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Species-specific Features of Blood Plasma Amino Acid Spectrum of Bats (Mammalia: Chiroptera) in the Urals

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Abstract—The amino acid spectrum of blood plasma in three bat species (*Myotis dasycneme*, *Pipistrellus nathusii*, *Vespertilio murinus*) inhabiting the Urals has been studied for the first time. The bats were trapped in the zone of their high abundance in Chelyabinsk oblast (2013–2014). Free amino acids were determined by liquid ion exchange chromatography (a total of 384 determinations). It has been shown that the plasma amino acid spectrum consists of 22 amino acids in subadult bats of all three species, but there are species-specific differences in their concentrations. The total amino-acid pool concentration in migratory *P. nathusii* and *V. murinus* exceeds that in resident *M. dasycneme* by factors of 2.9 and 1.8, respectively. Migratory species are characterized by a high concentration of plasma arginine: it is six times higher in *V. murinus* than in *M. dasycneme,* and in *P. nathusii* arginine accounts for 25.4% of the amino acid pool. The group of glucogenic amino acids is prevalent in the blood plasma of migratory species (75% in *V. murinus* and 79% in *P. nathusii*), while in *M. dasycneme* the total proportion of lysine, glycine, and glutamic acid is 2.3 times lower than in *P. nathusii* and 1.7 lower than in *V. murinus* ($p < 0.05$). These results provide evidence for significant differences in the contents of free blood plasma amino acids between migratory and resident bat species.

Keywords: bats, amino acids, blood
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Bats (Chiroptera: Vespertilionidae) are a specific and unique group of mammals that, in evolutionary terms, is a connecting link between aquatic and terrestrial–aerial ecosystems. They play a significant role in the ecosystems of entire continents [1–4] and are regarded as an indicator of ecosystem status [5–7]. In the course of evolution, bats have developed high ecological plasticity and strategy for reliable maintenance of homeostasis under extreme conditions. Meanwhile, they remain to be the least studied group of heterothermic vertebrates. The majority of publications on bats are of descriptive or faunistic type [8–12]. Various aspects of their biology and ecology have been investigated [13–17]. To date, the species composition of bats inhabiting the Urals and neighboring areas has been described in detail [18–21]. However, the physiological features of these ecologically flexible vertebrates capable of maintaining homeostasis under extreme conditions have not been studied sufficiently.

The bat fauna of the Urals comprises ecologically contrasting species, resident and migratory. The different directionality of their adaptive strategies is accounted for by the necessity to prepare for long-term hibernation in residents and for seasonal migrations to regions with a different climate in migrants. Considerable interspecific differences may be expected in the adaptation mechanisms of juvenile bats in the period of rapid growth and development. Data are available on modulating properties of free amino acids in structural, carbohydrate, and energy metabolisms [22, 23]. A key role in the development of adaptive changes in metabolic mechanisms of homeostasis in played by multifunctional amino acids [24–27].

The purpose of this study was to analyze the amino acid spectrum of blood plasma in three bat species inhabiting the Urals (*Myotis dasycneme*, *Pipistrellus nathusii*, *Vespertilio murinus*) and, for the first time, compare the concentrations of free AAs in resident and migrant bats.

MATERIAL AND METHODS

Bats were trapped and kept in the laboratory in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes

[28]. The experimental group consisted of young of the year (subadults) of three species: the pond bat (*Myotis dasycneme* Boie, 1825), a resident boreal species abundant and widespread in the Urals; the Nathusius' pipistrelle (*Pipistrellus nathusii* Keys, et Blas, 1839), a migratory mesophilic species widespread in the forest and forest–steppe zones of the Southern Urals and throughout the region; and the particolored bat (*Vespertilio murinus* Linnaeus, 1758), a migratory mesophilic species abundant and widespread in the Urals [19]. Differences between these species concern not only wintering grounds and preparation for hibernation but also foraging habits: [19]: *M. dasycneme* bats hunt over water bodies, 0.2–0.8 m above the surface, at distances of up to 50 m from the shore; *P. nathusii,* within and over tree crowns 10–15 m above the ground, sometimes diving to 1–2 m to catch prey; and *V. murinus,* in open areas along forest edges, clearings, glades, or over water bodies and ascend to altitudes of up to 720 m.

All bats were trapped in the second 10-day period of July (during the breeding season) in 2013 and 2014. Trapping was started at dusk and stopped at dawn. *Myotis dasycneme* bats were trapped at the margin of a birch forest on the shore of Lake Maloye Miassovo, near the village of Urazbaevo (Chelyabinsk oblast); *V. murinus* and *P. nathusii,* on the shore of Lake Bolshoy Kisegach.

