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Quantitative Design of the Skeleton in Bird Hatchlings: Does Tissue Compartmentalization Limit Posthatching Growth Rates?

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ABSTRACT Based on a detailed description of hatchling skeletons of the precocial buttonquail (*Turnix suscitator*) and the altricial budgerigar (*Melopsittacus undulatus*), this report presents the hypothesis that the rate of avian posthatching growth is limited by the quantitative design (i.e., relative volumes of cartilage, bone, and marrow) of the hatchling skeletons. A large portion of bone in the skeletal elements and fast growth are hypothesized to be mutually exclusive. This hypothesis is tested by morphometric techniques and by statistical comparison of morphometric and growth data. All predictions are met by the data, and the design of hatchling skeletons is described as determined by a tradeoff between tissue composition of skeletal elements and maximum rates of posthatching growth. The precocial design shows large bony areas that supposedly resist mechanical stress of locomotion; however, the relatively small cartilaginous areas exclude high growth rates. The altricial design shows the reverse relationship with small bony areas and a lack of locomotion on the one side but large cartilaginous areas and fast posthatching growth on the other side.

Diversification of life histories reflects evolutionary response to selection within limits established by internal structural and physiological constraints of the organism (Clutton-Brock and Harvey, '79; Brooks and McLennan, '91; Arnold, '92; Roff, '92; Stearns, '92). The wide spectrum of avian hatchlings ranging from precocial to altricial makes them a useful general model to study internal constraints. According to morphological, physiological, and behavioral traits, avian hatchlings may be classified into at least four developmental modes: precocial (e.g., Phasianidae), semiprecocial (e.g., Laridae), semialtricial (e.g., Ciconiidae), and altricial (e.g., Psittacidae, all passeriform birds).

It has long been recognized that birds vary considerably in their posthatching growth rates (Portmann, '35, '38, '54; Kramer, '53; Ricklefs, '83). Two-thirds of this variation can be statistically correlated to differences in body mass; developmental mode is thought to explain much of the remaining third. A comparison of posthatching growth rate constants (the rate of approach to asymptotic size; for details see Ricklefs, '67; '68) showed that precocial chicks attain adult size more slowly on average than altricial chicks with respect to the growth rate constant of the logistic equation (K_L). No precocial, semiprecocial, or semialtricial chick grows more rapidly than $K_L = 0.380\ day^{-1}$. Altricial birds reach distinctly higher values, with a mean of $0.360\ day^{-1}$, and a maximum at $0.742\ day^{-1}$. On average, they grow 2.0–3.2 times faster than species with other developmental modes. Previous studies have suggested that selection has maximized posthatching growth in altricial birds but not in precocials because growth rates of altricial species are skewed toward higher values (Ricklefs, '73, '79a, '83; Starck, unpub. obs.).

The analysis of avian growth has focussed on selective forces (Lack, '68; Case, '78; Werschkul and Jackson, '79; Sibly et al., '85) selecting for different growth patterns. Predation, limited energy supply, and sibling competition have been recognized as selecting for rapid growth of hatchlings. However, as some phenomena in posthatching growth, e.g., slow growth in precocial birds, cannot appropriately and exclusively be related to these selective forces, Ricklefs ('69a,b, '79b, '82) introduced the concept of "*internal constraints on postnatal growth rates*" to explain limits in avian growth. He showed in a series of ar-

ticles that growth rates are influenced by the degree of precocity, defined as the rate of acquisition of mature function (Ricklefs, '69b, '73, '79b). Thus the actual pattern of growth of an avian hatchling can be seen as balanced by internal constraints and external selection. However, internal constraints have been only vaguely determined. They might be seen in either "*supply organs*" such as the intestine or in "*demand organs*" such as muscle or bone. Several hypotheses discussed today are not mutually exclusive and address different aspects of contrained growth in birds. One hypothesis is that tissue function and growth are mutually exclusive (Ricklefs, and Weremiuk, '77; Ricklefs, '79b; Ricklefs et al., 1994). In that context, the degree of tissue maturity, measured in terms of its relative water content and enzyme activity (e.g., of muscle), has been shown to correlate with the dynamics of posthatching growth and is thought to limit the posthatching growth rates (Ricklefs and Webb, '85). A second hypothesis (Konarzewski, '88; Konarzewski et al., '89) focuses on "supply organs" and suggests a model that the intestines capacity to metabolize energy limits the posthatching growth. In a third hypothesis, different aspects of tissue composition of growing skeletons are said to limit posthatching growth rates. Carrier ('83) and Carrier and Leon ('90) suggest that maximum growth rates of skeletal elements are determined by an interplay between mechanical function and the rate at which ossified tissue can be deposited. Demands for high mechanical strength need dense bony tissue, which results in slower growth. Carrier and Auriemma ('92) suggest that the rate of wing bone elongation may act as a limiting factor for the length of fledging time in birds. In a fourth hypothesis, Starck ('89, '93) found that, besides considerable external developmental differences, the same skeletal elements are ossified in hatchlings of precocial and altricial birds. Although the pattern of ossified skeletal elements is almost identical in both developmental groups, he found considerable differences in the extend of cartilaginous and bony zones at hatching. To explain the differences in the extend of cartilaginous and bony zones, despite the constant patterns of development, Starck ('89, '93) suggested that it should be possible to establish a relationship between the quantitative skeletal tissue composition and the overall posthatching growth rate of birds.

The hypothesis by Starck ('89, '93) assumes that the dynamics of cell proliferation and differentiation of the skeleton determine the maximum possible posthatching growth rate of the body as a whole. Cartilage, as an ontogenetic precursor of enchondral bone, is a productive and fast-growing tissue. Embryonic and juvenile cartilage grows by appositional and interstitial cell division and does not depend only on local proliferation zones (Kirkwood et al., '89a; Shapiro, '92). The volume of a cartilaginous element therefore provides a measure for the actual number of proliferating cells and the rate of formation of new cartilage. In contrast, the histogenesis of bone is a slow process requiring several steps of differentiation, including activity and coordination of different types of cells. Supposing approximately similar cell-cycle parameters in growing cartilages of different species and at different times during development, cartilage volume is thought to determine maximum posthatching growth rate of chick body mass. Cartilage prebuilds the form and size of skeletal elements. Following with different cytokinetic dynamics, bone slowly replaces the precursor cartilage. Results of Carrier and Leon ('90) implicitly suggest that growth of a skeletal element and achieving functionality (mechanical strength) may be uncoupled. For those elements, which need early functionality, the deposition of bony material seems to be the growth rate limiting process.

This hypothesis leads to several predictions. First, the skeletons of altricial bird hatchlings, which are designed for fast growth, show a high portion of cartilage and a low percentage of bone. Conversely, precocial bird hatchlings, which locomote actively but grow slowly, should show a reverse relationship with a high portion of bone (indicative for functionality) and a comparatively lower portion of cartilage. This can be tested by measuring the tissue compartmentalization of skeletal elements of wing (humerus, radius, ulna) and leg (femur, tibiotarsus, tarsometatarsus) in precocial and altricial hatchlings and by relating the morphometric data to their pattern of posthatching mass increase. In short, the relative volume of the tissue compartments in hatchling skeletons may be used as a predictor for the dynamics of posthatching mass increase. Second, the relationship between tissue compartment and

posthatching growth rate of the bird, measured as increase in body mass, should be evident also in statistical analysis. Analysis of a large data set should reveal a strong correlation between growth rate and cartilage volume. Because this study compares only two species and few data are available from literature, the analysis undertaken here provides only preliminary conclusions. Third, ontogenetic development of any organ system has a time aspect and a size aspect. An alternative model to the cartilage/growth hypothesis may discuss (organ) size differences at hatching as caused by changes of timing and sequence of ossification during embryogenesis. This is exclusive with the cartilage/growth hypothesis, which suggests size differences in proliferation areas as causing differences in growth. Thus, there should be no time differences in the sequence ossifications that occur during embryogenesis.

The hypothesis that the potential cartilage proliferation at hatching determines posthatching growth rate is indirectly supported by earlier studies. Rogulska ('62) demonstrated that the degree of ossification differs significantly between precocial (high), semiprecocial (high-intermediate), and altricial (low) hatchlings. Kirkwood et al. ('89b) found in an allometric study that the rate of tarsometatarsal bone elongation is significantly higher in altricial birds than in precocials. Starck ('89, '93) showed that the timing and sequence of ossification during embryonic development are almost identical in precocial and altrical birds and that their young have approximately the same number of ossified elements at hatching.

