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Dwarf and giant geckos from the cellular perspective: the bigger the animal, the bigger its erythrocytes?

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Summary

1. Although evolutionary and ecological consequences of body size changes are relatively well understood, the proximate mechanisms of body size alteration at a cellular level are often surprisingly neglected, especially in vertebrates. The question of whether larger animals are made from larger cells is rarely tested in an explicit phylogenetic framework, i.e. among closely related species with known phylogeny.

2. Here we explore the relationship between erythrocyte and body size in a small gecko family (Eublepharidae) exhibiting large body size variation.

3. We found positive interspecific correlation of cell and body size. Assuming that size of other cell types changed in a similar way to red blood cell size, we can conclude than *c.* 15–20% of body size change in this group could be attributed to cell size variation.

4. As larger cells are generally more frugal than smaller cells, we hypothesise that a macro-evolutionary trade-off exists between body complexity and energetic efficiency.

5. We believe that knowing how particular animal lineages have solved this trade-off during body size evolution will help us explain much of the variation in ecophysiological traits among clades as well as within them.

Key-words: Allometry, cell size, *Eublepharis*, metabolism, macroevolution, phylogeny

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Introduction

The rapid evolution of body size has repeatedly been reported in various animal lineages. However, considerable change of body size is not a simple matter. As size changes, organisms must cope with the consequences of functional shifts in their systems. Many morphological, physiological, life history and behavioural traits clearly mirror this fact, and show regular alterations with changes in body size (Harvey & Pagel 1991) – probably the most frequently discussed example of such a regular alteration is the general allometry of basal metabolic rate (West, Brown & Enquist 2000). As a result, smaller organisms are seldom scaled-down versions of their larger relatives. From our point of view, one of the significant reasons for this observation is the restricted size of the basic organismal units – cells. Cells clearly cannot scale proportionally to body size over several orders of magnitude. It would be naive to assume that cells of an animal that is 500 times larger would themselves be 500 times larger. On the other hand, it would be equally naive – although usually much more acceptable – to assume *a priori* that cell size in variously sized animals does not differ between them at all.

At the cellular level, body size can be attributed to alteration in cell size, cell number or in an amount of the extra-cellular matrix. The importance of a particular proximate mechanism of size variation in individual animal lineages is poorly appreciated, although it definitely affects the functional transitions accompanied by size changes, at least because cells of different sizes behave differently (Goniakowska 1973; Mongold & Lenski 1996). The change in cell size, or more often the combination of changes in both cell size and cell number have been documented in different lineages of invertebrates (Partridge *et al*. 1994; Stevenson, Hill, & Bryant 1995). Erythrocyte size also correlates positively with body mass among birds (Gregory 2002 and references therein). On the other hand, it has been believed that intra- and interspecific changes in body size in mammals are entirely realized by changes in cell numbers, i.e. that the number of cells increases linearly with body size (Teissier 1939 *ex* Schmidt-Nielsen 1984; West *et al*. 2000; Trumpp *et al*. 2001). What mechanism determines evolutionary changes of body size in other vertebrates has remained largely unstudied. Nevertheless, it would be misleading to treat large animal groups (e.g. all mammals or reptiles) as uniform with respect

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to the body size–cell size relationship, because size diversification might take place independently within much narrower groups, and the role of cell size/cell number change could differ among particular lineages (Kozłowski, Konarzewski & Gawelczyk 2003). Therefore, it is important to investigate proximate mechanisms of body size variation at lower taxonomical levels using explicit phylogenetic framework. Here we analyse the size variation of red blood cells in eublepharid geckos. The family Eublepharidae is a monophyletic assemblage, probably a sister group of all the other gekkotan lizards (Han, Zhou & Bauer 2004). The phylogenetic relationships among the eublepharid species are relatively well-corroborated (reviewed in Kratochvíl & Frynta 2002). Most importantly for the present study, within this small family there is an amazing variation in body size – the largest species *Eublepharis angramainyu* Anderson et Leviton, 1966 is more than 20 times heavier than the smallest *Coleonyx brevis* Stejneger, 1893. The eublepharid ancestor was most probably a middle-sized gecko (Grismer 1988). During their phylogeny, eublepharids have thus radiated in body size in both directions. The aim of this paper is to test whether the evolutionary change in body and cell size are associated.