Animal body weight was measured with an Acculab PP-200dl1 electronic balance to an accuracy of ±0.1 g. Young (subadult) bats ($n = 34$) were distinguished from adults by the degree of ossification of the carpal and phalangeal epiphyses [29]. Subadults of each species significantly differed from the others in body weight ($Pr(|F_{ran}| > 303.2 = 0.0001$) and liver weight ($Pr(|F_{ran}| > 62.7 = 0.0001$). No significant differences in heart weight were revealed between *M. dasycneme* and *V. murinus* ($p = 0.19$), but both species significantly differed in this parameter from *P. nathusii* ($Pr(|F_{ran}| > 77.9 = 0.0001$) ($p = 0.000$). It should also be noted that the body weight of subadult *P. nathusii* was about half that of *M. dasycneme* and *V. murinus*: 5.7 ± 0.1 vs. 11.8 ± 0.2 and 10.5 ± 0.1 g, respectively.

Qualitative and quantitative analysis of blood plasma amino acids was performed using ion exchange chromatography in an AAA-339M automatic amino acid analyzer (Microtechna, Czech Republic). The animals were sacrificed by decapitation, blood samples (400–800 μL) were collected in sterile Vacutainer tubes with EDTA (BD, England), and processed following the standard procedure [30]. The blood was centrifuged in a K-23D centrifuge at 8000 rpm for 15 min; the supernatant (500 μL) was transferred to a new polyethylene tube, deproteinated by adding 100 μL of 30% sulfosalicylic acid, and the pH of the solution was brought to neutral with 200 μL of 7% LiOH. The sample was then supplemented with 100 μL of norleucine solution (2.5 μmol/L; BIO-LA-TEST kit, Lachema, Czech Republic) as an internal standard, centrifuged again at 10000 for 30 min, and the supernatant (400 μL) was applied onto the column of the amino acid analyzer. The entire spectrum of free amino acids in each sample was represented in the chromatograms. Their concentrations were determined and expressed in μmol/L and as a percentage of the total amino acid content. On the whole, 384 determinations were carried out.

The results were processed statistically with Statistica 6.0 (StatSoft, Inc.). The significance of differences in means between groups in a data set was estimated by computing the bootstrap mean and bootstrap standard error ($\bar{X}_{boot} \pm SE_{boot}$) at a 95% bootstrap confidence interval (95% CI_{boot}) [31]. Differences within the groups were assessed using Tukey's honest significance test (*P* value) and analysis of variance (ANOVA) with Fisher's *F*-test. Nonparametric multivariate analysis of variance (npMANOVA) and principal component analysis (PCA) were performed with vegan and ade4 packages in R 3.1.2 software [32, 33].

RESULTS AND DISCUSSION

The amino acid spectrum of blood plasma in subadult bats of three species consisted of 22 amino acids. Nonparametric MANOVA for variation in the free amino acid pool showed that their total concentration did not differ significantly between males and females (Table 1), but significant differences were revealed between the species (Table 2).

The total concentrations of free plasma amino acids in migratory *P. nathusii* and *V. murinus* exceeded that in resident *M. dasycneme* by factors of 2.9 and 1.8, respectively ($p < 0.05$). An elevated amino acid pool provides adequate substrate support for intensification of metabolism in migratory species. The concentrations of glucogenic amino acids (precursors for the formation of glucose) in *V. murinus* and *P. nathusii* were also higher than in *M. dasycneme*: 1114 ± 119 and 2147 ± 446 vs. 604 ± 70 μmol/L, respectively ($p < 0.05$). These amino acids prevailed over other metabolic groups in the amino acid pool of migratory species, with their proportion reaching 75% in *V. murinus* and 79% in *P. nathusii*.

The relative contents of blood plasma amino acids proved to be species-specific: the proportion of glutamic acid (the main collector of amino groups in amino acid metabolism) and glutamine (its amide derivative) in the total pool was 21% in *V. murinus*, 18% in *P. nathusii*, and 11% in *M. dasycneme*. Proline and citrulline were absent in the amino acid spectra of all three species, and only traces of asparagine, glutamine, cysteine, and tryptophan were detected in *M. dasycneme* (Table 2). The concentration of ornithine, which contributes to growth hormone production in subadults during their active development, did

Table 1. Two-way nonparametric multivariate ANOVA for species-related variation in free amino acid pool in blood plasma of subadult bats

Factors	Degrees of freedom *df*	Sum of squares	Mean squares	*F*-test	R^2	*p*
Species (1)	2	181.86	90.93	12.33	0.67	**0.001***
Sex (2)	1	7.89	7.89	1.07	0.03	0.35
1 × 2	2	9.65	4.82	0.65	0.03	0.73
Error	10	73.75	7.38		0.27	

The *p* values were determined by randomization; R^2 is the conditional coefficient of determination characterizing factor loadings; *differences between species are statistically significant.

not differ significantly between the three species ($p > 0.05$). According to our data, the total concentration of essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine) in the blood plasma of migratory *P. nathusii* in July reached 1313 μmol/L, exceeding that in resident *M. dasycneme* by a factor of 3.8 (Table 2).

