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Functional Activity of the Blood System in Two Migratory Bat Species of the Urals

L. A. Kovalchuk[a,*], V. A. Mishchenko[a,b], L. V. Chernaya[a], V. P. Snit'ko[c], and Academician V. N. Bolshakov[a]

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Abstract—Functional activity of the blood system was studied in two migratory Ural species, *Vespertilio murinus* Linnaeus, 1758 and *Pipistrellus nathusii* Keyserling et Blasius, 1839. A multivariate nonparametric ANOVA of red blood parameters showed significant interspecific differences ($p < 0.05$) between the migrating bats and the resident species pond bat. A certain genetically determined multidirectionality in the mobilization of emergency regulation mechanisms of the lymphoid blood system was observed in bats.

Keywords: bats, migratory species, peripheral blood, granulocytes

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INTRODUCTION

A total of 13 bat species inhabit the Urals. Nine of them spend the winter in summer habitats, while four migrate over great distances [1, 2]. The two-colored bat *Vespertilio murinus* Linnaeus, 1758 and Nathusius' pipistrelle *Pipistrellus nathusii* Keyserling et Blasius, 1839 have been studied most comprehensively among the migratory species [3]. Although migration directions, migration distances, and wintering sites of bats have not exactly been determined to date, bats are capable of traveling substantial distances [4, 5]. Numerous natural factors are encountered by migratory bats, the set including climatic changes [6, 7] and loss or fragmentation of habitats under anthropogenic pressure [8, 9]. This circumstance greatly affects their euribiotic status and survival [10–12]. Given that an optimal regulation of ecological and physiological processes is necessary for stable adaptation, it is of interest to comparatively study the blood system in migratory and resident species bats. Hematological parameters have previously been studied in the resident species pond bat *Myotis dasycneme* Boie, 1825 of the Ural fauna [13]. In this work, functional activity of the blood system was studied in migratory bats of the Urals.

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia*

[b] *Virom Federal Institute of Viral Infections, Federal Service for the Oversight of Consumer Protection and Welfare, Yekaterinburg, Russia*

[c] *South Ural Federal Research Center of Mineralogy and Environmental Geology, Ural Branch, Russian Academy of Sciences, Ilmen Nature Reserve, Miass, Russia*

**e-mail: kovalchuk@ipae.uran.ru*

MATERIALS AND METHODS

Our sample included bats from this underyearlings (*subadultus*) of two migratory species, Nathusius' pipistrelle *P. nathusii* and the two-colored bat *V. murinus*. The former is widespread in broadleaved and mixed woodlands with water bodies, while *V. murinus* tends to open spaces of the Ural forest–steppe zone and residential areas [1]. In summer, the species are associated with intrazonal biotopes (flood plain areas and banks of water bodies), which provide main foraging stations and accommodate breeding sites. Both of the species usually roost in natural refugia over daytime and often prefer shallow shelters (hollows of tree trunks, cavities, and human household buildings). Both species are capable of long-distance migrations and homing [14]. Ural migratory bats start returning from their wintering sites in the first decade of May [1, 2]. In early June, *P. nathusii* and *V. murinus* become abundant in their breeding sites. Cubs are born from the start to the end of the second decade of June; young bats leave their shelters from the end of the second decade to the middle third decade of July; and both of the species start migrating to their wintering sites from late August to early September [2].

Bats were captured and kept in the lab in compliance with guidelines of the Declaration of Helsinki on the protection of animals used for experimental and scientific purposes [15]. A total of 34 bats were captured for the study with mist nets on the banks of the Bol'shoi Kisegach Lake and near the Maloe Miassovo Lake (Chelyabinsk Oblast) in the second decade of July (a breeding season) from 2013 to 2015. The *V. murinus* population size is sufficiently high in the Ilmen Nature Reserve; the relative abundances of *V. murinus* and *P. nathusii* are 21.1 and 7.4%, respectively [2]. Comparisons were performed with *M. dasycneme*,