The objective of this report is to establish a relationship between two life history variables, the tissue compartmentalization of hatchling skeletons and posthatching growth rate, to explain differences between precocial and altricial hatchlings, which cannot be understand by adaptation or "heterochrony." The restricted number of species permits no far-reaching generalizations, but the data and comparisons presented here may help defining a research agenda in evolution of avain ontogenies.

MATERIALS AND METHODS

Species

In this study the barred buttonquail (*Turnix suscitator;* Turnicidae) has been investigated as a precocial species and the budgerigar (*Melopsittacus undulatus;* Psittacidae) as altricial. Hatchlings of both species were obtained from a breeding stock at the Anatomical Institute, (University of Giessen). Turnix chicks were hand-raised as described elsewhere (Starck, '91). Budgerigar chicks were raised in an aviary by their natural parents. Chicks of both species were weighed twice a day to describe posthatching increase in body mass. However, the growth curves are based on the early morning chick mass (before first feeding) allowing to calculate a close approximation of the daily net mass gain.

Curve fitting

The growth of nestlings and chicks has been analyzed by fitting empirical data to sigmoidal growth functions and using the growth rate constant K [day^{-1}] for comparison between species. The growth rate constant is a direct measure of the rate at which chicks approach asymptotic mass. Growth curves were fitted to the logistic growth function (budgerigar) or the Gompertz function (barred buttonquail). The Marquardt-Levenberg algorithm was applied to determine the parameters that minimize the sum of squares of differences between the dependent variable in the equation and the observation. Fitted curves were accepted when the absolute value of the difference between the square root of the sum of squares of the residuals from one iteration to the next was < 0.0001. Nonlinear regression of empirical data on fitted curves resulted in R^2 better than 0.99 (see Fig. 2). To gain an estimate of hatchling maturity and time independent growth rate exponential growth rates (EGR) were calculated and compared with the natural logarithm of relative size (W/A) as plotted in the inset in Figure 2.

Histology

Hatchlings were fixed in Bouin's fixative, dehydrated in ethanol and isopropanol, and embedded in Paraplast$^+$. Wings and legs of three or four individuals of each species were sectioned into series of 10 μm thickness. Sections were stained with either Azan Heidenhein stain or Rüdebergs stain (Tolouidin-Thionin). Wholemount specimens were stained with alzian blue and alizarin red and cleared with trypsin and glycerin following the procedure described by Dingerkus and Uhler ('77).

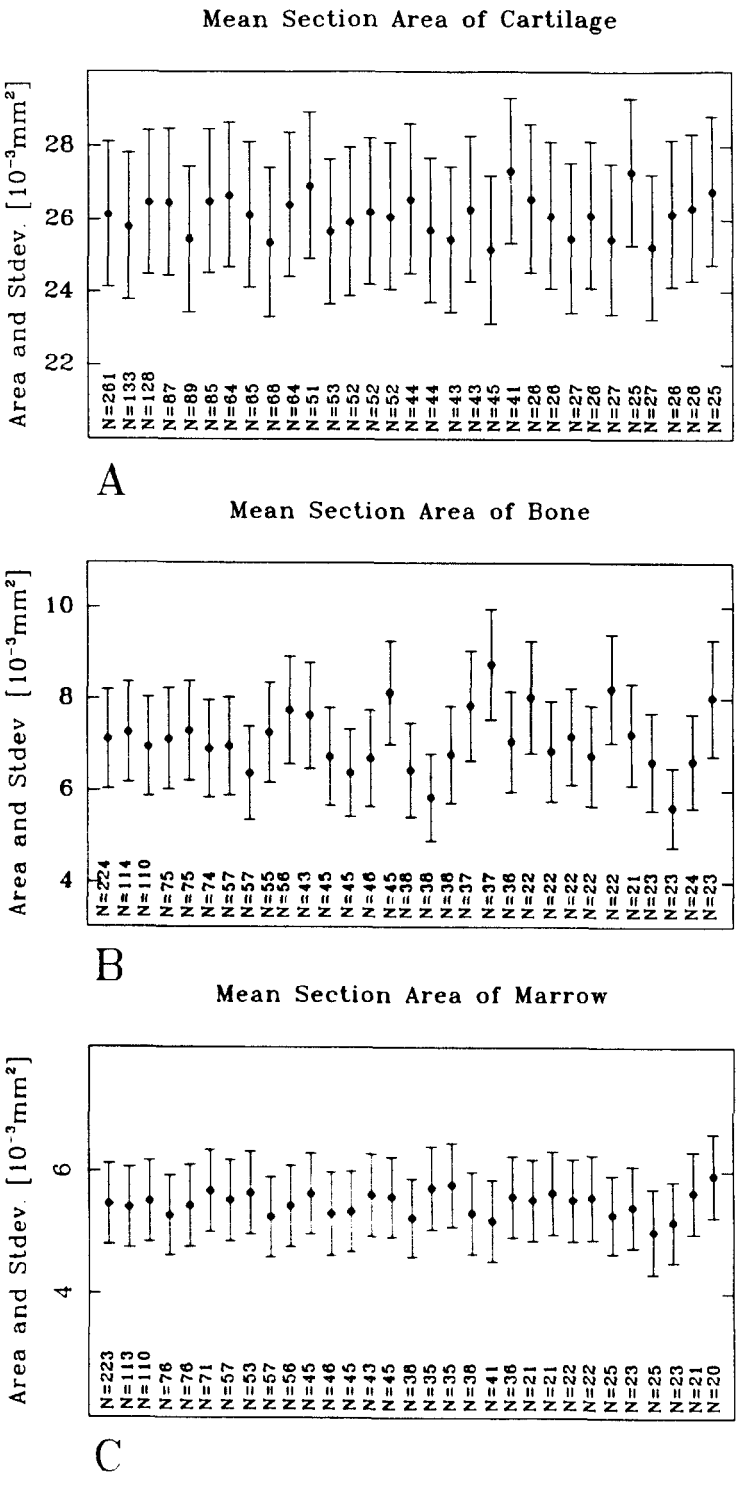

Fig. 1. Stereological error of morphometric measurements. Mean area (mm^2) and its standard deviation are given for a different numbers of traced slices ranging from 261 to 20. The high similarity in mean and standard deviation for the whole range of N indicates that measuring only 20–25 equidistant sections does not increase the stereological error. The morphometric error has been calculated for cartilage (**a**), bone (**b**), and marrow (**c**).

Morphometry and 3D reconstruction

Sections of skeletal elements were viewed using a Zeiss standard microscope supplied with a camera lucida. Tissue areas in each slice were traced on a digitizing tablet (Summagraphics Summasketch II Pro). The '92 update of the HVEM-3D program version 1.2 (Kinnamon et al., '86; Young et al., '87) has been used for data acquisition, measurement of section areas, and 3D reconstruction of the skeletal elements. This morphometric procedure produces three different kinds of error: (1) systematic hardware and software error, (2) stereological error, and (3) individual input error. The accuracy of the morphometric system (error source 1) has been tested measuring areas of known size (100 mm^2), and an error of $\pm 0.04\%$ has been determined with a standard deviation of $\pm 0.19\%$ for equal measurements. Error source 2 depends on the structure investigated, the position of the first section, and the number of sections measured (Hennig, '60; Zilles et al., '82; Starck and Kriete, '89). The magnitude of this error was tested for cartilage, bone, and marrow, and it was found that the variances of mean volumes do not change when the number of traced sections is reduced from 261 (all sections) to 20 (Fig. 1). Thus, measurement of 20–25 equidistant serial sections of wing and leg bones in bird hatchlings bears a stereological error of $< 1\%$. In this study, we have measured at least 40 sections per tissue compartment to make this error source negli-