Collagen is the main protein of the connective tissue (tendons, bones, cartilage, dermis) that makes it strong and elastic. It accounts for 25–35% of the total protein content in the body, and its composition includes 33% of glycine and 13% of alanine. Elastin contains many hydrophobic amino acids (glycine, alanine, and leucine). Increased concentrations of lysine, glycine, alanine, and glutamic acid are characteristic of all bat species. The total contribution of lysine, glycine, and glutamic acid to the pool of free plasma amino acids in migratory *P. nathusii* and *V. murinus* is 2.3 and 1.7 times greater than in resident *M. dasycneme,* respectively, which suggests that they are in increasing demand for the synthesis of connective tissue components (Table 2). It may well be that increased accumulation of the amino acid triad essential for the synthesis of collagen and elastin is characteristic of all migratory bat species.

A high content of plasma arginine is also characteristic of migratory species: its concentration in *V. murinus* proved to be six times higher than in *M. dasycneme,* and in *P. nathusii* arginine accounts for 25.4% of the amino acid pool (Table 3). Arginine has detoxification and antioxidant properties and stimulates growth hormone release from the pituitary in young animals [34, 35]. This amino acid in involved in the formation of muscle fibers formation and accelerates the healing of wounds and damaged bones and tendons, which probably helps in preparing for long-distance migration to wintering grounds [36, 37].

Using multivariate PCA for revealing interspecific differences in plasma amino acid spectrum, it has been found that the first and second principal component (PC1 and PC2) account for 30.4 and 24% of the total variance in the plasma amino acid pool (Figure 1, Table 3). All observations logically fall into in three groups (1) *M. dasycneme*, (2) *V. murinus*, and (3) *P. nathusii*. Amino acids contributing most to interspecific variation along PC1 are as follows: glutamine, valine, cysteic acid, glycine, isoleucine, methionine, taurine, aspartic acid, threonine, alanine, leucine, serine, and cysteine. With respect to these variables, resident *M. dasycneme* and two migratory species, *V. murinus* and *P. nathusii*, segregated into two groups along PC1. In *M. dasycneme,* amino acids contained in high proportions and strongly (significantly) correlated with PC1 include methionine, isoleucine, leucine, valine, taurine, and alanine; in *V. murinus*, these are cysteic and aspartic acids, threonine, serine, glutamine, glycine, and cysteine. This difference accounts for segregation between *V. murinus* and *M. dasycneme*. The amino acid pool of *P. nathusii* contains high proportions of arginine, tryptophan, and tyrosine. With respect to their contents (primarily of arginine), *P. nathusii* forms an individual group segregated from both *M. dasycneme* and *V. murinus*. Amino acids strongly correlated with PC2 include asparagine (–0.80), tryptophan (0.77), taurine (–0.62), glycine (–0.60), alanine (–0.65), phenylalanine (–0.62), ornithine (–0.53), and tyrosine (0.51). Thus, the results of multivariate PCA allow reliable differentiation of bat species in the space of the first two principal components, i.e., by the spectrum and proportions of blood plasma amino acids.

Thus, a comparative analysis of the amino acid spectrum of blood plasma has been performed for the first time in subadult bats of resident (*M. dasycneme*) and migratory (*P. nathusii* and *V. murinus*) species in the fauna of the Urals. The results show the role of nitrogen metabolism in providing the possibility of adaptive metabolic rearrangements related to active growth and organ formation during the important period of postnatal development. Significant interspecific differences have been revealed in the qualitative composition of free blood plasma amino acids in resident and migratory bats in summer. The pool of free plasma amino acids in *M. dasycneme* is significantly lower than in migratory species ($p < 0.05$). The level of accumulation of glucogenic amino acids in migratory *V. murinus* and *P. nathusii* is increased. This is a manifestation of metabolic strategy aimed at preparing the

