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GENERAL BIOLOGY

Comparative Analysis of the Amino Acid Spectrum of Blood Plasma in Chiroptera (*Vespertilio murinus* L., 1758 and *Myotis dasycneme* B., 1825) in the Fauna of the Ural Mountains

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Abstract—The article presents the results of a new comparative analysis of free amino acids in the blood plasma of representatives of insectivorous Chiroptera (Mammalia: Vespertilionidae) in the fauna of the Ural Mountains: the pond bat (*Myotis dasycneme* Boie, 1825*)* and the parti-colored bat (*Vespertilio murinus* Linnaeus, 1758*)*. This is the first study to show the species variability of free amino acids in resident and migratory species of bats from different ecosystems of the Ural Region.

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Chiroptera or bats (Chiroptera, Vespertilionidae) are the only group of mammals that have evolved the capacity for active flight and can adapt to the cold season at northern latitudes in two different ways: long hibernation or southward migration. Among 13 Chiroptera species of the Ural Mountains, there are seven hibernating and six migrating species [1].

In spite of the available studies on biology and ecology, bats are yet the least-studied group of vertebrates with respect to the strategies and mechanisms of adaptation to the sustained maintenance of homeostasis in extreme environments [2, 3].

It is known that polyfunctional free amino acids play a key role in adaptive modifications of the meta-

Table 1. Free amino acids (μmol/L) in the blood plasma of two bat species

AA	$\bar{x}_{boot}$ [95% CI_{boot}]		Permutation test for ANOVA ($p = Pr(\|F_{ran}\| \geq F_{obs})$)
	I. Pond bat ($n = 10$)	II. Parti-colored bat ($n = 8$)	
Threonine	61.5 [49.7–74.2]	101.3 [80.3–121.8]*	$Pr(\|F_{ran}\| \geq 8.96) = 0.01$
Methionine	5.8 [3.6–8.6]	11.9 [7.7–15.8]*	$Pr(\|F_{ran}\| \geq 5.23) = 0.04$
Isoleucine	20.3 [13–27.6]	45.9 [27.8–63.6]*	$Pr(\|F_{ran}\| \geq 5.62) = 0.03$
Leucine	29.6 [15.3–44.8]	111.7 [56.5–154.8]*	$Pr(\|F_{ran}\| \geq 9.07) = 0.004$
Histidine	11.4 [7.7–15.4]	26.9 [20.8–33.6]*	$Pr(\|F_{ran}\| \geq 16.07) = 0.001$
Phenylalanine	36.2 [28.1–43.4]	91.7 [80.5–109.9]*	$Pr(\|F_{ran}\| \geq 34.39) = 0.0001$
Tryptophan	42.6 [32.5–51.6]	14.2 [9.5–28.8]*	$Pr(\|F_{ran}\| \geq 18.54) = 0.001$
Arginine	37.4 [25.9–48.1]	50.9 [34.1–82.7]*	$Pr(\|F_{ran}\| \geq 11.7) = 0.06$
Valine	59.7 [39.1–80.3]	108.8 [67.7–153.6]*	$Pr(\|F_{ran}\| \geq 3.6) = 0.05$
Lysine	69.3 [53.3–88.6]	81.7 [63.3–100.6]	$Pr(\|F_{ran}\| \geq 0.3) = 0.43$
Total NA	373.8 [265–477]	645.0 [492.5–855.2]	
Free AA pool	1295.7 [976.5–1640.0]	1634.7 [1240.0–2123.0]	$Pr(\|F_{ran}\| \geq 0.73) = 0.17$

NA, nonessential amino acids, *$p < 0.05$ in comparing I and II; $\bar{x}_{boot}$, the arithmetic mean of the AA level; n, the number of animals in the group. [95% CI_{boot}], the confidence interval of bootstrap distribution; $p = Pr(|F_{ran}| \geq F_{obs})$, two-way ANOVA with permutation test (randomization).