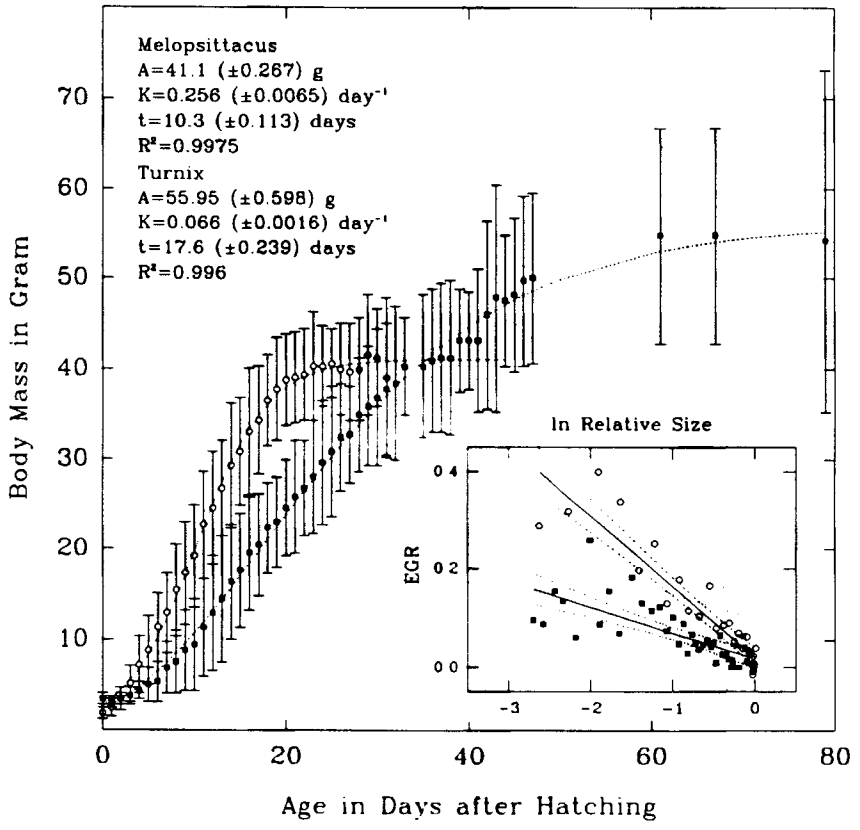

Fig. 2. Growth curves of the barred buttonquail (solid squares) and the budgerigar (open circles); vertical bars = standard deviation. Dotted lines represent the fitted curves for buttonquail (Gompertz function) and budgerigar (logistic function). Estimates for growth curve parameter (±s.e.) as obtained by nonlinear regression are given in the part of the graph. Inset: Exponential growth rate (EGR) as function of natural logarithm of relative size: $EGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ and plotted against relative size $\ln W/A = (\ln W_2 + \ln W_1)/2 - \ln A$. T = days after hatching, body mass at time t, A = asymptote size. Note that hatchlings of budgerigar hatch at about the same relative size as buttonquails, but reach adult size on a different growth trajectory.