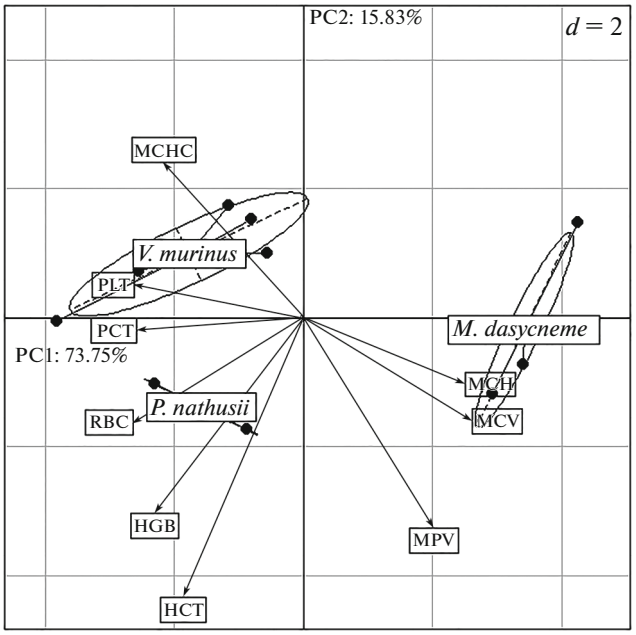

Fig. 1. Red blood cell parameters of bats in the space of two principal components, PC1 and PC2. Percent variance accounted for by a principal component is indicated. Arrows show the correlations between the principal components and the initial parameters. Ellipses show the 95% confidence areas.

which remains in the Urals for the winter and has a relative abundance of 45.3% in the Ilmen Nature Reserve.

Peripheral blood samples were collected in sterile BD Vacutainer vacuum tubes with EDTA (United Kingdom). The samples (400–800 μL) were tested using a BC-5800 hematology analyzer (Mindray, China). A white blood cell differential count (per 100 leukocytes) was performed using blood smears stained according to the Romanowsky–Giemsa procedure. A granulocyte-to-agranulocyte count ratio (rel. units) was calculated from the differential count and used as an integral leukocyte index (ILI) that reflects the physiological state and adaptive potential of an individual. The results were analyzed using the software package Statistica for Windows v. 10.0. Principal component analysis (PCA) was carried out in the R statistical environment (R 3.1.2, the Ade4 package) [16].

RESULTS AND DISCUSSION

Peripheral blood testing showed no significant interspecific difference in red blood cell count (p = 0.36), hemoglobin content (p = 0.12), platelet count (p = 0.60), and thrombocrit (p = 0.89) between the two migratory species, suggesting identical adaptation mechanisms for *P. nathusii* and *V. murinus*. High values of hemoglobin content, hematocrit, and red blood cell count were characteristic of bats, indicating that a high intensity of the respiratory function is characteristic of flying animals [14]. *Myotis dasycneme* significantly differed in certain red blood cell parameters. In comparison with *P. nathusii*, differences were observed in red blood cell count (RBC = 10.0 ± 0.03 T/L, p = 0.03), hemoglobin content (Hb = 148.9 ± 0.93 g/L, p = 0.03), hematocrit (HCT = 41.5 ± 0.05%, p = 0.03), mean corpuscular hemoglobin concentration (MCHC = 372.2 ± 1.8 g/L, p = 0.02), and mean corpuscular volume (MCV = 41.4 ± 0.07 fL, p = 0.03). In comparison with *V. murinus*, differences were observed in red blood cell count (RBC = 9.2 ± 0.6 T/L, p = 0.01), mean corpuscular volume (MCV = 36.8 ± 0.07 fL, p = 0.01), and mean corpuscular hemoglobin concentration (MCHC = 379.6 ± 7.3 g/L, p = 0.02) [18].

Figure 1 displays the three separate bat groups: the migratory species *V. murinus* and *P. nathusii* and the resident species *M. dasycneme*. Principal component analysis (PCA) showed that the first principal component (PC1) accounted for 73.75% of the total variance of red blood parameters and the second principal component (PC2), for 15.83%. In the case of PC1, the greatest contributions to the interspecific variation in red blood parameters were observed for red blood cell count (13.66%), mean corpuscular volume (13.22%), platelets count (13.42%), thrombocrit (13.07%), and mean hemoglobin content (12.27%).

The parameters showed significant coefficients of correlation with PC1: –0.95, 0.94, –0.94, –0.93, and 0.90, respectively (p = 0.001). PC1 values distinctly separate *M. dasycneme* from both of the migratory species. PC2 correlated strongly (–0.67) with hematocrit (contribution 36.86%) and to a lesser extent with hemoglobin concentration (contribution 18.01%), supporting the heterogeneity of individuals from the ecologically contrasting species.

Twice higher platelet counts were observed in the migratory species *P. nathusii* (PLT = 446.7 G/L) and *V. murinus* (PLT = 476.3 G/L) compared with *M. dasycneme* (PLT = 213.1 G/L), indicating that a greater volume is occupied in the whole blood by platelets, which act as effectors of the immune system. Platelets play a leading role in blood clotting and are involved in immune and allergic responses along with macrophages, neutrophils, and eosinophils [19]. When a blood vessel is damaged, platelets are the first to appear at the site of damage and act as an innate immunity factor. White blood cells of the migratory bat species included two cell groups, granulocytes (early neutrophils, band neutrophils, and segments neutrophils and eosinophils), which determine the innate immune responses, and agranulocytes (monocytes and lymphocytes), which are responsible for adaptive immune responses (Table 1).

