

A discriminating ability of haematological indicators: a comparative blood test of sympatric rodent species (*Myodes glareolus*, *Myodes rutilus* and *Craseomys rufocanus*)

Natal'ya A. Orekhova*, Yulia A. Davydova & Georgii Yu. Smirnov

ABSTRACT. Among representatives of the subfamily Microtinae (=Arvicolinae) — *Myodes glareolus*, *Myodes rutilus* and *Craseomys rufocanus* — an interspecies comparison was performed on haematological parameters characterising respiratory capacity of the blood, components of (non)specific immune defence and components of cellular haemostasis. *C. rufocanus* differs most strongly from the other two species by demonstrating higher counts of neutrophils, lymphocytes and platelets in peripheral blood and an increased number of “smaller” erythrocytes with high density of haemoglobin within the cell. The results are discussed in conjunction with the information available in the literature on basal metabolic rate of the species, their sociability and eurybionism, features of thermoregulation and phylogenetic relationships. The authors come to the conclusion that correct interspecific comparisons are possible only within the same reproductive-age groups (either immature under-yearlings or breeding voles that overwintered).

How to cite this article: Orekhova N.A., Davydova Y.A., Smirnov G.Y. 2022. A discriminating ability of haematological indicators: a comparative blood test of sympatric rodent species (*Myodes glareolus*, *Myodes rutilus* and *Craseomys rufocanus*) // Russian J. Theriol. Vol.21. No.1. P.24–37. doi: 10.15298/rusjtheriol.21.1.03

KEY WORDS: erythrocyte, leukocyte, and platelet indices, Microtinae.

Natal'ya A. Orekhova [naorekhova@mail.ru], Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, 8 Marta street 202, Ekaterinburg 620144, Russia; Yulia A. Davydova [davydova@ipae.uran.ru], Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, 8 Marta street 202, Ekaterinburg 620144, Russia; Georgii Yu. Smirnov [smirnovgy@gmail.com], Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, 8 Marta street 202, Ekaterinburg 620144, Russia.

Дифференцирующая способность гематологических показателей: сравнительный анализ крови симпатрических видов грызунов (*Myodes glareolus*, *Myodes rutilus* и *Craseomys rufocanus*)

Н.А. Орехова*, Ю.А. Давыдова, Г.Ю. Смирнов

РЕЗЮМЕ. Среди представителей подсемейства полеvoчки (Arvicolinae или Microtinae), *Myodes glareolus*, *Myodes rutilus*, *Craseomys rufocanus*, проведено межвидовое сравнение гематологических показателей, характеризующих дыхательную способность красной крови, компоненты (не)специфической иммунной защиты, а также клеточные элементы гемостаза. Красно-серая полевка (*C. rufocanus*) сильнее отличается от двух других видов, демонстрируя высокие уровни нейтрофилов, лимфоцитов и тромбоцитов в периферической крови, а также повышенное количество «более мелких» эритроцитов с высокой плотностью распределение гемоглобина в клетке. Полученные данные обсуждаются совместно с имеющейся в литературе информацией об уровне основного обмена у видов, степени их социальности и эврибионизма, а также особенностях терморегуляции и филогенетических взаимоотношений. Авторы приходят к выводу, что корректные межвидовые сравнения возможны только в рамках одних и тех же репродуктивно-возрастных групп (неполовозрелые сеголетки и участвующие в размножении перезимовавшие животные).

КЛЮЧЕВЫЕ СЛОВА: эритроцитарные, лейкоцитарные и тромбоцитарные индексы, Microtinae.

* Corresponding author

Introduction

The nature of species identification in terms of various sets of characteristics (morphophysiological, cytogenetic, biochemical and ethological) reflects the ways and mechanisms of evolutionary transformation of species under the influence of a continuously changing combination of environmental factors. Of particular interest is the assessment of the discriminating ability of haematological indicators. As a functional system, blood ensures timely delivery of oxygen and nutrients to cells and tissues and removes metabolic waste from organs and interstitial spaces. The blood occupies a special place in the emergence of a cooperative exchange of substances by forming communication channels through the synthesis and transport of intercellular-communication molecules, such as cytokines, autocoids, leukotrienes and prostaglandins (Jain, 1986; Hoffman *et al.*, 1999).