Material and methods

To solve this question, we took measurements of red blood cells in 27 members of 14 forms of eublepharids, including species with body size extremes for this clade: *Coleonyx brevis*; *C. elegans* (Gray, 1845); *C. mitratus* (Peters, 1845); *C. variegatus* (Baird, 1858); *Eublepharis* cf. *fuscus* (Börner, 1981); *E. macularius* (Blyth, 1854) – domesticated population; *E. macularius* – 'white' population; *E. macularius* – 'yellow' population; *E. angramainyu* – Syrian population; *E. angramainyu* – Iranian population; *Goniurosaurus araneus* (Grismer, Viets et Boyle, 1999); *G. luii* (Grismer, Viets et Boyle, 1999); *Hemitheconyx caudicinctus* (Duméril, 1851); *Holodactylus africanus* (Boetger, 1893). Individuals from white and yellow populations of *E. macularius* are the representatives of two morphologically different forms from Pakistan. We collected blood samples from two males of each form with the exception of *C. brevis*, where only a single female was available at the time of blood collection. Recent evidence has suggested, however, that there is no sexual dimorphism in erythrocyte size in reptiles (Uğurtaş, Sevinç & Yildırımhan 2003). All of the individuals came from breeding stocks or from the pet trade, except for *E. angramainyu*, collected by the authors and their co-workers in Iran and Syria. The animals that were sampled were adults which had been kept in our laboratory breeding room in common stable conditions at a temperature of 25–27 °C for at least 1 year before sample collection (for detailed description of the breeding conditions see Kratochvíl & Frynta 2002).

We measured the body mass and snout-to-vent length (SVL) of each individual before the collection of a small drop of blood from the humeral vessel. Moreover, we used maximal SVL for a given form as an alternative expression of body size. Estimation of maximal SVL was based on published sources and our morphometric dataset including about 1600 eublepharid individuals (cf. Kratochvíl & Frynta 2002). We dyed the dry smear using standard May–Grünwald and Giemsa–Romanovski solutions. The erythrocytes were photographed at the same magnification calibrated with a glass microscopic scale of known length (0·01 mm). On each slide the length, width and surface of the fixed red blood cells and their nuclei were taken for 50 randomly chosen erythrocytes using Image Tool for Windows vers. 3·0 (UTHSCSA 2002).

The $\log_{10}$-transformed values of body and cell size measurements were used in further analyses. In the majority of cases, the Kolmogorov–Smirnov test did not indicate violation of normal distribution for the red blood cell measurements. Thus, we took the midpoint of both individual means as an estimation of the form value for each measurement (with the exception of *C. brevis*). The relationships between body and cell measurements across forms were first checked using Spearman's non-parametric correlation in Statistica vers. 6·0 (StatSoft Inc. 2001). The relationship of the individual cell measurements was also visualised by principal component analysis (PCA). To test the hypothesis of scaling of individual erythrocyte measurement (C) on body size (S) we applied the power function in its $\log_{10}$-transformed form: $\log_{10} C = \log_{10} a + b \log_{10} S$ (Huxley 1932). For the regression slope estimations we employed the reduced major axis regression (RMA) model using RMA vers. 1·14b program (Bohonak 2002). Next, we used regression slopes to calculate contribution of cell size to body size variation (for detailed derivation of the relationship see Stevenson *et al*. 1995). Briefly, the allometric equation $C = a_1 S^{b1}$ describes the relationship between cell and body size. A similar equation $N = a_2 S^{b2}$ can be used to describe the allometric relationship between cell number (N) and body size. As body size is the product of cell size and cell number, $C \times N = a_1 a_2 S^{(b1+b2)} = S$. Thus, the sum of the exponents b_1 and b_2 should equal 1. The value of the coefficient b_2 then indicates the relative contribution of changes in cell size to changes in body size. Stevenson *et al*.'s estimation holds as long as measurements of cell and body size are in the same dimensions (e.g. length, area or volume).