Table 2. Free amino acid spectrum in bat blood plasma, μmol/L

Amino acid (AA)	I. *V. murinus* ($n = 8$)	II. *P. nathusii* ($n = 5$)	III. *M. dasycneme* ($n = 3$)	ANOVA with *Tukey's* test		
	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	I–II	I–III	II–III
Cysteic acid	36.0 ± 7.1	54.2 ± 12.5	6.3 ± 1.4*▲	$Pr(\lvert F_{ran}\rvert \geq 3.93) = 0.05$		
	[23.7–50.9]	[27.7–77.2]	[3.4–9.3]	0.29	0.04	0.04
Taurine	75.5 ± 11.7	82.4 ± 17.9	109.2 ± 6.0	$Pr(\lvert F_{ran}\rvert \geq 0.96) = 0.41$		
	[53.8–99.4]	[42.2–110.3]	[96.4–122]	0.76	0.15	0.43
Aspartic acid	53.9 ± 9.4	58.2 ± 15.3	16.3 ± 2.5	$Pr(\lvert F_{ran}\rvert \geq 2.16) = 0.15$		
	[38.9–74.6]	[28.2–87.9]	[10.9–21.7]	0.82	0.06	0.13
Threonine	103.2 ± 14.2	130.8 ± 27.2	49.5 ± 7.8	$Pr(\lvert F_{ran}\rvert \geq 2.47) = 0.13$		
	[75.9–131.5]	[76.7–182.5]	[33.0–65.9]	0.39	0.08	0.07
Serine	92.4 ± 18.9	138.6 ± 33.5	32.3 ± 5.1	$Pr(\lvert F_{ran}\rvert \geq 2.72) = 0.1$		
	[60.0–131.8]	[73.8–203.2]	[21.5–43.0]	0.25	0.11	0.09
Asparagine	8.1 ± 1.1	Traces*	4.3 ± 0.7▲	$Pr(\lvert F_{ran}\rvert \geq 14.95) = 0.001$		
	[5.8–10.3]		[2.9–5.7]	0.000	0.13	0.000
Glutamic acid	181.8 ± 25.5	315.6 ± 74.2	143.9 ± 28.7	$Pr(\lvert F_{ran}\rvert \geq 2.62) = 0.1$		
	[133.9–232.4]	[178.4–453.1]	[83.5–205.2]	0.09	0.46	0.21
Glutamine	133.3 ± 18.1	168.9 ± 39.9	Traces*▲	$Pr(\lvert F_{ran}\rvert \geq 6.06) = 0.01$		
	[99.4–170.0]	[90.5–247.6]		0.41	0.001	0.000
Glycine	259.2 ± 32.3	289.5 ± 62.5	112.4 ± 11.8	$Pr(\lvert F_{ran}\rvert \geq 2.44) = 0.13$		
	[191.8–317.6]	[165.9–405.4]	[87.0–137.5]	0.57	0.06	0.07
Alanine	134.6 ± 14.6	201.8 ± 41.3	131.5 ± 7.8	$Pr(\lvert F_{ran}\rvert \geq 1.84) = 0.2$		
	[107.2–163.9]	[114.3–278.3]	[114.7–148.1]	0.12	0.91	0.3
Valine	25.4 ± 3.8	93.8 ± 29.6*	71.2 ± 4.2*	$Pr(\lvert F_{ran}\rvert \geq 4.44) = 0.03$		
	[18.0–32.7]	[36.8–151.4]	[62.2–80.2]	0.02	0.01	0.65
Cysteine	9.8 ± 1.4	Traces*	Traces*	$Pr(\lvert F_{ran}\rvert \geq 18.91) = 0.001$		
	[7.2–12.9]			0.000	0.000	0.99
Methionine	11.7 ± 1.7	33.0 ± 13.7	15.5 ± 0.4	$Pr(\lvert F_{ran}\rvert \geq 1.9) = 0.12$		
	[8.2–15.0]	[7.1–60.2]	[13.5–17.4]	0.1	0.3	0.47
Isoleucine	19.5 ± 2.6	50.3 ± 11.0*	23.1 ± 0.5	$Pr(\lvert F_{ran}\rvert \geq 5.7) = 0.02$		
	[14.4–24.7]	[28.5–71.9]	[21.9–24.3]	0.01	0.46	0.22
Leucine	44.1 ± 8.8	98.2 ± 26.1	46.4 ± 0.3	$Pr(\lvert F_{ran}\rvert \geq 2.91) = 0.08$		
	[28.6–62.9]	[47.0–149.4]	[45.7–47.2]	0.06	0.88	0.28
Tyrosine	12.6 ± 3.0	38.4 ± 6.9*	12.8 ± 1.2▲	$Pr(\lvert F_{ran}\rvert \geq 8.17) = 0.02$		
	[7.3–18.8]	[25.4–52.6]	[10.4–15.3]	0.01	0.98	0.05
Phenylalanine	59.6 ± 16.3	32.6 ± 6.6	59.8 ± 2.1	$Pr(\lvert F_{ran}\rvert \geq 0.91) = 0.45$		
	[32.0–94.0]	[20.5–44.8]	[55.3–64.3]	0.3	0.99	0.07
Tryptophan	16.6 ± 1.8	50.7 ± 16.1*	Traces*▲	$Pr(\lvert F_{ran}\rvert \geq 5.67) = 0.01$		
	[12.8–19.9]	[26.5–87.7]		0.002	0.000	0.000
Ornithine	35.5 ± 7.5	37.6 ± 10.3	21.6 ± 1.3	$Pr(\lvert F_{ran}\rvert \geq 0.56) = 0.59$		
	[21.5–50.6]	[17.4–57.5]	[18.8–24.3]	0.88	0.31	0.4
Lysine	87.9 ± 8.1	105.0 ± 25.0	51.5 ± 3.8	$Pr(\lvert F_{ran}\rvert \geq 1.9) = 0.16$		
	[60.0–91.0]	[56.0–153.9]	[43.5–59.6]	0.25	0.17	0.23