Experimental data and theoretical concepts (Morrison & Tietz, 1957; Morrison, 1964; Kovalchuk & Tsvirenko, 1997; Kizhina *et al.*, 2020) indicate that as a result of a historically long process of differentiation of species in each region of a geographic range, a species-specific phenotype forms; it is closely related to the original ecological and climatic characteristics of the habitat. Therefore, there is a connection of the adaptive capacity of the blood with geographical characteristics (Kovalchuk & Yastrebov 2003; Bottaeva *et al.*, 2019), seasonal variability (Wiger, 1979; Wolk, 1981; Tarakhtii *et al.*, 2009), population dynamics (Lazutkin *et al.*, 2016), technogenic pollution (Topashka-Ancheva *et al.*, 2003; Tarakhtii & Mukhacheva, 2011; Tête *et al.*, 2015; Orekhova, 2018), hypoxia (due to burrowing, high altitudes or diving) and ambient temperature (Frase, 2002; Thomas & Ono, 2015; Kusumoto, 2015).

According to these literature data, within the order Rodentia, species specificity of haematological indicators is difficult to analyse due to the heterogeneity of the compared samples in terms of many exogenous factors (habitat, year, season of capture and others) and endogenous parameters (sex, reproductive status, invasions and infections). Such heterogeneity can mask interspecies differences. We believe that it is necessary to study closely related species with similar ecological and morpho-physiological parameters to identify the range of “purely haematological” interspecific differences. Voles as a taxon can be regarded as “specialists” in maximising biological production, ensuring sustainable existence of the species at upper trophic levels (McNab, 1992). The present study was aimed at comparing three species of voles — *Myodes glareolus* Schreber, 1780, *Myodes rutilus* Pallas, 1779 and *Craseomys rufocanus* Sundevall, 1846 — by peripheral-blood indices. The analysed groups of animals were stratified by capture site, year of capture and reproductive status.

Material and methods

Sites of capture

We used materials obtained in 2020 during the monitoring of small mammals in the Visim State Nature

Biosphere Reserve, which protects an area of southern taiga in the Middle Ural Mountains (Sokolov & Syroechkovsky, 1988). The entire study area belongs to a temperate continental climatic zone. The average annual air temperature is -0.1°C , the absolute minimum is -50°C , the absolute maximum is 37°C , the average temperature of the warmest month (July) is 16.6°C , and the average temperature of the coldest month (January) is -16.8°C . The average annual precipitation is 505 mm (range: 350 to 700 mm). In terms of physical and geographical zoning, the study area is within the southern taiga subzone of the Middle Ural low mountains. The vegetation cover is represented by indigenous southern taiga fir-spruce herb-dwarf shrub forests and their derivatives (birch, aspen, grey alder, dwarf shrub, green moss and grasses). The site of capture of the voles (57.37°N , 59.77°E , 538 m above sea level) is located in the highest southeastern part of the reserve and is characterised by low-mountain relief with elevation differences of 250–300 m and a maximum altitude of 699 m (Bolshoi Sutuk mountain).

The animals

We examined representatives of three species of rodents of the subfamily Arvicolinae: the bank vole (*M. glareolus*), the red-backed vole (*M. rutilus*) and the grey red-backed vole (*C. rufocanus*). Taxonomic references are presented according to Wilson & Reeder (2005). For the grey red-backed vole, the term *Myodes rufocanus* has been used previously; however, in the accepted fractional vole system (Abramson & Lisovsky, 2012), the *Craseomys* rank was raised to generic. For more than 2 decades, *M. glareolus* has been dominant in this area, and captures of *C. rufocanus* and *M. rutilus* have been rare (Kshnyasev & Davydova, 2021).

