Cell size is positively correlated between different tissues in passerine birds and amphibians, but not necessarily in mammals

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We examined cell size correlations between tissues, and cell size to body mass relationships in passerine birds, amphibians and mammals. The size correlated highly between all cell types in birds and amphibians; mammalian tissues clustered by size correlation in three tissue groups. Erythrocyte size correlated well with the volume of other cell types in birds and amphibians, but poorly in mammals. In birds, body mass correlated positively with the size of all cell types including erythrocytes, and in mammals only with the sizes of some cell types. Size of mammalian erythrocytes correlated with body mass only within the most taxonomically uniform group of species (rodents and lagomorphs). Cell volume increased with body mass of birds and mammals to less than 0.3 power, indicating that body size evolved mostly by changes in cell number. Our evidence suggests that epigenetic mechanisms determining cell size relationships in tissues are conservative in birds and amphibians, but less stringent in mammals. The patterns of cell size to body mass relationships we obtained challenge some key assumptions of fractal and cellular models used by allometric theory to explain mass-scaling of metabolism. We suggest that the assumptions in both models are not universal, and that such models need reformulation.

Keywords: erythrocytes; cellular architecture; metabolic rate; scaling

1. INTRODUCTION

Any substantial change of body mass in a lineage must be caused by alteration of cell size, cell number or a combination of the two. Because cell size differs between tissues in the same organism, we have to ask whether the sizes of different cell types evolve independently or in concert. If it is the latter, evolution is more

constrained in changing body size through alterations of cell size. Animal bodies would change by roughly rescaling all types of cells, which creates a size correlation between different cell types.

Evolutionary coupling between cell size and body mass is postulated to play an important role in shaping the mass-scaling of metabolism (Davison 1955; Szarski 1983; Kozłowski *et al.* 2003). With increasing cell size, the cell surface area/volume ratio decreases and a smaller fraction of metabolism is spent on maintaining ionic gradients across cell membranes. In a group of closely related organisms with differences in body mass attributed entirely to cell size change, the metabolic rate should scale with body mass with the exponent $b = 0.67$ dictated by the changing surface area/volume ratio of the cells. The exponent should become 1.0 if cell size remains unaltered and body mass varies entirely owing to differences in cell numbers. Then the question is whether the component of body size change realized through the change in cell sizes is sufficient to explain the usually negative allometries of metabolism in nature ($0.6 < b < 0.8$; Glazier 2005)?

A concurrent model (West *et al.* 1997) predicts the unique metabolic scaling exponent 0.75 from the fractal nature of the supplying system. One of its key assumptions is body mass-invariance of capillary length and diameter. Given that adequate gas exchange requires the short axis of the erythrocyte to be about 25 per cent more than capillary diameter (Snyder & Sheafor 1999), West *et al.* implicitly assume mass-invariance of erythrocyte size. Consequently, if organism-wide rescaling of cell sizes exists in nature, other cell types should not change their size with the organisms' mass. Savage *et al.* (2007) attempt to link fractal and cellular models by considering either body mass-scaling or mass-invariance of cell sizes in mammals, and show that both patterns are present depending on cell type. This result requires verification for other taxa, and testing whether mass-scaling of cell size supports a cellular model and to what extent it challenges the assumptions of a fractal model.

Measuring cell size is extremely laborious, which explains the paucity of available data and the lack of conclusive evidence for the arguments. Here, we analyse the sparse data on cell size in tissues of passerine birds, amphibians and mammals to test whether cell size for different cell types is intercorrelated and related to body mass. In particular, we examine whether erythrocyte size correlates with the size of other cells and with body mass. The existence of these intercorrelations in different taxa would indicate the need to reconcile some key assumptions of the fractal model. To test the cellular model, we calculate exponents of mass-scaling of cell sizes, assess the corresponding exponents of metabolism predicted by the model and compare the predicted exponents with their actual values from the literature.

2. MATERIAL AND METHODS

We used published and unpublished data on birds, amphibians and mammals collected by the group founded by Henryk Szarski, mostly at Nicolaus Copernicus University (Toruń, Poland, TG hereafter), and two datasets for mammals extracted from Morgado *et al.* (1990) by Savage *et al.* (2007) and from Levi (1925) by Szarski

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Table 1. Results of PCA of cell size in amphibian, bird and mammalian tissues (on raw data and phylogenetic contrasts). (Loadings in bold are greater than 0.5. *n* is the number of species used.)