Table 2. (Contd.)

Amino acid (AA)	I. *V. murinus* ($n = 8$)	II. *P. nathusii* ($n = 5$)	III. *M. dasycneme* ($n = 3$)	ANOVA with *Tukey's* test		
	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]	I–II	I–III	II–III
Histidine	26.7 ± 2.3	30.4 ± 6.3	12.6 ± 0.05*▲	$Pr(\|F_{ran}\| \geq 3.15) = 0.05$		
	[22.8–31.5]	[21.3–44.4]	[12.5–12.7]	0.63	0.01	0.05
Arginine	84.3 ± 11.4	686.2 ± 157.1*	14.4 ± 1.3*▲	$Pr(\|F_{ran}\| \geq 13.7) = 0.004$		
	[61.6–105.9]	[391.5–986.0]	[11.7–17.2]	0.0001	0.001	0.001
AA pool	1488.5 ± 161.7	2701.3 ± 555.4*	934.7 ± 67.7*▲	$Pr(\|F_{ran}\| \geq 4.83) = 0.02$		
	[1206.8–1834.3]	[1614.7–3781.7]	[790.4–1079.0]	0.05	0.05	0.04

Statistically significant differences ($p < 0.05$) between I and II or I and III are indicated by an asterisk; between II and III, by a black triangle.

Table 3. Results of principal component analysis of blood plasma free amino acids in subadult bats of three species: coefficients of correlation between 22 amino acids and principal components PC1 and PC2 (the Ade4 R package)

AA, μmol/L ($i = 22$)	Loadings, a_{ij}			Contribution to PC = $(a_{ij}^2 \times 100)/\lambda_j$, %		
	Principal components, $j = 1, 2, 3$					
	1	2	3	1	2	3
Cysteic acid	**–0.75*****	0.13	0.25	**8.35*****	0.31	2.14
Taurine	**0.64*****	**–0.62****	0.08	**6.21*****	**7.28****	0.23
Aspartic acid	**–0.64*****	–0.35	0.02	**6.09*****	2.29	0.01
Threonine	**–0.6****	**–0.45***	0.26	**5.34****	**3.87***	2.28
Serine	**–0.56****	–0.07	**0.71*****	**4.69****	0.09	**17.25*****
Asparagine	–0.16	**–0.8*****	–0.33	0.39	**12.27*****	3.69
Glutamic acid	0.3	–0.19	0.15	1.34	0.69	0.71
Glutamine	**–0.92*****	0.02	–0.11	**12.56*****	0.00	0.4
Glycine	**–0.69*****	**–0.6****	–0.07	**7.18*****	**6.76****	0.17
Alanine	**0.61****	**–0.65*****	–0.08	**5.56****	**8.05*****	0.22
Valine	**0.85*****	–0.2	0.1	**10.68*****	0.78	0.33
Cysteine	**–0.52****	**–0.46***	**–0.55****	**4.1****	**4.07***	**10****
Methionine	**0.68*****	0.05	–0.37	**6.85*****	0.05	4.67
Isoleucine	**0.69*****	0.01	0.32	**7.12*****	0.00	3.48
Leucine	**0.65*****	–0.17	0.07	**6.32*****	0.55	0.17
Tyrosine	**0.41***	**0.51***	–0.2	**2.5***	**4.99***	1.28
Phenylalanine	0.24	**–0.62****	0.26	0.89	**7.37****	2.31
Tryptophan	–0.32	**0.77*****	–0.34	1.56	**11.17*****	3.95
Ornithine	–0.07	**–0.53****	**0.58****	0.08	**5.43****	**11.27****
Lysine	0.17	**–0.49***	**–0.81*****	0.45	**4.6***	**22.24*****
Histidine	–0.34	–0.34	**–0.61****	1.73	2.22	**12.68****
Arginine	0.001	**0.95*****	–0.13	0.00	**17.18*****	0.53
	PC eigenvalues, λ_j			Variance explained by PC, %		
	6.69	5.27	2.98	30.41	23.96	13.54