These species are closely associated with forest and forest-steppe zones; they inhabit several mountain systems, rising to the mountains' upper belts, but there are some habitat differences. *M. rutilus* is more typical of indigenous boreal taiga of the lower sub-belt; these voles go far to the north up to the coasts of the Polar Basin, where they are colonising tundra biotopes (Chernyavsky *et al.*, 2003). *M. glareolus* is more typical of mixed forests of the upper sub-belt. This species penetrates tundra and steppe only through floodplain forests of river valleys. It does not occur on bare soil, and an ample ground cover seems necessary (Konig & Claus, 1973). *C. rufocanus* in the Urals is a relatively stenotopic species; its main habitats are rocky placers-curums and forest formations growing on them. This species can inhabit other biotopes only when its population is large (Berdyugin, 1996).

All three species have similar dietary spectra but differ from each other in the proportion of various types of food in their diet. For example, *C. rufocanus* diet is characterised by a predominance of vegetative parts of plants rather than seeds. Seeds of herbaceous and woody plants are dominant in the diets of *M. glareolus* and *M. rutilus* for all seasons. At the same time, *M. rutilus* is considered a plant polyphage: it supplements its diet with lichens and berries (Hansson, 1985).

The animals were caught in the spring–autumn period in 2020 (the year of a population peak) using wooden live traps. Trapped animals were kept in a vivarium for 1–3 days at room temperature under the natural photoperiod to standardise their living conditions. Food and water were available *ad libitum*. The animals were euthanised by decapitation. Trunk blood from each animal was collected immediately into tubes with sodium citrate as an anticoagulant (5.0% [w/v], 0.2 ml citrate/ml blood). Using an Abacus junior vet (Austria) haematology analyser, the following parameters of erythrocytes (red blood cells; RBCs), leucocytes (white blood cells; WBCs) and platelets were measured and calculated:

A) RBCs: haemoglobin concentration (HGB), the RBC count, mean corpuscular haemoglobin (MCH = HGB/RBC count), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCHC = HGB/RBC count × MCV) and red cell distribution width (RDW-SD).

B) WBCs: the total WBC count and absolute and relative numbers of lymphocytes (LYM), monocytes (MID) and granulocytes (GRA).

C) Components of haemostasis: the platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW-SD).

The species affiliation was identified by means of exterior, craniological and odontological features. Stratification into reproductive-age groups (individuals that overwintered as well as immature and sexually mature under-yearlings; only the first two groups of the three were compared in this study) was carried out by analysing the reproductive and dental systems (Olenev, 2002). Sample sizes for the three species of voles (stratified by sex, reproductive-age groups and capture season) are shown in Table 1.

Statistical calculations were performed in STATISTICA version 8.0 and STATGRAPHICS version 8.0 software (StatSoft, 2012). For pairwise comparisons of the analysed groups of animals, the nonparametric Mann-Whitney *U* test and Kruskal-Wallis *H* test were performed; the latter does not require normally distributed data. Multivariate analysis of haematological similarities among the analysed groups of animals was performed by principal component and classification

analysis (PCCA). This technique transforms multivariate data of intercorrelated variables into a new mutually uncorrelated dataset by means of linear variables called principal components (PCs). Thus, PCCA reduces the total dataset's dimensionality and reveals clusters of analysed groups of animals in the PC scale range (Mather, 1987). The PCs were interpreted by means of the module of one-way analysis of variance (ANOVA) with two factors, namely species affiliation and reproductive-age status. Associations between PC values and haematological parameters were evaluated by correlation analysis.

To take into account the possible influence of seasonality and sex, statistical preprocessing of the available data was conducted. No major differences were found between males and females (Appendix 1) and between seasons (Appendix 2, 3).