group	cell type	raw data			phylogenetic contrasts		
		PC1	PC2	PC3	cPC1	cPC2	cPC3
birds (Nitecki) <i>n</i> = 6	hepatocytes	0.990	0.123	−0.001	0.977	0.179	−0.007
	proximal kidney tubules	0.997	−0.028	0.058	0.989	−0.085	−0.100
	duodenal epithelium	0.988	0.139	0.067	0.976	0.187	−0.108
	skin epithelium	0.991	0.005	−0.132	0.976	0.051	0.210
	tracheal chondrocytes	0.957	−0.290	0.022	0.898	−0.438	−0.004
	thyroid follicles	0.997	0.042	−0.013	0.992	0.072	0.010
explained proportion of variation		0.97	0.02	0.004	0.94	0.05	0.01
amphibians (TG) <i>n</i> = 8	kidney (nephron)	0.974	−0.118	−0.193	0.911	−0.266	0.315
	intestinal epithelium	0.966	−0.200	0.164	0.912	−0.262	−0.316
	epidermis	0.945	0.325	0.031	0.798	0.602	0.002
explained proportion of variation		0.93	0.05	0.02	0.77	0.17	0.07
mammals (Morgado) <i>n</i> = 9	adipocytes (skin)	0.729	−0.064	−0.580	0.013	−0.650	0.723
	cerebellar granule neurons	0.925	−0.173	0.283	−0.676	0.482	0.533
	cerebellar Purkinje neurons	0.932	−0.054	0.338	−0.430	0.651	0.573
	fibroblasts	−0.256	−0.809	0.496	−0.789	0.228	−0.513
	fibrocytes	0.285	−0.636	−0.682	−0.566	−0.697	0.298
	glomerular epithelium	−0.085	−0.946	0.236	−0.917	−0.201	−0.223
	goblet cells	0.800	0.319	−0.118	0.468	−0.246	0.705
	Henle-loop cells	−0.225	−0.560	−0.216	−0.002	−0.718	−0.164
	hepatocytes	−0.400	0.162	0.483	0.320	−0.008	−0.228
	proximal convoluted tubules	0.586	−0.780	0.173	−0.966	−0.080	0.159
	sebaceous gland cells (skin)	0.699	0.416	0.466	0.211	0.870	0.216
explained proportion of variation		0.37	0.29	0.17	0.34	0.27	0.20
mammals (TG) <i>n</i> = 8	hepatocytes	0.343	−0.914	0.206	0.128	−0.972	−0.164
	proximal kidney tubules	−0.065	0.418	0.903	0.056	−0.117	0.989
	duodenal epithelium	0.800	0.542	−0.002	0.883	0.358	0.119
	skin epithelium	0.878	0.328	−0.248	0.891	0.329	−0.191
	thyroid follicles	0.861	−0.442	0.242	0.780	−0.614	0.039
explained proportion of variation		0.46	0.32	0.20	0.44	0.31	0.21
mammals (Levi) <i>n</i> = 8	Purkinje cells (cerebellum)	0.843	0.445	−0.076	0.917	−0.205	−0.001
	neuron (small ganglion)	0.829	0.311	−0.372	0.807	−0.207	−0.466
	hepatocytes	−0.354	0.628	0.551	0.622	0.544	0.413
	adrenals fascicular zone	−0.313	0.710	−0.546	0.316	0.749	−0.460
	pancreas	−0.705	0.593	−0.133	0.012	0.838	0.142
explained proportion of variation		0.507	0.454	0.595	0.794	−0.290	0.332
explained proportion of variation		0.40	0.29	0.19	0.44	0.29	0.12

(1985). The data on six species of passerine birds were published by Nitecki (1972). They were collected by one person, and the tissues were taken from the same individuals, increasing the accuracy of data on birds. TG's data on amphibians and mammals (restricted to closely related groups, rodents and lagomorphs) were compiled from different papers and MSc theses. Not all same-species measurements were taken from the same individuals or made by the same person. Thus, the amphibian and mammalian data are less precise. Original data are given in the electronic supplementary material.

All analyses used original data and phylogenetically independent contrasts. Contrasts were calculated following the method in Phenotypic Diversity Analysis Programmes (Garland *et al.* 1999) with Nee's transformation (Purvis 1995) using the trees in the electronic supplementary material. We calculated correlations between cell size and body mass, and size correlations between cell types (see the electronic supplementary material). Mass-scaling exponents of cell size for different tissues were calculated from slopes of log cell volume – log body mass least-squares regressions. Mean exponents in each animal group were calculated from exponent values in different tissues. The means were tested against zero with the *t*-test. To examine intercorrelations between cell sizes and to reduce the problem of multiple comparisons, we performed principal component analysis (PCA) on the most complete data subsets on cell size. Phylogenetically controlled PCA was carried out on correlation

coefficients (calculated through origin) of standardized contrasts from cell volumes (Garland *et al.* 1992). Our principal components (PCs) identify tissue associations with highly correlated cell sizes. PC scores measure the combined cell sizes in such groups. Loadings indicate the strength and direction of tissue contributions to PCs. Negative loadings indicate an inverse relationship between PC scores and the cell size of contributing tissues. PC scores were used to examine correlations of cell size with body size and erythrocyte size.