Asterisks indicate significance level: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; boldface indicates that the contribution of an amino acid to the principal components exceeds the average contribution defined as unity divided by the number of variables.

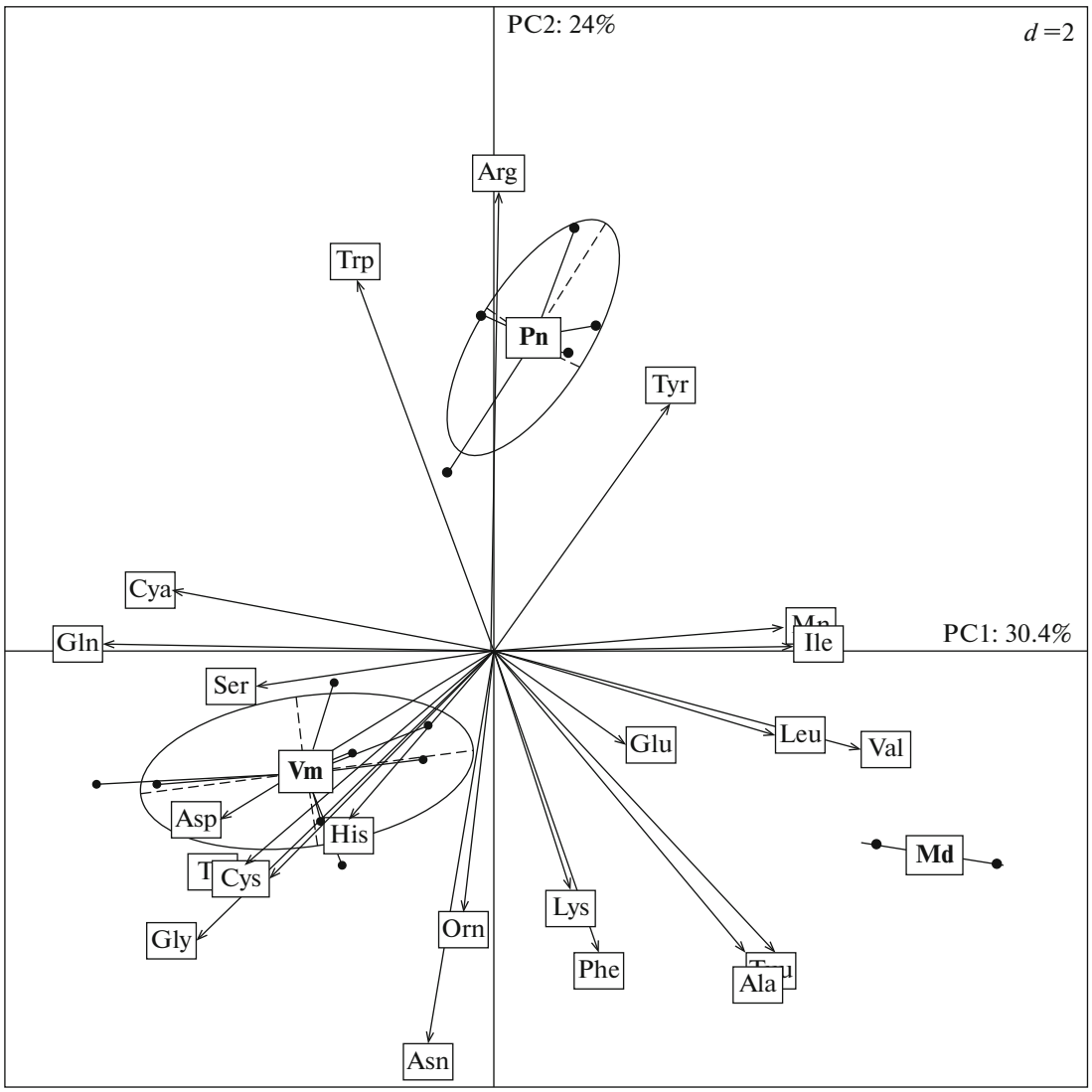

Fig. 1. Principal component analysis of blood plasma amino acid spectrum in three bat species (contributions to principal components, percentages of the total pool): *Vm*, *Vespertilio murinus*; *Md*, *Myotis dasycneme*; *Pn*, *Pipistrellus nathusii*. Arrows show correlations of PC1 and PC2 with initial parameters (amino acids); ellipses delimit 95% confidence regions.

bats for long-distance flight to wintering grounds, which may be characteristic of all migratory bat species.