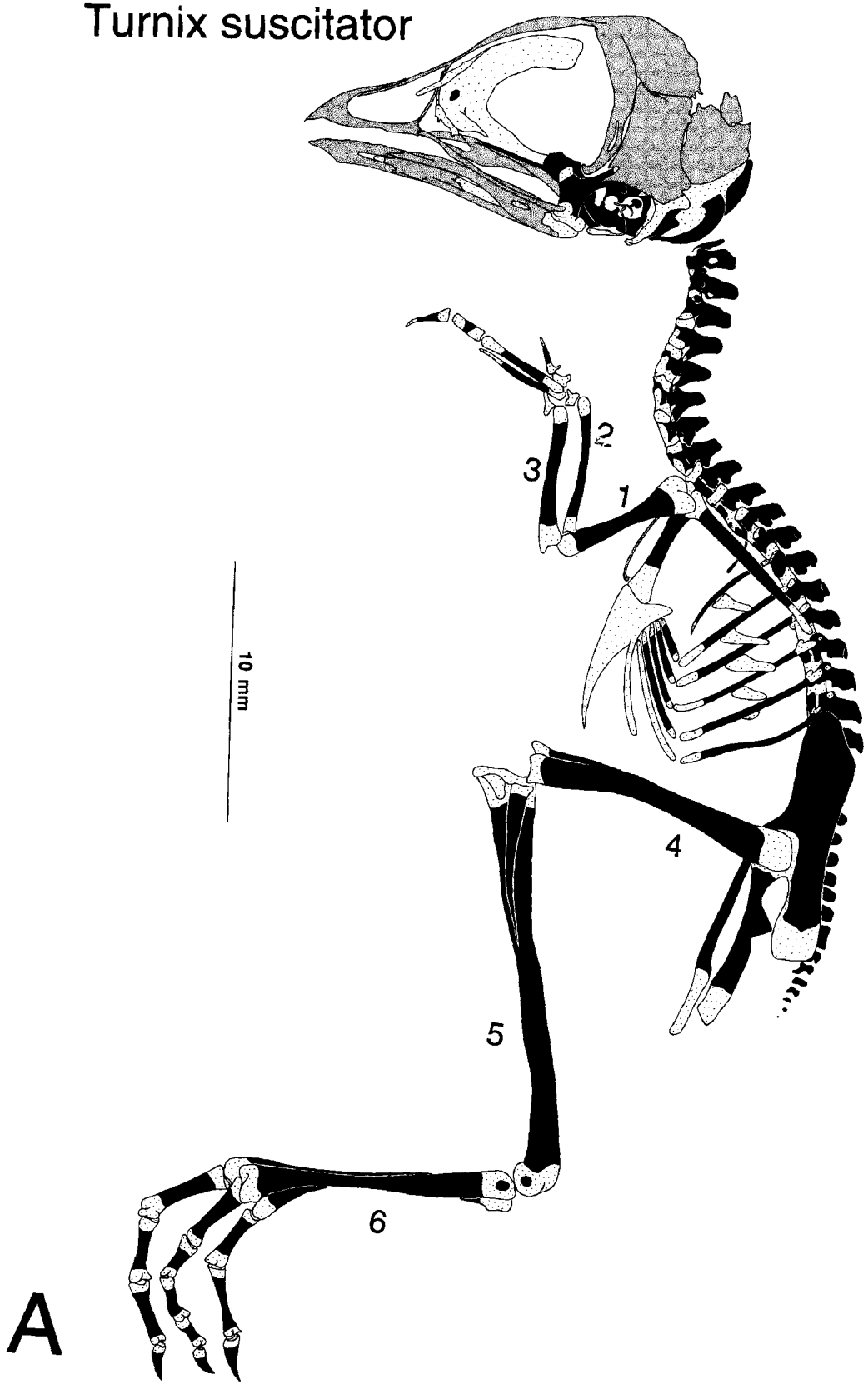

Figure 3

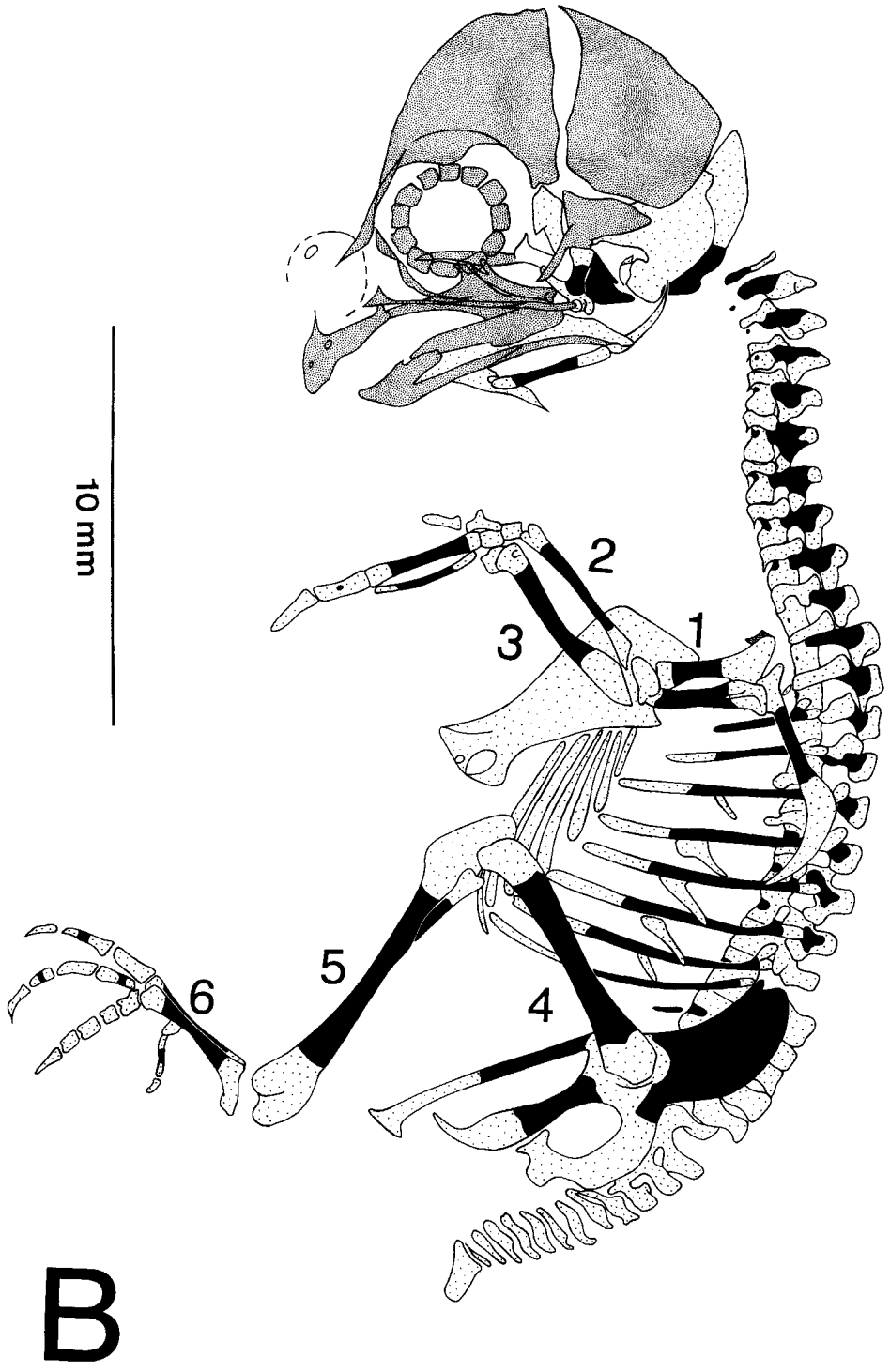

Fig. 3. Hatchling skeleton of the buttonquail (**a**) and the budgerigar (**b**). Redrawn from cleared specimens.

gable. Error source 3, the individual input error, cannot be determined exactly. An input difference of ~ 1% between different individuals tracing slices has been found. However, this error source cannot be resolved and will be included in the variances of morphometric data. All statistical calculations have been performed using CSS Statsoft Inc®.

RESULTS

Growth patterns

The barred buttonquail is one of the smallest precocial species with a mean hatchling mass of 3.42 gm (N = 76). Chicks hatch after an embryonic period of 13–14 days. Their posthatching growth is best described by the Gompertz function (Fig. 2) with growth rate constant K_G of 0.066 day^{-1} (species mean). Transformed to logistic values, following Ricklefs ('73), posthatching growth rate constant for body mass were determined as K_L = 0.133 day^{-1} in the male and K_L = 0.129 day^{-1} in the female. The asymptotic body mass of males (55 gm) and females (80 gm) is reached after a period of 45–50 days (Fig. 2) ($t_{10} - t_{90}$ interval = 42 days; for details of posthatching development of *Turnix suscitator,* see Starck, '91). Budgerigars hatch as altricial chicks after an embryonic period of 18 days. Their mean hatchling mass is 1.82 gms (N = 101), and they reach asymptotic body mass of 42 gms at a growth rate constant of K_L = 0.256 day^{-1} within 20 days ($t_{10} - t_{90}$ interval = 19 days, Fig. 2). Thus in this comparison the embryonic period of the altricial species is 4 days longer than that of the precocial, and the altricial chick hatches with only half the size of the precocial chick. However, during posthatching development the altricial species reaches asymptotic body mass in about half the time required by the precocial chick. Using the natural logarithm of relative size as a measure of maturity at a given time and as a time-independent scale for comparison of growth rates shows that hatchlings of buttonquail and budgerigar have about the same relative size (compared to adult size) at hatching. However, at a given relative size exponential growth rates of budgerigars are about twice as high as those of buttonquail (inset in Fig. 2).

Hatchling skeleton

The ossification patterns of buttonquail hatchlings and budgerigar hatchlings are depicted in Figure 3a,b. The number of ossifications and occurrence of homologous bony elements at hatching show only minor differences between both species. They involve the vertebral centra and lateral processes of the vertebrae, which are ossified through the entire vertebral column down to the pygostyle in the turnix. Ossification has just started in the cervical and thoracal vertebrae of the budgerigar. The sacral and caudal vertebrae are not yet ossified, and the caudal vertebrae are not yet fused to build the pygostyle.

The sternal ribs are ossified in the buttonquail but still cartilaginous in the budgerigar. The ossification of the clavicula occurs early in embryogenesis as dermal bone in both species. It is rudimentary in the hatchling budgerigar. However, it is present and ossified at time of hatching and not yet fused to the coracoid bone as in adults.

The ossification patterns of the wing and the leg are almost identical in both species. However, the distal phalangal elements of the wing and the foot are ossified at hatching in the buttonquail but not in the budgerigar. In the budgerigar, the 4th toe is not yet ossified. Hatchlings of both species have developed only a perichondral bony sheath around the shaft of any element of the wing and the leg (Fig. 4a,b). Enchondral ossification has not begun at this time of development. A peculiarity is found in hatchlings of buttonquails, where two distinct ossification centers are recognized in the distal end of the tibiotarsal bone and the proximal part of the tarsometatarsal bone. They represent tarsal elements (astragalus) that are incorporated into the distal part of the tibiotarsus and the proximal part of the tarsometatarsus, respectively. However, these ossifications are not found in all hatchlings of buttonquails and thus might follow rather variable ossification patterns. They do not represent an "epiphysis" type of ossification center as known from mammalian species.

The turnix and the budgerigar show the same pattern of ossification in the dermal bones of the skull. Some differences, however, are found in the skulls enchondral ossifications. The ethmoidal region of a turnix hatchling has begun to ossify as mesethmoidal bone. The basicranium and the otic region differ in the supraoccipital bone, exoccipital bone, and the basisphenoid complex, which are ossified in the turnix but not yet in the budgerigar. The prootic and the supraoccipital bones are also still lacking in the budgerigar.

TABLE 1. Embryonic days when first signs of ossification are found[1]

	Humerus	Radius	Ulna	Femur	Tibiotarsus	Tarsometarsus
Buttonquail	7 (32)	7 (32)	7 (32)	7 (32)	7 (32)	7 (32)
Budgerigar	8–8.5 (30–31)	8–8.5 (30–31)	8–8.5 (30–31)	8–8.5 (30–31)	8–8.5 (30–31)	9 (32)

[1]Normal stages in parentheses.

The hyal skeleton differs in the ossification of the stapes of the columella auris and ossification centers in the basihyale I and the urohyale, which has begun in the buttonquail but not in the budgerigar.

In summary, only few and minor differences are recognized by comparing the pattern of ossifications already present at hatching in skeletons of hatchlings of a precocial and an altricial species. Especially the wing and shoulder girdle, as well as the leg and pelvic girdle, show the same pattern of ossifications.

DEVELOPMENTAL SCHEDULE

The time from the first appearance of ossifications of the wing and the leg to hatching is listed for both species in Table 1. It is obvious from the table that ossification begins at day 8.5 of embryogenesis in the budgerigar, which is 1.5 days later than in the buttonquail, where earliest ossifications appear at embryonic day 7. However, although ossification begins later, budgerigar embryos have 9.5 days to develop a hatchling skeleton, whereas buttonquails have only 7 days to develop a distinctly larger skeleton. It must be pointed out that these differences in timing do not represent heterochrony in a sense that the altricial species would be "delayed" in development. On the contrary, the budgerigar has more time to develop a smaller skeleton. When considering embryonic normal stages rather than physical time, the differences are no longer evident and ossification begins in stages 30–32 in both species (Starck, '89).

HISTOLOGY

The cartilagineous areas of wing and leg skeleton occupy the proximal and distal caps of each element. In the humerus as well as radius and ulna of the budgerigar, the cartilage extends through the central part of the skeletal element, where the bone marrow occupies only a small cavity (Fig. 4a, 4b). In turnix, the cartilage has been removed from the central part of the element and is replaced by a (comparatively large) cavity for the bone marrow. Bone is developed as perichondral ossification surrounding the shaft of each element. No enchondral ossifications have been developed in either species until hatching (Fig. 4).