As species data are not independent, we also carried out a phylogenetically controlled analysis using the method of independent contrasts (Felsenstein 1985). We generated the independent contrasts of transformed body and erythrocyte measurement using COMPARE vers. 4·5 (Martins 2003), which employs the slightly altered phylogenetic hypothesis of eublepharids based on morphological and molecular characters (Kratochvíl & Frynta 2002). We replenished the character matrix by new molecular data on 12S and 16S rRNA sequences in several forms, especially in *E.* cf. *fuscus*, and all mentioned forms of *E. angramainyu* and *E. macularius*.

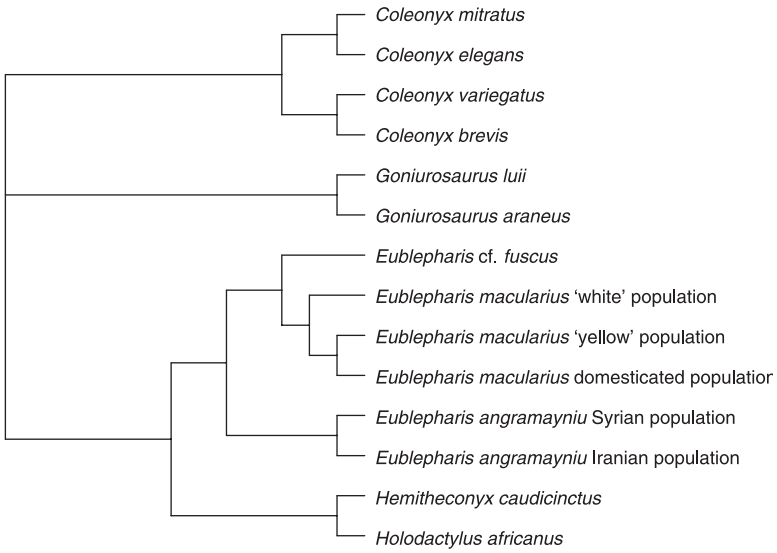

Fig. 1. Phylogetic tree of eublepharid geckos used for comparative statistical analyses. The relationships between forms are largely based on Kratochvíl & Frynta (2002), their character matrix was replenished by new molecular data in several forms.

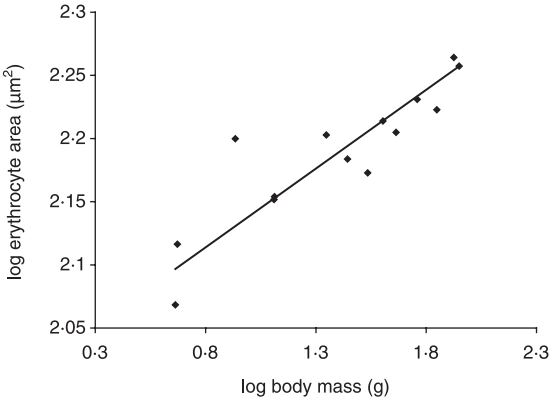

Fig. 2. Scatterplot of the red blood cell area vs. body mass among 14 populations of eublepharid geckos. Depicted line is the estimated RMA regression line.

The computed phylogeny revealed the sister position of *E.* cf. *fuscus* to the clade of all *E. macularius* populations, where 'white' population was sister to 'yellow' population and the domesticated population. The clade of *E.* cf. *fuscus* and *E. macularius* was sister to both *E. angramainyu* populations. The remaining splitting stayed the same as in our original paper (Fig. 1). The new cladogram will be described in detail elsewhere. Computing independent contrasts, we took a position of *Coleonyx*, *Goniurosaurus* and *Eublepharis – Hemitheconyx – Holodactylus* clade as unresolved, and set all branch lengths to 1. The diagnostic proposed by Garland, Harvey & Ives (1992) revealed that the contrasts were appropriately standardized. All correlations and regressions using contrasts were computed through the origin.