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REFERENCES

1. Wilkinson, G.S. and South, J.M., Life history, ecology and longevity in bats, *Aging Cell,* 2002, no. 1, pp. 124–131.
2. Hutterer, R., Ivanova, T., Meyer-Cords, C., and Rodrigues, L., *Bat Migrations in Europe: A Review of Banding Data and Literature,* Bonn: German Agency for Nature Conservation, 2005.
3. Kunz, T.H., Braun, De., Torrez, E., Bauer, D., et al., Ecosystem services provided by bats, Ann, N.Y. Acad. Sci., 2011, vol. 1223, pp. 1–38. doi 10.1111/j.1749-6632.2011.06004.x
4. *Bats in the Anthropocene: Conservation of bats in a Changing World,* Voigt, C.C. and Kingston, T., Eds., Springer Int. Publ., 2016. http://link.springer.com/book/10.10007/978-3-319-25220-9.
5. Mickleburgh, S., Waylen, K., and Racey, P., Bats as bushmeat: A global review, *Oryx,* 2009, vol. 43, pp. 217–234.
6. Boye, P. and Dietz, M., *Development of Good Practice Guidelines for Woodland Management for Bats,* English

Nature Research Reports, no. 661, The Bat Conservation Trust, September 2015.

7. Zukal, J., Pikula, J., and Bandouchova, H., Bats as bioindicators of heavy metal pollution: History and prospect, *Mamm. Biol.–Z. Säugetierkd.,* 2015, vol. 80, pp. 220–227.
8. Il'in, V.Yu., Bats (Chiroptera: Vespertilionidae) on the southeast of the Russian Plain, *Extended Abstract of Doctoral (Biol.) Dissertation,* Penza, 1999.
9. Barclay, R.M.R., Ulner, J., Mackenzie, C.J.A., et al., Variation in the reproductive rate of bats, *Can. J. Zool.,* 2004, vol. 82, pp. 688–693.
10. Brunet-Rossinni, A.K. and Austad, S.N., Aging studies on bats: A review, *Biogerontology,* 2004, vol. 5, pp. 211–222.
11. Dietz, C., Helversen, O., and Nill, D., *Bats of Britain, Europe and Northwest Africa,* London: A & C Black, 2009.
12. Kruskop, S.V., New data on the bat fauna of Con Dao Islands, *Russ. J. Theriol.,* 2011, vol. 10, no. 2, pp. 37–46.
13. Ransome, R.D. and McOwat, T.P., Birth timing and population changes in greater horseshoe bat colonies (*Rhinolophus ferrumequinum*) are synchronized by climatic temperature, *Zool. J. Linn. Soc.,* 1994, vol. 112, pp. 337–351.
14. Wojciechowski, M.S., Jefimow, M., and Tegowska, E., Environmental conditions, rather than season, determine torpor use and temperature selection in large mouse-eared bats (*Myotis myotis*), *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.,* 2007, vol. 147, pp. 828–840.
15. Dietz, M. and Org, A., Thermoregulation of tree dwelling temperate bats: A behavioural adaptation to force life history strategy, *Folia. Zool.,* 2011, vol. 60, pp. 5–11.
16. Mishchenko, V.A., Koval'chuk, L.A., Snit'ko, V.P., et al., The hematopoietic system of the pond bat, *Myotis dasycneme* (Boie, 1825), living in the Urals, *Plecotus et al.,* 2014, no. 17, pp. 18–29.
17. Kovalchuk, L., Mishenko, V., Chernaya, L., et al., Haematological parameters of pond bats (*Myotis dasycneme* Boie, 1825; Chiroptera: Vespertilionidae) in the Ural Mountains, *Zool. Ecol.,* 2017, vol. 27, no. 2, pp. 168–175. doi 10.1080/21658005.2017.1305153.
18. Snit'ko, V.P., The fauna of bats (Mammalia, Chiroptera) in the Southern Urals, *Extended Abstract of Cand. Sci. (Biol.) Dissertation,* Yekaterinburg, 2004.
19. Bol'shakov, V.N., Orlov, O.L., and Snit'ko, V.P., *Letuchie myshi Urala* (Bats of the Urals), Yekaterinburg: Akademkniga, 2005.
