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## THE RELATIONSHIP BETWEEN BODY LENGTH AND FEMUR BONE THICKNESS IN *Lacerta agilis boemica* AND *L. strigata*. IMPLICATIONS FOR GROWTH INFERENCES FROM SKELETOCHRONOLOGICAL DATA

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### INTRODUCTION

Mark-recapture and experimental studies — the main tool to obtain data on growth and longevity of animals — are very time-consuming. A promising alternative tool to get such data for reptiles and amphibians is investigation of growth layers in their bone tissue. This method provides not only an accurate age determination, but also a quantitative estimation of the pattern of bone growth (Smirina, 1974; Castanet et al., 1977; Castanet and Smirina, 1990; Hemelaar, 1988; Castanet and Baez, 1991; etc.). Due to a generally high correlation between the bone thickness and body size, inferences about body growth are also possible

(Smirina, 1983; Marunouchi et al., 2000 and references therein) provided that the relationship between the body size and the size of growth mark (bone thickness) is known in detail.

### MATERIAL AND METHODS

Snout-vent length (*SVL*) and femur bone thickness (*D*, measured as the mean of the minimal and maximal diameter on transverse sections in the middle of the femur diaphysis) were recorded for 320 adults and yearlings of *Lacerta agilis* (subspecies *L. a. boemica*) and a related species *L. strigata* from five lowland and mountain localities in the eastern North Caucasus (Table 1, Fig. 1). The relationship between *SVL* and *D* in homogenous (for species, sex and locality) samples was examined with an allometric

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**TABLE 1.** Characteristics of the Regression Lines of the Femur Diameter on the Snout-Vent Length in Male and Female Samples of *Lacerta agilis boemica* and *L. strigata* from Eight Populations in the Eastern North Caucasus\*

Samples			<i>n</i>	Slope, <i>a</i> **		Y-intercept, <i>ln b</i> **		<i>r</i> ***
Species	Locality	Sex						
<i>agilis</i>	Kostek	M	24	1.069	(0.976 – 1.162)	–5.271	(–6.333) – (–4.210)	0.981
<i>agilis</i>	Kostek	F	30	1.000	(0.851 – 1.149)	–4.539	(–6.222) – (–2.857)	0.933
<i>agilis</i>	Sergokala	M	19	1.158	(0.976 – 1.340)	–6.246	(–8.301) – (–4.191)	0.956
<i>agilis</i>	Sergokala	F	18	1.154	(0.969 – 1.339)	–6.285	(–8.370) – (–4.200)	0.957
<i>agilis</i>	Khuchni	M	28	1.022	(0.862 – 1.183)	–4.711	(–6.527) – (–2.895)	0.932
<i>agilis</i>	Khuchni	F	25	0.995	(0.894 – 1.096)	–4.438	(–5.578) – (–3.298)	0.973
<i>agilis</i>	Termenlik	M	22	0.938	(0.858 – 1.019)	–3.774	(–4.683) – (–2.866)	0.983
<i>agilis</i>	Termenlik	F	29	0.903	(0.825 – 0.982)	–3.439	(–4.315) – (–2.563)	0.977
<i>agilis</i>	Kuli	M	10	0.969	(0.557 – 1.381)	–4.145	(–8.839) – (–0.548)	0.887
<i>agilis</i>	Kuli	F	13	1.131	(1.035 – 1.226)	–6.061	(–7.150) – (–4.972)	0.992
<i>strigata</i>	Kostek	M	26	1.023	(0.905 – 1.141)	–4.744	(–6.081) – (–3.408)	0.965
<i>strigata</i>	Kostek	F	21	1.037	(0.903 – 1.171)	–4.959	(–6.485) – (–3.434)	0.966
<i>strigata</i>	Sergokala	M	14	0.958	(0.819 – 1.097)	–3.993	(–5.574) – (–2.411)	0.974
<i>strigata</i>	Sergokala	F	13	0.642	(0.370 – 0.913)	–0.519	(–3.590) – (–2.552)	0.843
<i>strigata</i>	Khuchni	M	26	1.111	(0.970 – 1.252)	–5.713	(–7.309) – (–4.117)	0.957
<i>strigata</i>	Khuchni	F	24	1.003	(0.884 – 1.121)	–4.558	(–5.899) – (–3.218)	0.966

\* Natural log-transformed data,  $\ln D = a \ln SVL + \ln b$ ; \*\* Estimation and the 95% confidence interval in parenthesis; \*\*\* All correlations significant at  $P < 0.001$ .

formula,  $D = aSVL^b$  which is a general form of relative growth of different parts of the body (Gould, 1966; Mina and Klevezal, 1976; Smirina, 1983).

**RESULTS**

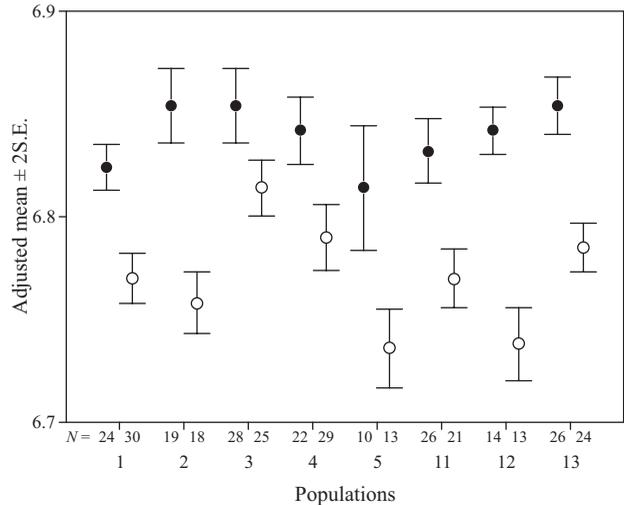
Linear regressions,  $\ln D = a \ln SVL + \ln b$  as well as product-moment correlation coefficients,  $r$  between  $\ln D$  and  $\ln SVL$  were calculated for each sample (Table 1). The correlation coefficients amounted 0.84 – 0.98, with 14 of the 16 values exceeding 0.9. High correlation between the  $SVL$  and  $D$  (0.80 – 0.98) remained even after truncating the data to the  $SVL$  range of 76 – 100 mm which was well represented in all study samples.