All procedures performed with animals complied with the ethical standards of the Institute of Plants and Animals of Ecology, Ural Branch of the Russian Academy of Sciences (Protocol No. 3 dated 18/12/2014).

Results

Graphic illustration of interspecies variation of blood parameters, depending on reproductive-age status, is shown in Figures 1–3. The highest RBC count was found in *C. rufocanus*, while *M. glareolus* had the lowest number of RBCs in peripheral blood and lower saturation of these cells with haemoglobin (MCHC). *M. rutilus* exhibited increased MCV and MCH (Fig. 1). Differences in leucocytes manifested themselves primarily in total numbers of lymphocytes and granulocytes (*M. glareolus* < *M. rutilus* < *C. rufocanus*) and monocytes (*C. rufocanus* < *M. rutilus* ≤ *M. glareolus*; Fig. 2). *C. rufocanus* showed the highest total WBC count. Regarding the three indicators of haemostasis (Fig. 3), the grey red-backed vole had the largest number of platelets (*M. rutilus* ≤ *M. glareolus* < *C. rufocanus*). The red-backed vole had fewer platelets and the smallest platelet volume (*M. rutilus* < *M. glareolus* ≈ *C. rufocanus*) and the smallest platelet width distribution (*M. rutilus* < *M. glareolus* < *C. rufocanus*).

Table 1. Sample size in three species of voles (*Myodes glareolus*, *Myodes rutilus*, and *Craseomys rufocanus*) from two reproductive-age groups for hematological analysis (females and males [f/m] was carried out in the spring — autumn period in 2020).

Reproductive-age groups	Catching season	Species		
		<i>Myodes glareolus</i> [f/m]	<i>Myodes rutilus</i> [f/m]	<i>Craseomys rufocanus</i> [f/m]
Immature under-yearlings	Spring	—	—	3 [1/2]
	Summer	34 [15/19]	6 [1/5]	4 [3/1]
	Autumn	49 [16/33]	9 [2/7]	7 [3/4]
Individuals that overwintered	Spring	18 [8/10]	5 [1/4]	9 [5/4]
	Summer	2 [0/2]	2 [1/1]	—
	Autumn	4 [2/2]	—	1 [0/1]