Results

The size of eublepharid erythrocytes varied across the taxa. For example, the red blood cell surface ranged from approximately 117 μm² in *Coleonyx brevis* to 184 μm² in Iranian *Eublepharis angramainyu*, encompassing an increase of about 36%. Original data are provided in Table 1.

We found an interspecific correlation between body size and erythrocyte dimensions (Table 2, Fig. 2). The close relationship among individual cell size measurements was visualised by PCA, where PC1 encompassed the overwhelming majority of variation (over 85%). PC1 could be interpreted as a generalised erythrocyte size (Fig. 3). Body size measurements added into PCA

Table 1. Means of body and cell size dimensions in 14 populations of eye-lid geckos. For abbreviations see legend to Fig. 3

Population	SVL (mm)	Mass (g)	Max SVL (mm)	EL (μm)	EW (μm)	EA (μm²)	NL (μm)	NW (μm)	NA (μm²)
Coleonyx brevis	61	4·6	66·6	15·856	9·074	117·033	6·347	3·524	19·945
Coleonyx elegans	91	12·9	107·7	18·111	9·540	141·803	6·320	3·843	21·014
Coleonyx mitratus	83·5	12·95	96·1	18·161	9·593	142·569	6·656	3·872	21·999
Coleonyx variegatus	62	4·7	69	17·690	9·251	130·747	6·570	3·288	18·497
Eublepharis angramainyu Iranian population	149	84·2	154	20·848	10·889	183·752	7·623	4·349	28·308
Eublepharis angramainyu Syrian population	162·5	88·95	163	20·761	10·742	180·874	7·406	4·308	27·766
Eublepharis cf. *fuscus*	111	34·25	125·2	19·097	9·682	148·888	7·254	3·990	24·886
Eublepharis macularius domesticated population	119·5	40·2	137·8	19·753	10·277	163·620	6·982	4·242	25·223
Eublepharis macularius yellow population	141·3	70·5	145·5	19·692	10·424	167·022	7·073	4·054	25·156
Eublepharis macularius white population	123·5	46·05	147·3	19·638	10·030	160·274	7·370	4·162	26·335
Goniurosaurus araneus	110	22·25	117·2	19·936	9·918	159·508	7·414	4·322	26·760
Goniurosaurus luii	114·5	27·75	122·8	19·526	9·402	152·689	7·226	4·273	26·312
Hemitheconyx caudicinctus	132	57·45	144·5	19·352	10·824	170·204	7·255	4·574	28·019
Holodactylus africanus	73	8·6	78·6	19·677	9·806	158·436	6·406	4·145	22·057

Table 2. Coefficients of allometric scaling of cell dimensions on three different body size measurements. 95% confidence intervals are presented in RMA slopes and intercepts. Asterisks denote slopes significantly different from zero at $P < 0{\cdot}05$. For abbreviations see legend to Fig. 3

Dependent variable	Log mass			Log SVL			Log max SVL		
	Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2
log EL	1·188 ± 0·039	0·068 ± 0·024*	0·65	0·844 ± 0·162	0·217 ± 0·078*	0·66	0·814 ± 0·173	0·227 ± 0·081*	0·64
log EW	0·907 ± 0·026	0·062 ± 0·016*	0·77	0·583 ± 0·118	0·203 ± 0·057*	0·73	0·523 ± 0·167	0·228 ± 0·080*	0·67
log EA	2·025 ± 0·048	0·118 ± 0·028*	0·80	1·423 ± 0·200	0·379 ± 0·095*	0·79	1·345 ± 0·253	0·409 ± 0·118*	0·75
log NL	0·748 ± 0·029	0·068 ± 0·019*	0·72	0·390 ± 0·137	0·224 ± 0·067*	0·72	0·331 ± 0·171	0·247 ± 0·081*	0·68
log NW	0·494 ± 0·043	0·084 ± 0·029*	0·65	0·066 ± 0·194	0·270 ± 0·095*	0·66	0·017 ± 0·209	0·288 ± 0·099*	0·65
log NA	1·198 ± 0·051	0·156 ± 0·046*	0·85	0·498 ± 0·185	0·440 ± 0·091*	0·86	0·404 ± 0·235	0·474 ± 0·111*	0·83