The cartilage is spatially organized and can be divided into several zones, each representing different functional stages. A thin layer of collagen-rich fibrocartilage covers the articulation facet. The fibrocartilage layer is equivalent to that in adults and supposedly serves protective functions for the articulation facet and as insertion site for tendons. The hyaline cartilage occupies the largest portion of the cartilaginous compartment in the hatchlings. It is characterized by relatively small cells surrounded by a large interstitial matrix. It forms the proximal and distal ends ("epiphysis") of the skeletal elements. Few blood vessels enter from the exterior periostium, mostly from the tip of the element and not from the side.[1] The hyaline cartilage borders the proliferation zone, which extends as a horizontal plate through the middle of the cartilaginous cap. The proliferation zone is characterized by weakly staining, small flat cells and a lack of the interstitial matrix. It is only a few cell layers thick and proliferates cells (chondroblasts) toward the distal and the medial part of the cartilage. However, the hyaline cartilage forming the articulation is functional and grows very slowly, mostly by appositional growth (see below). Elongation of the skeletal element comes through chondroblast proliferation toward the shaft. Here, an extended zone of columnal cartilage is found. In this zone the cells are large, show only a small interstitial matrix, and are arranged in columns. Chondrocytes of the columnal cartilage are supposed to undergo mitosis, thus also contributing to bone elongation. Together with the proliferation disk,

[1]The cartilaginous caps of skeletal elements of birds have no independent ossification centers as in mammals and ossify from the central cavity of the bone. In that, they follow the typical sauropsid ossification pattern and should not be described as true epiphyses (Lubosch, '36). Most developmental studies, however, cover only the embryonic period until hatching. Thus later development of epiphyses might have been missed. Occasional descriptions of epiphyses in half-grown subadult fowl (Kirkwood et al., 1989a) or megapode chicks (Starck, unpub.) ask for more detailed investigation, especially in late embryonic and postnatal stages.

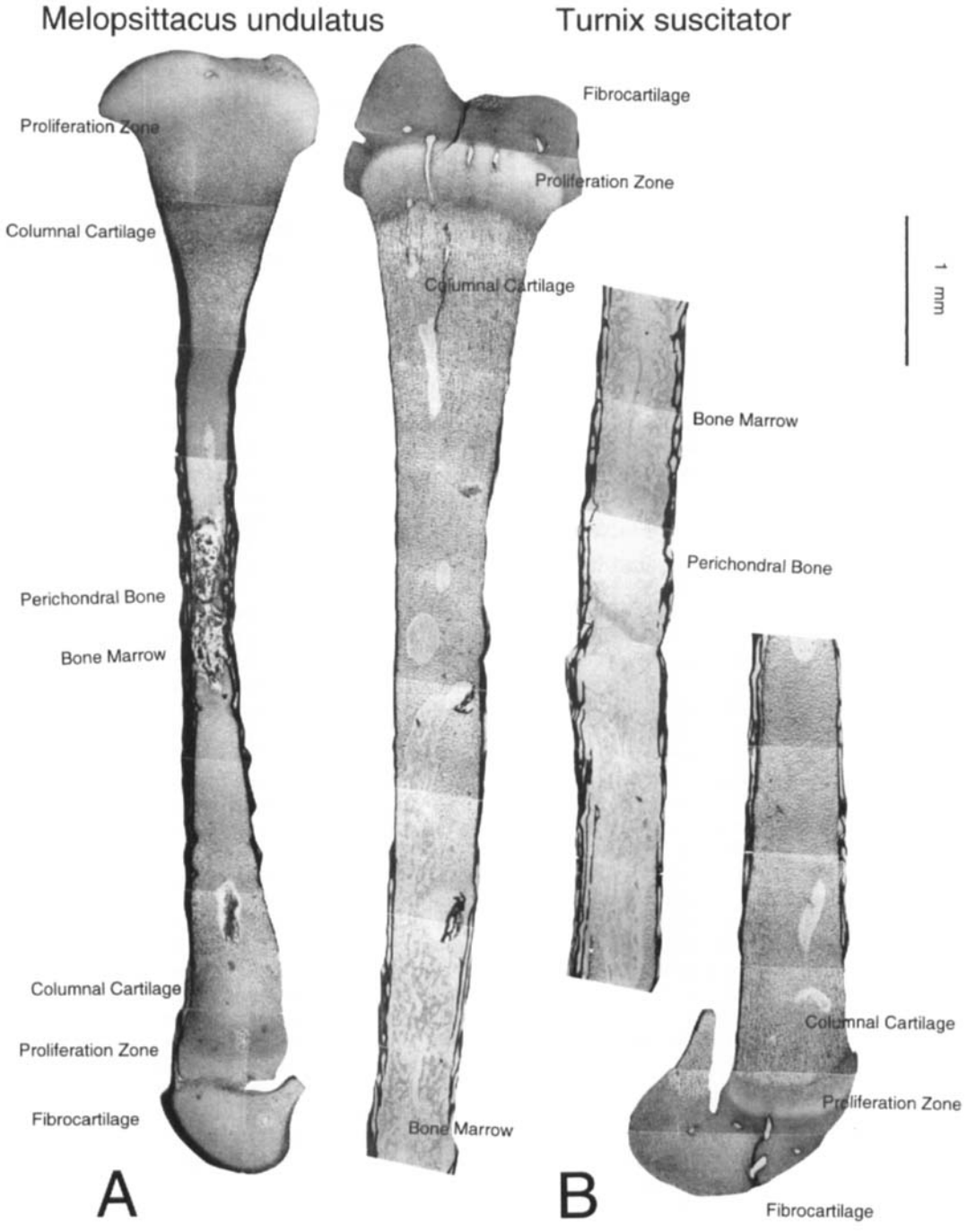

Fig. 4. Longitudinal section through the tibiotarsus of (**a**) budgerigar (paraffin histology, 10 μm, Azan (Domagk) stain); (**b**) buttonquail (Paraffin histology, 10 μm, Rüdeberg stain).

TABLE 2. Tissue volumes of the skeleton of Turnix suscitator *hatchlings*

Element	Tissue	Mean [mm³]	N	Standard deviation	Variance
	Cartilage	1.23	7	0.391	0.1526
Humerus	Bone	0.273	7	0.100	0.0100
	Marrow	0.257	7	0.041	0.0017
	Cartilage	0.221	6	0.036	0.0013
Radius	Bone	0.046	6	0.018	0.0003
	Marrow	0.064	6	0.006	0.0000
	Cartilage	0.553	6	0.042	0.0018
Ulna	Bone	0.183	6	0.107	0.0114
	Marrow	0.122	6	0.015	0.0002
	Cartilage	3.55	6	0.434	0.1883
Femur	Bone	1.26	6	0.904	0.8169
	Marrow	1.11	6	0.185	0.0341
	Cartilage	4.90	6	1.164	1.3540
Tibiotarsus	Bone	2.46	6	1.679	2.8182
	Marrow	1.78	6	0.302	0.0909
	Cartilage	4.07	6	0.516	0.2658
Tarsometatarsus	Bone	1.04	6	0.118	0.0141
	Marrow	0.75	6	0.392	0.1541

this zone represents the significant part of bone elongation. More centrally the columnal cartilage degrades, becomes removed by chondroclast activity, and is replaced by bony tissue and marrow cavity.

The differentiation of the cartilaginous areas is essentially the same in the precocial buttonquail and the altricial budgerigar (Fig. 4a,b). Minor differences are recognized in the extent each area occupies. Especially the growth zone appears to be larger in the buttonquail than in the budgerigar.

At this stage of differentiation, bone is deposited as a sheath surrounding the central part of the skeletal element as perichondral bone. Although chondroclast activity can be found at the central parts of the proximal and distal cartilaginous caps, no enchondral ossification has yet developed. The bony sheet is somewhat thicker in the turnix than in the budgerigar. It consists of primarily deposited bone matrix, and no osteons are found in either species.

The central cavity of each of the skeletal elements is filled with erythropoetic marrow. The marrow has not been investigated for its histological differentiation as it does not contribute to bone elongation. It occupies "empty space" within skeletal elements of the hatchling bird and serves the demands of a high blood cell proliferation. Later during posthatching development, the erythropoetic organ is replaced either by pneumatization or a fat body. Only small amounts of red bone marrow remain in adults.

QUANTITATIVE DESIGN

The absolute and relative portions of cartilage, bone, and marrow have been measured in the humerus, radius, and ulna of the wing skeleton, and femur, tibiotarsus, and tarsometatarsus of the leg skeleton. The results are presented in Table 2 and Figure 5 for the buttonquail and Table 3 and Figure 6 for the budgerigar, respectively. A comparison of the tissue volumes shows that for all skeletal elements, the precocial buttonquail has developed 2–10 times the tissue volume compared to the budgerigar; e.g., the humerus has developed 1.23 mm^3 of cartilage in the buttonquail but 0.72 mm^3 in the budgerigar, the bony compartment comprises 0.27 mm^3 and 0.05 mm^3, respectively, and the marrow 0.26 mm^3 and 0.02 mm^3 (data for all skeletal elements are listed in Tables 2 and 3).