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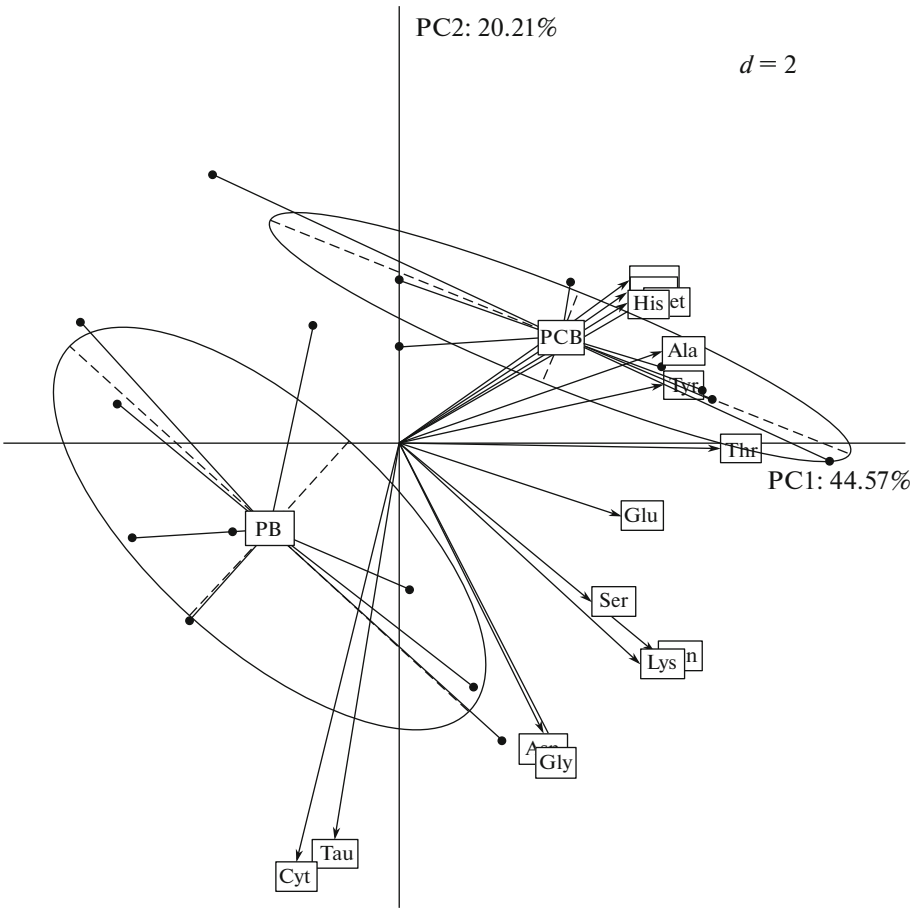

Fig. 1. Free amino acids in the blood plasma (μmol/L) of female bats of two species in the space of the first two principal components. PC1, PC2 are the principal component axes; % is the variance percentage of the data explained by the principal component; the arrows show the correlation between principal components and initial indices (amino acids); ellipses, 95% CI; PB, pond bat; PCB, parti-colored bat.

bolic and molecular mechanisms of homeostasis maintenance in organisms [4, 5]; hence, this study was aimed at investigating the amino acid spectrum of blood plasma in ecologically different Chiroptera species in the fauna of the Urals: parti-colored bat (*Vespertilio murinus* L., 1758) and pond bat (*Myotis dasycneme* B., 1825).

Both species are fairly common in the area of the South and Middle Urals. The pond bat is most abundant in the forest and forest–steppe zone; it is a typical hibernating species for caves, rock fractures, and other underground shelters. This species begins hibernating in the last third of September to early October and usually wakes up in late April. The parti-colored bat is a migratory species arriving at breeding grounds in the second half of May. Its migrations to wintering areas begin from the second half of August [1]. This is the first study on free amino acids in the blood plasma of bats inhibiting the Urals.

The animals were captured in the second third of July (the period of population reproduction) in 2013–2015 in Chelyabinsk oblast. Pond bats were captured in the vicinity of Lake Maloe Miassovo, and parti-colored bats were captured on the coast of Lake Bolshoy Kisegach. Free amino acids (AAs) were measured in the blood plasma of females by ion exchange chromatography with an AAA-339M amino acid analyzer (Microtechna, Czech Republic). The free AA concentration was expressed in μmol/L and percentage of total content. The results were processed with Statistica 6.0. Nonparametric multivariate analysis of variance and principal component analysis (PCA) in the R software environment (R 3.1.2, Vegan and Ade4 packages) were also used [6].