A higher neutrophil count was characteristic of the migratory species (Table 1), being probably related to nonspecific defense against toxins and virus and bacterial infections. The heterophil content was significantly increased due to an increase in mature seg-

Table 1. Leukocyte composition of the peripheral blood in bats

Parameter	I. *M. dasycneme*	II. *V. murinus*	III. *P. nathusii*
	$\overline{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]		
Neutrophils, %	39.78 ± 1.42 [37.00–42.50]	54.02 ± 3.30* [47.20–60.20]	66.00 ± 0.47*▲ [65.00–67.00]
• early, %	2.49 ± 1.14 [0.50–4.75]	8.01 ± 1.29* [5.20–10.40]	7.52 ± 2.10 [3.00–12.00]
• band, %	25.99 ± 3.19 [18.75–30.00]	24.63 ± 3.02 [18.80–30.60]	38.00 ± 1.88*▲ [34.00–42.00]
• segments, %	11.19 ± 2.89 [6.00–16.75]	21.37 ± 2.46* [17.60–27.00]	20.50 ± 0.24* [20.00–21.00]
Lymphocytes, %	56.02 ± 1.83 [6.00–16.75]	43.41 ± 3.05* [37.60–49.20]	32.00 ± 0.01*▲ [31.05–32.08]
Monocytes, %	2.50 ± 0.76 [0.75–3.75]	1.59 ± 0.78 [0.40–3.40]	1.00 ± 0.00 [1.00–1.01]
Eosinophils, %	1.25 ± 0.42 [0.50–2.00]	1.00 ± 0.28 [0.40–1.60]	1.00 ± 0.47 [0.10–2.00]
Granulocytes, %	41.01 ± 1.80 [37.50–44.50]	54.90 ± 3.40* [48.00–61.40]	67.01 ± 0.90*▲ [65.12–69.03]
Agranulocytes, %	58.50 ± 2.10 [54.50–62.50]	44.90 ± 3.50* [38.60–52.01]	33.01 ± 0.01*▲ [32.09–33.01]
ILI, rel. units	0.8	1.2	2.0

Differences (*) between I and II, I and III, or (▲) II and III were significant at ($p < 0.05$); $\overline{X}_{boot} \pm SE_{boot}$, mean ± standard error of the mean of the bootstrap distribution; [95% CI_{boot}], confidence interval of the bootstrap distribution.

mented forms in both *V. murinus* ($p < 0.05$) and *P. nathusii* ($p < 0.05$); this ensured both suppression of inflammatory responses and active nonspecific defense against pathogens (Table 1).

The identity of adaptive mechanisms in the two migratory species is supported by the fact that no significant difference in differential count was observed with respect to eosinophils ($p = 0.99$) and monocytes ($p = 0.99$), which produce proinflammatory cytokines serving as endogenous regulators of hematopoiesis and cell-mediated immune responses [20].

Many diverse functions are performed by cytokines, the set including the regulation of innate and adaptive immunity, activation of inflammatory responses, and stimulation of hematopoiesis [20]. A high granulocyte proportion is characteristic of the white blood cell composition in the migratory species: 55% in *V. murinus* and 67% in *P. nathusii*. Granulocytes form the first line of defense against infections and toxins and ensure the survival of bats outside of their native range (Table 1). High ILI values observed in *V. murinus* (ILI = 1.2) and *P. nathusii* (ILI = 2.0) compared with *M. dasycneme* (ILI = 0.8) confirm the higher reactivity of the natural innate immunity system. The system facilitates the adaptive responses to ensure the euribiotic status of *V. murinus* and *P. nathusii*, which migrate over great distances.

Our study of the functional activity of the blood system indicate that *V. murinus* and *P. nathusii* can be used to evaluate their adaptive potential, which facilitates long-distance seasonal migrations, and provide a basis for monitoring the species abundance and population sizes in bats in order to preserve biodiversity and to rationally use animal resources.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animals were captured and kept in the lab in compliance with guidelines of the Declaration of Helsinki on the protection of animals used for experimental and scientific purposes. Animal studies were approved by the Ethics Com-

mittee at the Institute of Plant and Animal Ecology (protocol no. 11 dated April 29, 2022).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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