The differences between both species become more obvious when calculating the relative portions each tissue contributes to the total size of the skeletal element at hatching. As shown in Figure 5b, the cartilaginous compartments ranges between 54.4% and 69.5% in the buttonquail. In the budgerigar, they range between 81.2% and 90.0% (Fig. 6b). The bony component ranges between 15.5% and 25.3% in the buttonquail but 7.2% and 13.3% in the budgerigar. The marrow comprises between 12.3% and 20% in turnix and between 2.8% and 5.5% in the budgerigar (see Tables 2 and 3 for standard deviations and variances).

The traced serial sections and the morphometric data given in Tables 2 and 3 have been used to calculate 3D reconstructions of all skeletal elements. The reconstructions represent direct quantitative and topographic visualizations of the gathered data. Figure 7a–d shows the distribution and extension of carti-

TABLE 3. Tissue volumes of the skeleton of Melopsittacus undulatus *hatchlings*

Element	Tissue	Mean [mm³]	N	Standard deviation	Variance
	Cartilage	0.721	6	0.1234	0.01522
Humerus	Bone	0.057	6	0.0121	0.00015
	Marrow	0.024	6	0.0125	0.00016
	Cartilage	0.129	6	0.0227	0.00052
Radius	Bone	0.022	6	0.0047	0.00002
	Marrow	0.008	6	0.0044	0.00002
	Cartilage	0.378	6	0.0774	0.00599
Ulna	Bone	0.035	6	0.0084	0.00007
	Marrow	0.022	6	0.0077	0.00006
	Cartilage	2.074	6	0.3102	0.09622
Femur	Bone	0.232	6	0.0701	0.00491
	Marrow	0.071	6	0.0305	0.00093
	Cartilage	2.258	6	0.5000	0.25000
Tibiotarsus	Bone	0.227	6	0.0364	0.00133
	Marrow	0.070	6	0.0445	0.00198
	Cartilage	0.845	6	0.1215	0.01477
Tarsometatarsus	Bone	0.095	6	0.0253	0.00064
	Marrow	0.030	6	0.0081	0.00007

laginous and bony areas for selected elements of turnix and budgerigar. Comparison of the 3D reconstructions shows the differences in tissue sizes between both species. Because the 3D reconstructions are magnified to approximately the same size, they visualize differences in relative tissue volumes.

CARTILAGE AND POSTHATCHING GROWTH RATE

Data on the quantitative portion of cartilage in skeletal elements of avian hatchlings are available for the domestic fowl (*Gallus gallus f.dom.*), the common black-headed gull (*Larus ridibundus*), and the rook (*Corvus frugilegus*) (Rogulska, '62). Because these data were obtained from linear measurements, the cubic roots of the volume data provided in this report are used for comparison (Cartilage index). For each species, a mean value of cartilage index has been calculated from all skeletal elements; increasing values indicate increase of the cartilaginous compartment. Cartilage indices have been related to growth rate indices, which are the residuals of a least-squares regression of growth rate constant on body mass (N = 539 species; Starck unpub.). The residuals are a measure for posthatching growth rate, corrected for the allometric effects of body mass. Values above zero indicate high growth rates and values below zero low growth rates. Results of the comparison of cartilage index with growth rate index given in Figure 8. An increase in the cartilage portion relates to an increase of growth rate index. Although this in accordance with the above predictions of the bone growth hypothesis, this comparison should be considered cautiously only as preliminary support. More data are necessary to confirm the tendency shown in Figure 8.

DISCUSSION AND CONCLUSIONS

The hatchlings of the precocial barred buttonquail and the altricial budgerigar show almost identical qualitative patterns of ossifications. Especially in the skeleton of wings and legs, both species have the same number of individual ossifications. A lack of ossification in the most distal phalangal element in budgerigar is the only difference between both species. Only few publications report the ossification patterns of avian hatchling skeletons, and almost nothing is known about the quantitative design of the skeletons. Hatchling skeletons of the domestic fowl (*Gallus gallus f. dom.*), domestic pigeon (*Columba livia f. dom.*), and great crested grebe (*Podiceps cristatus*) have been described by Schinz and Zangerl ('37). Erdmann ('39) described the skull development of domestic fowl. Maillard ('48) studied the embryonic development of the skeleton in the northern skua (*Catharacta skua*). The development of skeletons of common black-headed gull (*Larus ridibundus*) and the mew gull (*Larus canus*) has been described by Schumacher and Wolff ('66a,b) and compared to that of the domestic fowl. Rogulska ('62) reports on the skeletons of embryos and hatchlings of the rook (*Corvus frugilegus*), the common black-headed gull, and domestic fowl, and presents quantitative data of skeletal tissue. In addition to the species in this study, Starck ('89, '93) surveyed the time pattern of devel-

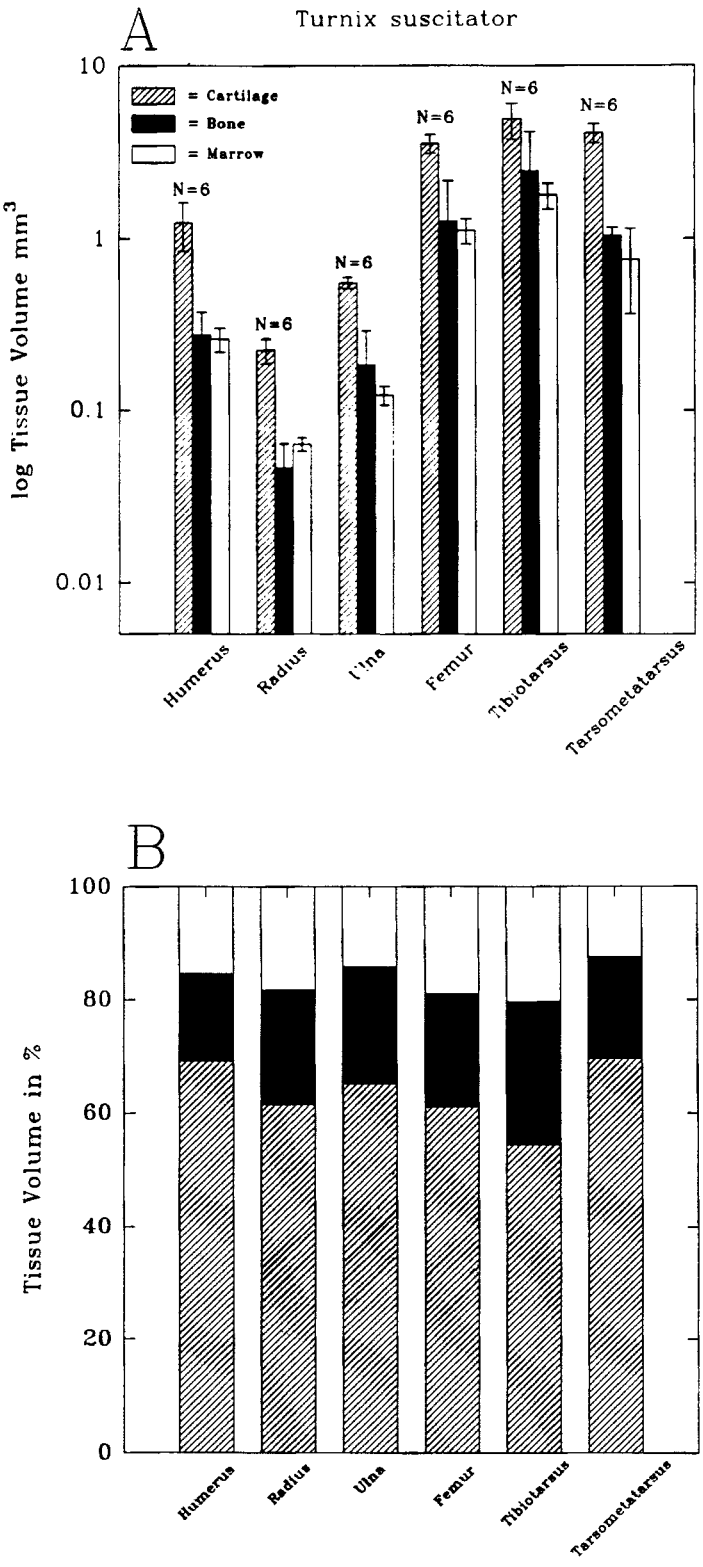

Fig. 5. *Turnix suscitator*, Tissue volumes (**a**) Absolute values ± (s.e. + morphological error term as derived from Figure 1 for each tissue component); (**b**) as percentage of skeletal element volume.

