3.0779 - 0.9263x$, $R^2 = 0.799$, $p = 0.000001$, $n = 17$ species) and by using PIC ($y = 2.74997 - 0.7992x$, $R^2 = 0.791$, $p < 0.0001$, $n = 16$ contrasts) is essentially identical: body size explains 79% of the variation of the log relative thickness of the OCL. The relative

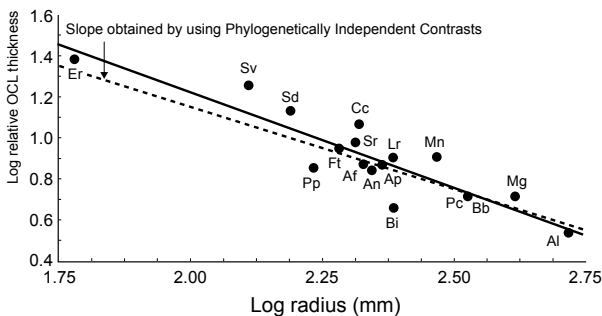


Fig. 1. Logarithmic regression of the relative thickness of the outer circumferential layer (OCL) on the radius of the femoral shaft (solid line). The scaling equation has also been constructed by using Phylogenetically Independent Contrasts (dotted line). Af – *Asio flammeus*, Al – *Alectura lathamii*, An – *Accipiter nisus*, Ap – *Anas platyrhynchos*, Bb – *Buteo buteo*, Bi – *Bubulcus ibis*, Cc – *Corvus corone*, Er – *Erithacus rubecula*, Ft – *Falco tinnunculus*, Lr – *Larus ridibundus*, Mg – *Macronectes giganteus*, Mn – *Megapodius nicobariensis*, Pc – *Phasianus colchicus*, Pp – *Perdix perdix*, Sd – *Streptopelia decaocto*, Sr – *Scolopax rusticola*, Sv – *Sturnus vulgaris*.

OCL thickness shows a negative allometry with respect to body size, that is, smaller birds have relatively thicker OCL than bigger birds (Fig. 1), which implies that small birds have a longer period of slow residual growth than do large birds. If real, the apparent absence of OCL in the ratites (Chinsamy 1995) may possibly indicate a complete phasing out of the residual growth phase in largest birds. Thus, all birds of similar size appear to have similar relative thickness of the OCL, apparently regardless of their developmental mode (Table 1).

Size dependence of the OCL thickness seems unlikely to be adaptive in terms of mechanical design because this could not be demonstrated even for the entire cortex of avian tubular bones (Currey 2002). It is more likely to be a side effect of terminal growth dynamics as imposed by the growth rate in the preceding main phase. The overall growth rate is known to be size dependent, with small birds growing faster than large birds (Starck & Ricklefs 1998).

The phenomenon of residual growth is evidenced by the lines of arrested growth in the OCL of many birds (Klevezal et al. 1972, van Soest & van Utrecht 1978, Lewis 1979, Nelson & Bookhout 1980, Koubek & Hrabec 1984) but otherwise remains poorly known. Postmaturational residual growth has recently been demonstrated in Pied Flycatchers *Ficedula hypoleuca*, which grow at a very low rate for two years past sexual maturity and so does their tarsal length (Dale et al. 2002). The OCL is deposited at a much slower rate than is the fibro-lamellar core (Castanet et al. 1996, de Margerie et al. 2002), which suggests that its thickness may reflect the amount of residual growth. This conclusion is in general agreement with the proposition that slow growth is conducive to enhanced ossification (Blom & Lilja 2004), but raises a question of why the amount of residual growth would be larger in smaller birds, which grow faster (Starck & Ricklefs 1998). We feel that the answer lies in a more abrupt termination of fast growth. However, since the relationship between the overall growth rate and ossification processes are only beginning to be understood at any developmental stage (Blom & Lilja 2004), more dedicated studies of residual growth with precisely aged individuals are needed in order to test this hypothesis.

ACKNOWLEDGMENTS

We thank Marek Konarzewski (University of Białystok) and Ken Campbell (Los Angeles County Museum) for constructive reviews, Łukasz Paško

(University of Wrocław) for discussion of statistical methods and Storrs Olson (Smithsonian Institution) for pointing to us the striking variation in terminal growth stages among the cranes. A. E. and A. C. gratefully acknowledge the support from a Poland-RSA collaboration grant for 2003–2004.

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STRESZCZENIE

[Zmienność grubości blaszki okalającej zewnętrznej w kościach kończyn ptaków]

Rdzenne części kości ssaków, ptaków i teropodów zbudowane są z szybko odkładanej kości włóknisto-blaszkowatej (fibrolamellar bone) i otoczone są beznacyniową warstwą (blaszką) okalającą zewnętrzną (OCL), w której u ptaków często znajdują się gęsto upakowane pierścienie przyrostowe. Obecna praca jest pierwszą analizą względnej grubości OCL u ptaków, tzn. stosunku jej grubości bezwzględnej do promienia trzonu (diafyzy). Stwierdzono, że względna grubość OCL zależna jest w 79% od wielkości ciała ptaka i wykazuje ujemną allometrię (-0.799), tzn. jest mniejsza u większych ptaków, co może wyjaśnić dotychczasowy brak jednoznacznego stwierdzenia OCL u Ratitae. OCL odkładana jest pod koniec wzrostu, stąd Autorzy wysuwają hipotezę, że jej grubość skorelowana jest z długością okresu resztkowego wzrostu, który zachodzi przynajmniej u niektórych ptaków po osiągnięciu w przybliżeniu ostatecznych rozmiarów ciała, a nawet po osiągnięciu dojrzałości płciowej. Uzyskane wyniki sugerują, że resztkowy wzrost wyrażony jest mocniej u mniejszych ptaków.