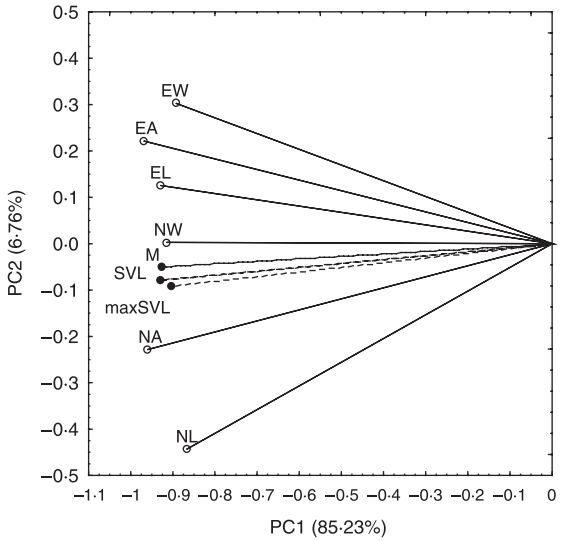

Fig. 3. Visualisation of coefficients for the first two principal components of erythrocytes measurements in 14 populations of eublepharid geckos, depicting the close relationship among individual cell size measurements. When body size measurements were added into the erythrocyte PCA space, they fell within the cell size variables and were strongly correlated with PC1. EW, erythrocyte width; EL, erythrocyte length; EA, erythrocyte area; NW, nucleus width; NL, nucleus length; NA, nucleus area; SVL, snout-to-vent length; M, body mass; maxSVL, maximum SVL for a given population.

space projected to the middle of the cell size variables and strongly correlated with PC1 (Spearman's correlation coefficient of PC1 with SVL and body mass, respectively: $r = -0{\cdot}8945$; with maxSVL $r = -0{\cdot}8857$, $P < 0{\cdot}0001$ in all cases; Fig. 3). When the effect of phylogeny was taken into account, the correlations, except those concerning red blood cell length, remained significant (Table 3).

Table 3. Results of independent contrasts analysis on relations between cell and body size measurements. 95% confidence intervals are given. Asterisks denote slopes significantly different from zero at $P < 0{\cdot}05$. For abbreviations see legend to Fig. 3

Dependent variable	Log mass		Log SVL		Log max SVL	
	Slope	R^2	Slope	R^2	Slope	R^2
log EL	0·027 ± 0·035	0·16	0·090 ± 0·107	0·18	0·088 ± 0·110	0·17
log EW	0·049 ± 0·022*	0·61	0·148 ± 0·070*	0·59	0·144 ± 0·080*	0·53
log EA	0·076 ± 0·044*	0·49	0·240 ± 0·134*	0·50	0·233 ± 0·140*	0·46
log NL	0·056 ± 0·026*	0·59	0·165 ± 0·085*	0·55	0·167 ± 0·090*	0·55
log NW	0·058 ± 0·036*	0·46	0·182 ± 0·109*	0·48	0·192 ± 0·110*	0·50
log NA	0·120 ± 0·037*	0·77	0·364 ± 0·120*	0·76	0·373 ± 0·120*	0·76

The allometric slope of linear dimensions was in the range 0·14–0·19. Assuming that size of other cell types correlates with red blood cell size, we can conclude that *c.* 15–20% of body size change could be attributed to changes in cell size.

Discussion

We chose red blood cells as the representatives of generalised cell size because they are fully differentiated, more

or less uniform in shape, and they do not enter cellular division. Red blood cells function in the essential distribution of respiratory gasses into all parts of an organism. Therefore, we can expect that larger erythrocytes would mean larger blood vessels and consequently larger cells of all the adjacent tissues. At least across several bird species, erythrocyte size strongly correlates with the size of cells in other tissues (Gregory 2002). Moreover, as described above, erythrocytes can be sampled from living individuals relatively non-invasively, i.e. without severely harming the experimental animals. On the other hand, in spite of the obvious advantages of erythrocytes, it can be argued that these cells are designed for very specialised function, and consequently their size may be under different selection than that of other cell types.