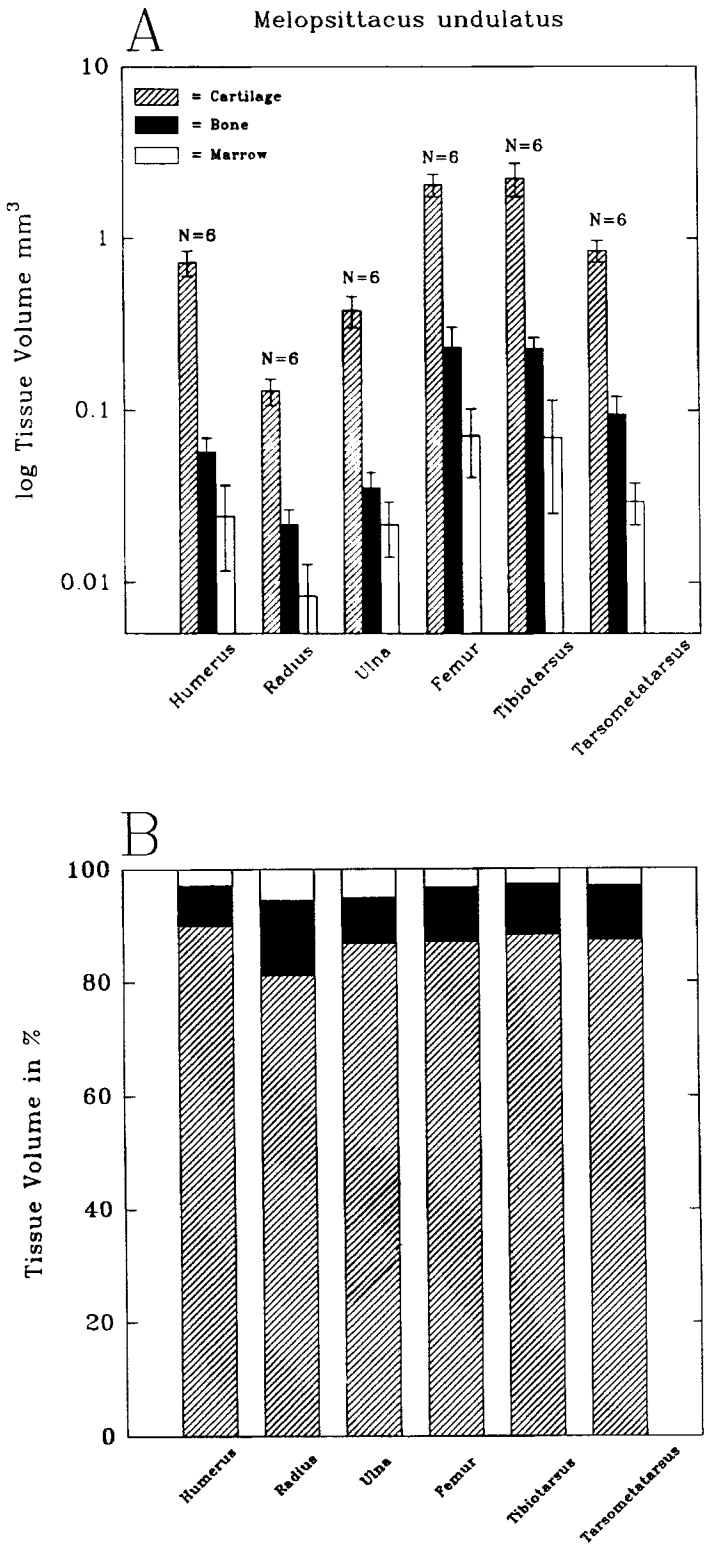

Fig. 6. *Melopsittacus undulatus*, Tissue volumes (**a**) Absolute values ± (s.e. + morphological error term as derived from Figure 1 for each tissue component); (**b**) as percentage of skeletal element volume.

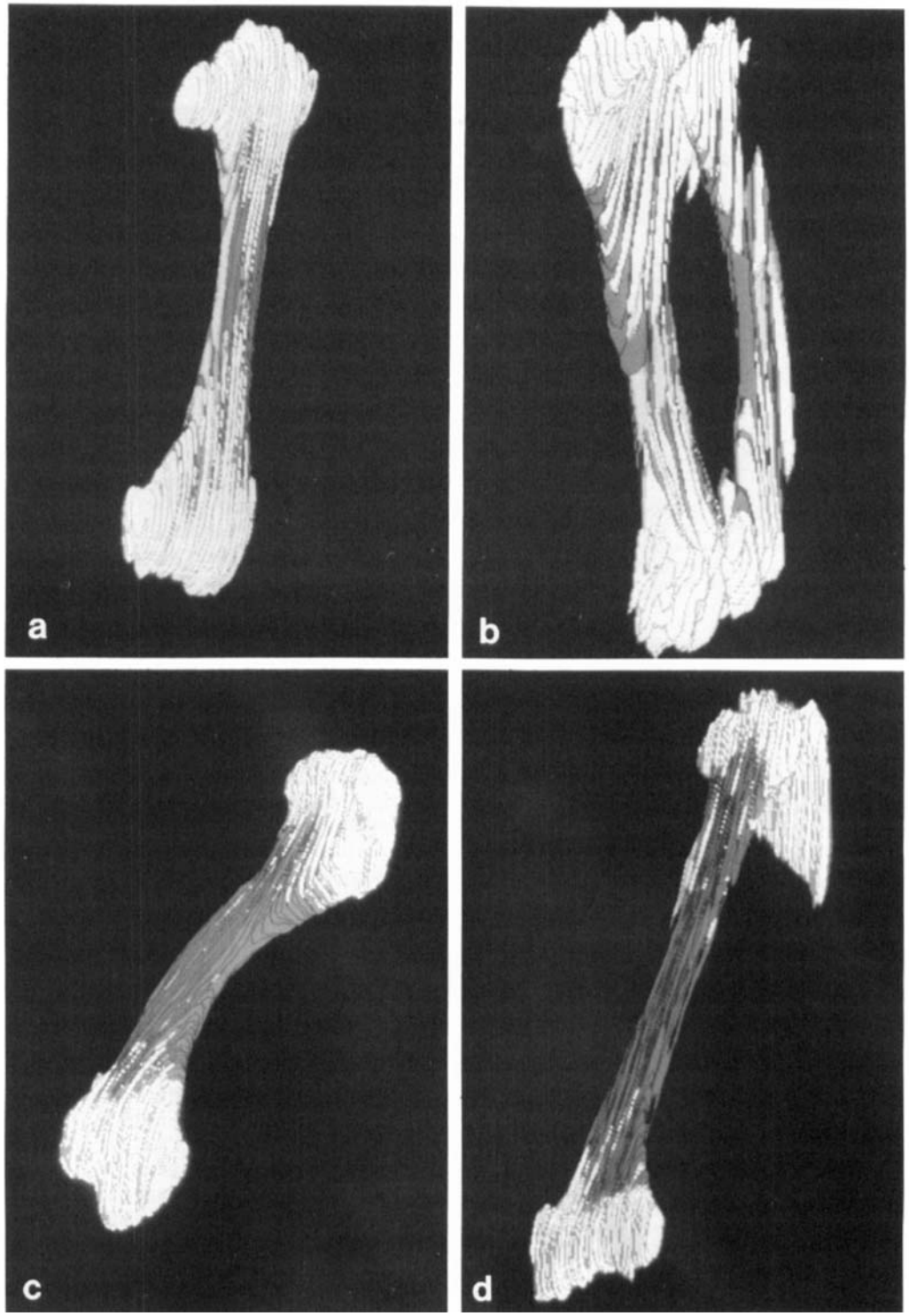

Fig. 7. 3D reconstruction of morphometric data: (**a**) humerus (buttonquail), (**b**) radius (right) and ulna (left) (budgerigar), (**c**) femur (budgerigar), (**d**) tibiotarsus (buttonquail). The proximal end of all elements is oriented to the top of the figures. All elements are adjusted to the same size to visualize the relative size of the tissue compartments. Cartilage is represented by light grey, bone is dark. Dark lines on the 3D reconstruction represent edges of digitized section.