The amino acid spectrum of blood plasma of the bat species studied is represented by 23 AAs (Table 1). We did not observe any significant interspecies differences between *Myotis dasycneme* B., 1825 and *Vespertilio murinus* L., 1758 in the total concentration of free AAs. The absence of proline was shown for both species. The trace amounts of citrulline were found in the parti-colored bat. With regard to the percentage of metabolic groups in the common pools, glycogenic AAs were predominant: 70% (1144.2 μmol/L) in *Ves-*

pertilio murinus L., 1758 and 69% (894.0 μmol/L) in *Myotis dasycneme* B., 1825. In summer (which is quite short in the Urals), in the period of active growth and development of bats, an enhanced accumulation of these AAs is a predictor of activation and processes of the synthesis of collagen and elastin that form the basis of connective tissue by 25–35%. Thus, glycine is one-third of all amino acid residues of collagen [4]. Our data suggest that this is exactly why the maximum contribution of glycine to the total pool of free AAs in blood plasma was observed: 14% in *Vespertilio murinus* L., 1758 and 17% in *Myotis dasycneme* B., 1825. In addition, the very high content of essential AAs (EAs) was recorded for the parti-colored bat in this period (40%, Table 1). The total content of essential EAs in the blood plasma of *Vespertilio murinus* L., 1758 exceeded that of *Myotis dasycneme* B., 1825: 1.7-fold for threonine; 2.0-fold for methionine; 2.3-fold for isoleucine; 3.8-fold for leucine; 2.4-fold for histidine; 2.5-fold for phenylalanine; and 1.8-fold for valine (Table 1). It seems that the enhanced accumulation of these amino acids reflects the metabolic strategy of animals preparing for long-haul flights to wintering areas and can be characteristic of all migratory species. Identification of differences in the amino acid spectrum of blood plasma in the studied bat species by multivariate analysis (PCA) at a 95% confidence interval showed that 44.57% of total variance of the amino acid pool of bat blood plasma was accounted for by the first principal component (PC1) and 20.21% was accounted for by the second principal component (PC2, Fig. 1). The values of the first and second principal components showed significant interspecies differences between the bats in the blood content of free AAs (Fig. 1). Threonine, tyrosine, glutamine and alanine made the maximum contribution to the interspecies AA variability with regard to PC1: the PC1 correlation coefficient for the content of these AAs was 0.97, 0.8, 0.79, 0.8, respectively (Fig. 1). It is important to note the high percentages of taurine, lycine, citrullin, and aspartate (21.21, 12.94, 23.78, 11.49%, respectively) and a rather high coefficient of correlation between their levels and the second principal component PC2. We have recorded a high content of glycogenic glycine (22.76%) with the highest coefficient of negative correlation (–0.53) with the third principal component PC3. The principal component analysis made it possible to visualize the species specificity of amino acid spectrum in blood plasma of the two bat species of the Ural fauna, confirming the results of statistical analysis of AA levels in blood plasma.

The results of this research allowed us to estimate the metabolic specificity of essential and nonessential AAs in *Vespertilio murinus* L., 1758 and *Myotis dasycneme* B., 1825 and to reveal the species specificity of the AA spectrum in blood plasma of the resident and migratory Chiroptera species.

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REFERENCES

1. Bol'shakov, V.N., Orlov, O.L., and Snit'ko, V.P., *Letuchie myshi Urala* (Bats of the Urals), Yekaterinburg, 2005.
2. Geiser, F., Westman, W., McAllan, B.M., and Brigham, R.M., *J. Comp. Physiol.,* 2006, vol. 176, pp. 107–116.
3. Kovalchuk, L., Mishenko, V., Chernaya, L., Snitko, V., and Mikshevich, N., *Zool. Ecol.,* 2017, vol. 27, no. 2, pp. 168–175.
4. Lehninger, A., *Principles of Biochemistry,* New York: Worth, 1982.
5. Chernaya, L.V., Kovalchuk, L.A., and Nokhrina, E.S., *Dokl. Biol. Sci.,* 2016, vol. 466, pp. 42–44.
6. Chessel, D., Dufour, A.B., and Thioulouse, J., *R News,* 2004, no. 4, pp. 5–10.

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