We found a clear correlation between changes in red blood cell size and body size. As there is evidence that the eublepharid ancestor was a medium-sized gecko (Grismer 1988), eublepharids have thus radiated in body and red blood cell size in both directions. The smallest and the largest eublepharid species are then derived, and they possess the smallest and largest erythrocytes, respectively (Table 1). Assuming that the size of other cell types changes in the same way as the size of the red blood cells, the cell size may contribute about 15–20% to evolutionary changes in body size. Our analysis shows a strong allometric relationship between erythrocyte and body size. The larger forms of our geckos have absolutely larger erythrocytes; however, their red blood cells are relatively smaller.

Although the overwhelming majority of variation in body size in eublepharid lizards could be explained by changes in cell numbers, the observed changes in cell size should have an impact on lizard physiology. As the large cells are economically more frugal (Goniakowska 1973; Mongold & Lenski 1996; Fig. 3 in West, Woodruff & Brown 2002), animals with larger cells should have relatively smaller standard metabolic rate (Szarski 1983; Kozłowski *et al*. 2003). Indeed, two dwarf eublepharid species – *C. brevis* and *C. variegatus* – have elevated levels of mass-standardised basal metabolism compared to their larger relatives (Dial & Grismer 1992). The level of metabolism without any doubt influences species ecology and life-history. To exemplify, we have previously shown that of four examined species of eublepharid geckos (*C. brevis*, *C. elegans*, *C. mitratus*, *E. macularius*), the smallest *C. brevis* has the largest growth rate (Kratochvíl & Frynta 2003).

Which mechanism could lead to the observed correlation between body and cell size? We hypothesise that from the macro-evolutionary perspective a trade-off could exist between body complexity and energetic efficiency that may affect body size evolution. Complex and large bodies could be made from either large or small cells. Nevertheless, as mentioned above, small cells are economically disadvantageous – they are associated with a large standard metabolic rate and a wasteful way of life. During evolution, large organisms, with the exception of animals with extreme energetic demands of, e.g. actively flying animals such as birds or bats, should thus tend to increase their cell size. Bodies of dwarfs could be made either from small cells at a cost of higher metabolic rate and larger energetic demands, or from large cells at a cost of complexity. Tiny Bolitoglossine salamanders possess large cells and have simplified brains in comparison with their larger relatives (Roth, Nishikawa & Wake 1997). Small size together with large cells could potentially result even in an organ loss. A classical example is the loss of the fifth digit in plethodontid salamanders (Alberch & Gale 1985). During the miniaturisation process, individual lineages have found different solutions to the proposed macro-evolutionary trade-off. For example, fine-grained morphology was maintained due to cell size minimalisation in loriciferans, small marine invertebrates (Minelli 2003). On the other hand, appendicularians are marine animals with very small, simplified bodies made from cells of enormous size (Minelli 2003).

We suggest that variation in cell size as a proximate mechanism of the variation in body size has been somewhat neglected, although it should be seriously taken into account also in vertebrates. The belief, that, e.g. 'all mammals are built from essentially the same "fundamental" cellular materials with the number of cells increasing linearly with body size' (West *et al*. 2000: 89) is unsubstantiated until careful phylogenetic study of cell and body size relationship within particular lineages is made. For the sake of illustration, a recent database on cell size managed by T. R. Gregory (2001) shows considerable variance (from 2·1 to 10·8 μm) in the dry red blood cell diameter even in mammals – interestingly, erythrocytes of both elephant species are near the upper margin. This variance suggests that the importance of cell size changes during body size evolution should not be omitted in any vertebrate group. We believe that knowing how particular animal lineages have solved the trade-off between energetic efficiency and complexity during body size evolution will help explain much of the variation in ecophysiological traits among clades as well as within them. The aim of our paper is to stimulate further phylogenetic studies of the role of cell number vs. cell size in vertebrate size variation.

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