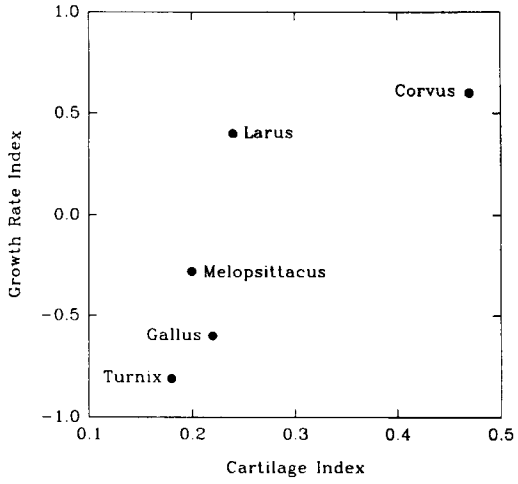

Fig. 8. Comparison of cartilage index (cartilage as percent of element length) and growth rate indices (gallus, larus, and corvus from Rogulska, '62); growth rate indices are residuals from least-squares regression growth rate constant vs. body mass (Starck, unpub.).

opment of the skeleton in altricial Java sparrow (*Padda oryzivora*) and domestic pigeon (*Columba livia f. dom*), semialtricial Eurasian kestrel (*Falco tinnunculus*), American kestrel (*F. sparverius*), and collared falconet (*Microhierax caerulescens*), and precocial European quail (*Coturnix c. coturnix*) and Muscovy duck (*Cairina moschata f. dom.*). All studies report almost the same pattern of ossifications in the hatchlings as described in detail here. Unpublished data on the altricial European starling (*Sturnus vulgaris*) and the semiprecocial Forster's tern (*Sterna forsteri*) also fit the description given above. The temporal and spatial pattern of ossifications, especially of the wing and the leg, is obviously independent of the developmental mode. In other words, when birds hatch, their skeletons have the same developmental stage and show no differences in respect of timing and topography of ossifications. Some altricial species have even more time to develop hatchling skeletons than precocial species, and these skeletons are relatively smaller. These observations are in agreement with the third hypothesis concerning cartilage/growth hypothesis noted earlier, which excludes heterochrony.

In spite of the qualitative similarity, the quantitative differences are striking. The hypothesis predicts large tissue volume for cartilage and small volume for bone in altricial hatchlings and the reversed relationship in precocials. This prediction is met by the data presented in this report. The tissue volume of cartilage ranges between 80% and 95% in the altricial budgerigar and between 60% and 85% in the precocial buttonquail. The differences are present for all elements except for the radius, where the relative volumes are similar in the bone and cartilage compartments.

Rogulska ('62) points out that the developmental sequence and timing of ossification are essentially the same in all species she studied but that the quantitative differences occur during embryogenesis. Mean values for the degree of ossification at hatching are given between 79% in the fowl, 77% in the gull, and 53% in the rook. Although these data are based on linear measurements of the bony shaft and relative to the length of each skeletal element at hatching, they show the same result of a higher portion of osseous areas in the actively moving hatchlings of precocials (fowl) and semiprecocials (gulls), and a considerably lower portion in the altricial (rook).

The design of the avian hatchling skeletons seems to be balanced by phylogenetically old, genetically determined time patterns and the cytokinetics of cartilage proliferation. (1) The sequence of occurrence of skeletal elements and the time patterns of ossification in birds are constant and apparently cannot be changed between species or between developmental modes. (2) Despite constancy in time patterns, precocial chicks have relatively larger osseous areas than altricials, even when time for ossification is shorter. The quantitative differences between precocial and altricial species must therefore be established by mechanisms different from heterochrony. It is highly probable that differences in cartilaginous cell proliferation, either through changes of proliferation rates or size changes of the proliferation zones, contribute to the quantitative differences of the skeletons. (3) The small cartilage-large bone design of the precocials correlates with their ability to locomote at hatching and their relatively slow growth. Conversely, the large cartilage-small bone design of altricials is found in correlation with a lack of locomotion but fast growth. These relationships are thought to represent a developmental trade-off that allows an avian hatchling either to locomote or to grow fast. Locomotion demands a high degree of ossifi-

cation to strengthen the skeleton mechanically. Simultaneously, the decrease of cartilage volume causes a decrease in capability for fast growth. On the other side, fast growth demands large cartilaginous areas, which lower the mechanical strength of the skeleton and thus its function in locomotion. (4) Preliminary statistical comparison of relative cartilage portion in hatchling skeletons and posthatching growth rate indices shows a positive correlation of growth rate indices with increasing cartilage portions. It supports the hypothesis that the volume of cartilage determines the posthatching growth rate and represents an internal constraint on posthatching growth. However, the comparison is based on five species only and needs improvement by more species. A detailed relationship cannot be presented on such a poor data base, and the establishment of a mathematical relationship between cartilage volume and posthatching growth rates remains a task for future studies.

The data presented here generally support the hypothesis. However, it is based on the assumption that cell proliferation rates are constant and comparable within and between species. The data presented cannot provide information about this assumption. Present research is focussing on the cytokinetics of cartilage and bone cell proliferation. Furthermore, a detailed topography of cartilage proliferation needs to be established to see whether interstitial growth of cartilage contributes a significant part to growth and the cartilage volume is an appropriate measure for proliferation.

The comparison of precocial (ancestral) and altricial (derived) developmental mode allows for an interpretation in terms of evolutionary theory. The similarity of the hatchling skeletons of the precocial buttonquail and the altricial budgerigar is in sharp contrast to the differences of their external appearance. The ossification pattern seem to have persisted as phylogenetically old traits that have not changed even when developmental mode shifted from precocial to altricial (Starck, '89). The quantitative differences described for the species are therefore thought to be adaptive in a tradeoff between tissue composition and fast growth. As shown above, heterochronic effects or neoteny must be excluded to account for any of these differences.

The cartilage/growth hypothesis fits to a growing body of knowledge on the functional design of avian hatchlings. Earlier studies (Ricklefs, '69b, '73, '79b; Ricklefs and Weremiuk, '77; Choi et al., 1993, Ricklefs et al., 1994) have shown that a general tradeoff between tissue maturity and growth determines the developmental mode of a bird hatchling because once a cell is differentiated, it can no longer undergo proliferation. This inability limits the growth capacity of the tissue. Thus tissue functionality and tissue growth are held to be mutually exclusive. Kirkwood et al. ('89a) showed that the size of the cartilaginous growth zones of the long bones of terrestrial vertebrates is correlated with the rate of elongation. Carrier and Leon (1990) suggested that the mechanical strength of the bone determines growth rates. Studying the semiprecocial California gull, they found that the fast-growing wing bones of these species are composed of mechanically weak flexible tissue. Mechanical strength was achieved after the wings had reached final length. The leg bones were composed of more dense material and grew much slower. An attempt to describe the rate of wing elongation as a constraint on fleding time (Carrier and Auriemma, '92) revealed a significant relationship in some avian families, but remained ambiguous in others. However, these studies may offer a link between factors determining wing and leg elongation and the tissue composition data presented here, although a final relationship remains to be elaborated.

The intestine capacity model of Konarzewski ('88) and Konarzewski et al. ('89) adds another aspect in that functionality, e.g., the gut's capacity to assimilate energy, has been shown to limit growth of altricial chicks.

In conclusion, the developmental stage of avian hatchlings seems to be determined by the general relationship between growth capacity and functionality of an organ. Tissue functionality and fast growth are incompatible. Cartilage growth seems to be an exception because even differentiated cells can undergo cell divisions and contribute to (interstitial) tissue growth. However, it is possible to assume a topographic separation between functional and proliferating parts of growing tissues. For example, growth of the intestine depends on cryptcell proliferation, whereas functional resorptive cells are positioned on the villi. Also, a separation of proliferation zones and functional areas in parenchymatous organs, e.g., liver, would make it necessary to modify the present hypotheses about tissue maturity and organ growth as

being mutually exclusive. However, the size of the proliferation zones and the cell cycle parameters would finally determine the growth capacity of any organ. A detailed knowledge of the topography of growth of each organ system will therefore be necessary to improve present hypotheses about the tradeoff between tissue maturity and growth.

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