Slopes (exponent of the allometric equation) varied about unity: in only three of the 16 study samples, 95% confidence intervals were above or below unity (Table 1). This suggests a linearity of the relationship between body length and bone thickness in the two species, at least within the  $SVL$  range of ca. 45 – 105 mm.

Heterogeneity of regression slopes in different study samples was not significant (interaction  $\ln SVL \times \text{Sex}$ :  $F_{1,317} = 0.13$ ,  $P = 0.72$ ; interaction  $\ln SVL \times \text{Population}$ :  $F_{7,317} = 1.53$ ,  $P = 0.15$ ), so the usual ANCOVA was used for further analysis. The effect of sex, species and locality on the bone thickness when its correlation with  $SVL$  is statistically removed was assessed with a three-factor ANCOVA (Table 2). To maintain orthogonality of data, only sympatric populations were included in this analysis. The effects of Sex, Locality and their interaction were highly significant ( $P < 0.001$ ), whereas all effects involving Species were non-significant or only marginally significant ( $0.01 < P < 0.05$ ) (Table 2).

To examine the effect of locality (population) on the femur diameter relative to  $SVL$  in more details we conducted a single-factor ANCOVA for each species-sex combination separately. All five populations of *L. agilis* were included in the analyses. In both species the effect of locality was more pronounced in females (*L. agilis*:  $F = 12.00$ ,  $df = 4$ ,  $P < 0.001$ , coefficient of intraclass correlation  $r_1 = 0.335$ ; *L. strigata*:  $F = 9.67$ ,  $df = 2$ ,  $P < 0.001$ ,  $r_1 = 0.325$ ;) than in males (*L. agilis*:  $F = 3.36$ ,  $df = 4$ ,  $P < 0.013$ ,  $r_1 = 0.096$ ; *L. strigata*:  $F = 2.52$ ,  $df = 2$ ,  $P = 0.088$ ,  $r_1 = 0.072$ ).

To visualize the pattern of sexual, interlocality and interspecific differences for femur diameter relative to  $SVL$ , adjusted means of  $\ln D$  from an ANCOVA which involved all 16 samples are presented graphically (Fig. 1). In both species, males had consistently higher values than those of females, but the extent of sex differences did vary



**Fig. 1.** Adjusted means of ANCOVA with  $\ln D$  as the dependent variable, sample as the factor, and  $\ln SVL$  as the covariate. Means (solid circles, males; open circles, females)  $\pm 2$  standard errors (vertical bars) are indicated. Populations: 1 – 5, *L. agilis*; 11 – 13, *L. strigata*. Localities: 1, 11, Kostek (50 m above sea level); 2, 12, Sergokala (600 m); 3, 13, Khuchni (600 m); 4, Termenlik (960 m); 5, Kuli (1900 m).

among localities (Fig. 1). The latter circumstance was reflected in a highly significant Sex  $\times$  Locality interaction of the three-factor ANCOVA considered above. In both species, the interlocality variation in the extent of sex differences is largely determined by the samples of Sergokala exhibiting particularly strong sex differences (Fig. 1). In both sexes of both the species, samples from Khuchni exhibited the highest adjusted mean for  $\ln D$  among the five populations of *L. agilis* and the three populations of *L. strigata* (Fig. 1). This has a  $(1/5)^2 \times (1/3)^2 \approx 0.0045$  chance of occurring at random which significantly differs from 0.5 (binomial test,  $P < 0.001$ ).

**TABLE 2.** Analysis of Covariances of the Femur Diameter for Sympatric Populations of *Lacerta agilis boeica* and *L. strigata*

Source	df	F	P
$\ln SVL$	1	2827.965	0.000
Species	1	4.701	0.031
Sex	1	242.841	0.000
Locality	2	21.221	0.000
Species $\times$ Sex	1	4.031	0.046
Species $\times$ Locality	2	2.354	0.097
Sex $\times$ Locality	2	9.053	0.000
Species $\times$ Sex $\times$ Locality	2	0.567	0.568

**Notes.** The main effects are sex, species, and locality; the covariate is  $SVL$ . Morphometric data are log-transformed.

## DISCUSSION

Strong and linear interdependence between the *SVL* and femur diameter in the study species enables a quantitative estimation of the pattern of body growth from bone growth using simple back-calculation formulas (Marunouchi et al., 2000; Roitberg and Smirina, in preparation). However, the pattern of this relationship was found to vary not only between sexes but also among populations, especially in females. This provides some problems for use of all regression-based formulas because we should calculate the regression parameters separately for each combination of sex and population whereas the corresponding samples might be insufficiently representative.

Apart from its methodological consequences, the pronounced interlocality variation for femur bone thickness relative to *SVL* revealed in the study species is noteworthy itself. A concordance of this variation in the two species, along with the lack of between-species differences within localities, argues for its exogenous (environmental) determination.

Comparable studies, in which the phalanx of a frog, *Rana temporaria* (Ryser, 1996) and the humerus of a newt, *Notophthalmus viridescens* (Caetano and Leclair, 1996) were used, revealed no or moderate interlocality variation for the relationship between the body length and bone thickness.

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