20. Pervushina, E.M., Spatial and biotopic distribution of bats (Chiroptera, Vespertilionidae) in the southern forest zone of the Middle Urals, *Russ. J. Ecol.,* 2010, vol. 41, no. 2, pp. 183–185.
21. Orlova, M., Orlov, O., Zhigalin, A., and Mishenko, V., Comparative analysis of vespertilionid bats (Chiroptera: Vespertilionidae) infestation with gamasid mites of the genus *Macronyssus* Kolenati, 1858 during hibernation in the Urals and Western Siberia, *Zool. Ecol.,* 2015, vol. 25, no. 4, pp. 1–5.
22. Meister, A., *Biochemistry of the Amino Acids,* New York: Academic, 1957.
23. Garaeva, S.N., Redkozubova, G.V., and Postolati, G.V., *Aminokisloty v zhivom organizme* (Amino Acids in the Living Organism), Chisinau: Akad. Nauk Moldovy, 2009.
24. Zapadnyuk, V.I., Kuprash, L.P., Zaika, M.U., and Bezverkhaya, I.S., *Aminokisloty v meditsine* (Amino Acids in Medicine), Kiev: Zdorov'ya, 1982.
25. Koval'chuk, L.A., *Ekologo-fiziologicheskie aspekty adaptatsii k usloviyam tekhnogennykh ekosistem* (Ecophysiological Aspects of Adaptation to the Conditions of Technogenic Ecosystems), Yekaterinburg: Ural. Otd. Ross. Akad. Nauk, 2008.
26. Chernaya, L.V., Kovalchuk, L.A., and Nokhrina, E.S., Seasonal variability of free amino acids in tissues of the medicinal leech (*Hirudo verbana* Carena, 1820), *Russ. J. Ecol.,* 2015, vol. 46, no. 4, pp. 385–387.
27. Chernaya, L.V., Kovalchuk, L.A., and Nokhrina, E.S., Role of the tissue free amino acids in adaptation of medicinal leeches *Hirudo medicinalis* L., 1758 to extreme climatic conditions, *Dokl. Biol. Sci.,* 2016, vol. 466, no. 1, pp. 42–44. doi 10.1134/S0012496616010129
28. *European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes,* Strasbourg, March 18, 1986. http://conventions.coe.int/treaty/commun/quevoulezvous.
29. Strelkov, P.P., Sex ratio during the breeding season in adult individuals of migratory bat species (Chiroptera, Vespertilionidae) in Eastern Europe and neighboring territories, *Zool. Zh.,* 1999, vol. 78, no. 12, pp. 1441–1454.
30. Williams, A.P., General problems associated with the analysis of amino acids by automated ion-exchange chromatography, *J. Chromatogr.,* 1986, vol. 373, no. 2, pp. 175–190.
31. Shitikov, V.K. and Rozenberg, G.S., *Randomizatsiya i butstrep: statisticheskii analiz v biologii i ekologii s ispol'zovaniem R* (Randomization and Bootstrap: Using R for Statistical Analysis in Biology and Ecology), Tolyatti: Kassandra, 2014.
32. Anderson, M.J., A new method for non-parametric multivariate analysis of variance, *Austral. Ecol.*, 2001, vol. 26, pp. 32–46.
33. Chessel, D., Dufour, A.B., and Thioulouse, J., The ade4 package-I: One-table methods, *R News,* 2004, no. 4, pp. 5–10.
34. Wu, G. and Morris, S.M., Arginine metabolism in mammals, in *Metabolic and Therapeutic Aspects of Amino Acids in Clinical Nutrition,* Cynober, L.A., Ed., Boca Raton, FL: CRC Press, 2004, pp. 153–167.
35. Tong, B.C. and Barbul, A., Cellular and physiological effects of arginine, *Mini Rev. Med. Chem.,* 2004, vol. 4, no. 8, pp. 823–832.
36. Witte, M.B. and Barbul, A., Arginine physiology and its implication for wound healing, *Wound Rep. Reg.,* 2003, vol. 11, pp. 419–423.
37. Wu, G., Jaeger, L.A., Bazer, F.V., and Rhoads, J.M., Arginine deficiency in preterm infants: Biochemical mechanisms and nutritional implications, *J. Nutr. Biochem.,* 2004, vol. 15, no. 8, pp. 442–451.

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