3. RESULTS

Birds and amphibians differed from mammals in the structure of intercorrelations between cell types (table 1). Birds and amphibian tissues formed a single major PC with high loadings of the same sign, revealing positive cell size correlations between tissues. Mammalian tissues clustered in three PCs (tissue groups), indicating that not all cell types had correlated sizes. Within each group, cell size usually correlated positively between tissues (high loadings with the

Table 2. Correlation matrix of erythrocyte size, body mass and scores of PCs: conventional analysis (normal font) and with phylogenetically independent contrasts (in italics). (Significant correlations are in bold. PCs define groups of tissues with correlated cell sizes (table 1). PC scores measure cell size in these groups. Only PCs explaining greater than 10% variance were used. *n* reports the number of species used.)

group	trait	log body mass	erythrocyte	PC1	PC2	PC3
birds (Nitecki) <i>n</i> = 6	log body mass		0.987 0.969	0.986 0.965		
	erythrocyte	<i>p</i> < 0.001 <i>p</i> < 0.002		0.995 0.988		
	PC1	<i>p</i> < 0.001 <i>p</i> < 0.002	<i>p</i> < 0.001 <i>p</i> < 0.001			
amphibians (TG) <i>n</i> = 8	erythrocyte			0.739 0.342	0.147 0.480	
	PC1		<i>p</i> = 0.036 <i>p</i> = 0.41			
	PC2		<i>p</i> = 0.73 <i>p</i> = 0.23			
mammals (Morgado) <i>n</i> = 9	log body mass		−0.243 0.245	0.876 −0.262	0.244 −0.317	−0.025 0.729
	erythrocyte	<i>p</i> = 0.53 <i>p</i> = 0.53		0.528 0.304	0.064 −0.774	−0.558 −0.065
	PC1	<i>p</i> < 0.002 <i>p</i> = 0.50	<i>p</i> = 0.14 <i>p</i> = 0.43			
	PC2	<i>p</i> = 0.53 <i>p</i> = 0.41	<i>p</i> = 0.87 <i>p</i> = 0.014			
	PC3	<i>p</i> = 0.95 <i>p</i> = 0.026	<i>p</i> = 0.12 <i>p</i> = 0.87			
mammals (TG) <i>n</i> = 8	log body mass		0.854 0.834	0.876 0.396	0.244 −0.554	0.025 −0.286
	erythrocyte	<i>p</i> = 0.007 <i>p</i> = 0.010		0.612 0.474	0.529 −0.517	−0.280 −0.014
	PC1	<i>p</i> = 0.002 <i>p</i> = 0.33	<i>p</i> = 0.11 <i>p</i> = 0.24			
	PC2	<i>p</i> = 0.53 <i>p</i> = 0.15	<i>p</i> = 0.18 <i>p</i> = 0.19			
	PC3	<i>p</i> = 0.95 <i>p</i> = 0.49	<i>p</i> = 0.50 <i>p</i> = 0.97			
mammals (Levi) <i>n</i> = 8	log body mass		−0.016 0.667	0.807 0.674	0.160 −0.293	−0.255 −0.196
	erythrocyte	<i>p</i> = 0.97 <i>p</i> = 0.071		−0.191 0.549	0.308 −0.016	0.490 0.471
	PC1	<i>p</i> = 0.016 <i>p</i> = 0.067	<i>p</i> = 0.65 <i>p</i> = 0.16			
	PC2	<i>p</i> = 0.70 <i>p</i> = 0.48	<i>p</i> = 0.46 <i>p</i> = 0.97			
	PC3	<i>p</i> = 0.54 <i>p</i> = 0.64	<i>p</i> = 0.22 <i>p</i> = 0.24			

same sign), with a few exceptions. Some tissues contributed simultaneously to two PCs. PCA on contrasts produced similar results, but some tissues, especially in mammals, changed their quantitative (loading value) and qualitative (loading sign) impacts on PCs. In mammals (Morgado), PCs on contrasts changed in relative importance (cPC1 corresponds to PC2, cPC2 to PC3, cPC3 to PC1).

An index of cell sizes based on raw PC1 was positively correlated with body mass in birds and mammals (table 2). It was impossible to define a single size in indeterminately growing amphibians, and this precluded such an analysis. Note that in mammals (Levi), the increase in the PC1 index with body mass corresponded to an increase in the size of

tongue epithelium, Purkinje and neuronal cells, but a decrease in pancreatic cell size (table 1). The correlation between erythrocyte size and body mass (table 2) was positive in birds and only in the taxonomically most uniform mammalian dataset (rodents and lagomorphs, TG); it became nearly significant in mammals (Levi) after phylogenetic corrections (*p* = 0.071). Erythrocyte size appeared to be a reliable predictor of PC1 only in the birds (*p* < 0.001) and in amphibians (*p* = 0.036), but not in mammals. Contrasts for the examined variables correlated similarly (table 2), except for erythrocyte size versus PC1 (amphibians), and body mass versus PC1 (mammals, TG). In mammals (Morgado), contrasts for erythrocyte size correlated with cPC2, and a positive

Figure 1. Mean mass-scaling exponents of cell volumes in different tissues of birds and mammals; whiskers show minimum and maximum values. Means were tested against zero with a *t*-test. Open squares, raw; filled squares, contrasts.

correlation between cPC3 and mass contrasts corresponded to the correlation between PC1 and mass on raw data. Cell volume in tissues scaled with body mass, with exponents ranging from -0.09 to 0.280 (-0.067 to 0.281 for contrasts). Mean exponent values were significantly higher than zero in birds and mammals (Morgado) (figure 1).

4. DISCUSSION

We demonstrated that cell sizes in different tissues, including erythrocytes, are highly intercorrelated in passerine birds and amphibians, which indicates that some insufficiently recognized epigenetic mechanisms determining cell size relationships are very conservative in these groups. The correlation of erythrocyte size with the size of other cell types in birds and amphibians also has practical implications. Blood samples can be taken in the field without killing, and erythrocytes are easy to measure. The cellular architecture of mammals seems different. Variation of their cell sizes is not entirely captured by a single PC, but splits between three PCs (table 1), and erythrocyte size is usually unrelated to the size of other cell types (table 2). In birds, our measure of cell sizes across tissues (PC1) and erythrocyte area correlates strongly with body mass. A similar increase in erythrocyte size with body mass was earlier reported by Starostová *et al.* (2009) in geckos. In these groups, at least, this evidence invalidates the assumption of mass-invariance of erythrocyte size in West *et al.*'s (1997) fractal model. A positive correlation between erythrocyte size and body mass was also reported in mammals when phylogeny was considered (Promislow 1991), but it was not detected when the phylogenetic context was ignored (Savage *et al.* 2007). According to our results, erythrocyte size increases with body mass only in the phylogenetically uniform dataset on rodents and lagomorphs (TG), and the increase becomes nearly

significant in mammals (Levi) after phylogenetic corrections. Interestingly, only the size of cell types forming PC1 correlates with body mass in mammals; the cells involved in the remaining PCs stay mass-invariant (in mammals (Morgado), body mass correlates with the PC1 on raw data, and with the PC3 in contrasts, but similar tissues contribute to both PCs). Thus, the size of erythrocytes and of other cell types may depend on body mass in mammals, but it is not a norm.

Cell size seems to play a different role in the body mass evolution of the studied animals. The exponent of mass-scaling of cell volumes in different tissues is on average positive in all groups, but only in passerine birds and in Morgado's mammals is the mean exponent value significantly larger than zero (figure 1). Mass-scaling exponents of cell size did not exceed 0.3 in individual tissues, which means that changes in cell number also contributed to evolutionary differentiation of body masses, especially in mammals. According to the cellular model (Davison 1955; Kozłowski *et al.* 2003), an increase in cell volume with body mass to the power 0.3 should be associated with a metabolic scaling exponent b equal to 0.9. Apparently, this value of the metabolic exponent is much higher than the values actually observed in nature (b usually ranges from 0.6 to 0.8 in birds and mammals; Glazier 2005). Low exponents of the mass-scaling of erythrocytes and metabolism were also reported in a group of gecko species (Starostová *et al.* 2009). These pieces of evidence suggest that metabolic differences between species may be only partly determined by the cell surface/volume ratio, and mostly by other body size-related factors such as membrane permeability and the density and activity of mitochondria.

The empirical data reveal that the patterns of mass dependence of cell sizes in different animal groups are inconsistent with the assumptions of fractal and

cellular models. This calls for revision of the theories. Qualitative differences in the cellular architecture of amphibians and birds versus mammals point to the importance of the phylogenetic context in future metabolic studies, and warn against formulating universal explanations. We believe that further studies integrating the levels of cell and organism are required if we are to untangle the knot of causality in the relationships between body size, metabolism and cell traits.

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