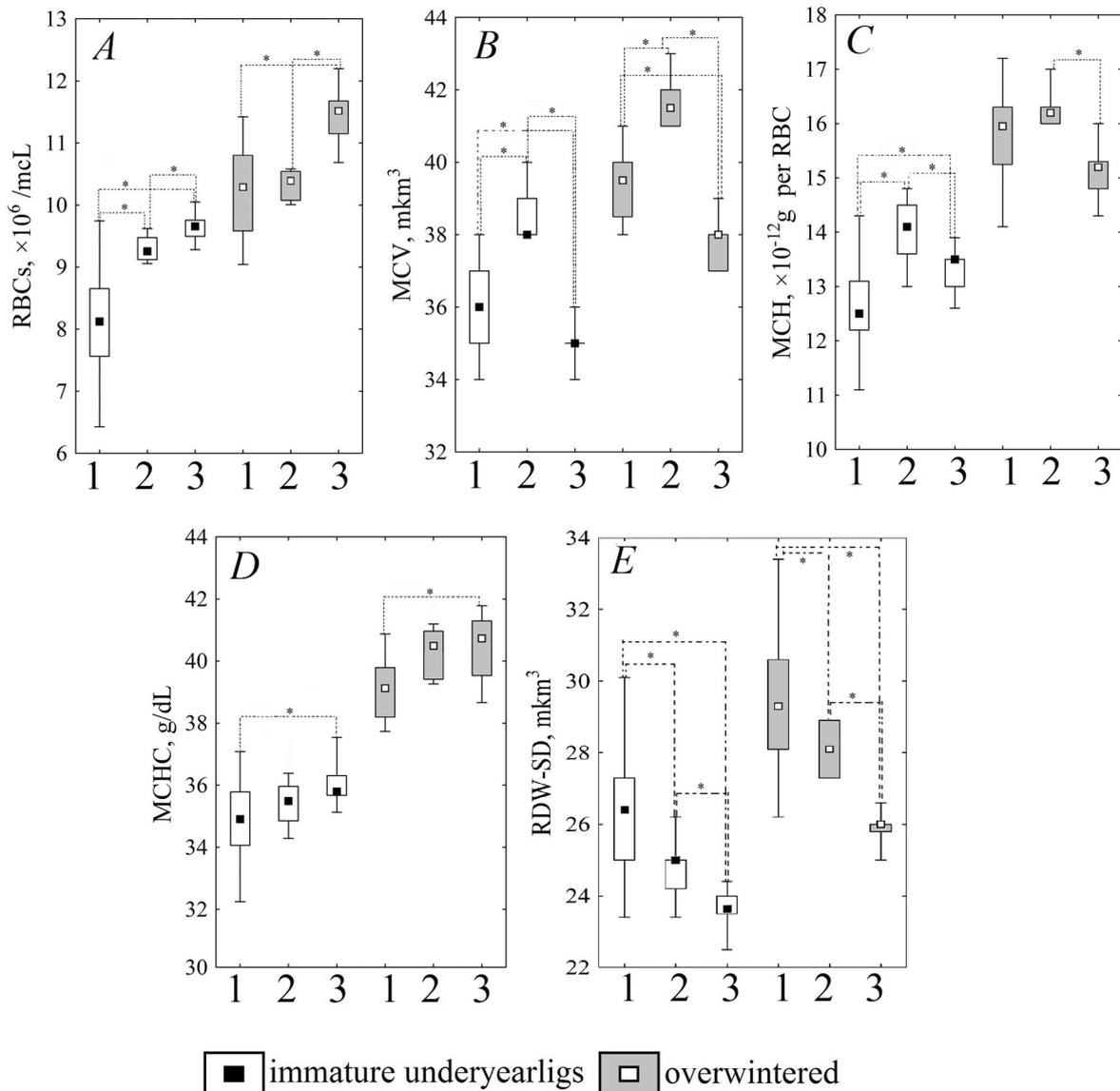


Fig. 1. Erythrocyte indices (median, minimum–maximum, and 25%–75% quartiles) of individuals of three species of voles from two reproductive-age groups: 1 — *M. glareolus*; 2— *M. rutilus*; 3 — *C. rufocanus*; * — The significance level (p) for pairwise comparison of samples is given in accordance with the *U*-test: * < 0.05. A — red blood cells (RBCs) count; B — mean corpuscular volume of RBCs; C — mean corpuscular haemoglobin of RBCs; D — mean corpuscular haemoglobin content of RBCs; E — variation of the RBCs volume distribution.

The interspecific variation of voles of the two reproductive-age status groups (voles that overwintered and sexually immature under-yearlings) can be represented graphically via a multivariate comparison of data by PCCA (Fig. 4). Judging from the close relation between the PC values and haematological data (Tab. 2), the main contribution to the variance of PC-2 is made by MCH, MCV and mean platelet volume. The PC-1 axis is based on correlations with other indicators. All three species, when reproductive-age status was taken into account, were quite well separated in the plane of the two principal components (Fig. 4). The similarity of

C. rufocanus with *M. glareolus* is obvious in PC-2, and *M. rutilus* forms the most distant cluster, a phenomenon that is explained by the three indicators. The distance between *M. glareolus* and *M. rutilus* in terms of PC-2 is smaller than that between these species and *C. rufocanus*.

The interspecies differences were found to be slightly greater for immature under-yearlings. A comparison of interspecific and ontogenetic variation levels is presented in Figure 5 in a joint analysis of the two reproductive-age status groups. According to the close relation between the PC values and haematological data (Tab. 3), the main contribution to the variance of PC-1 is made by

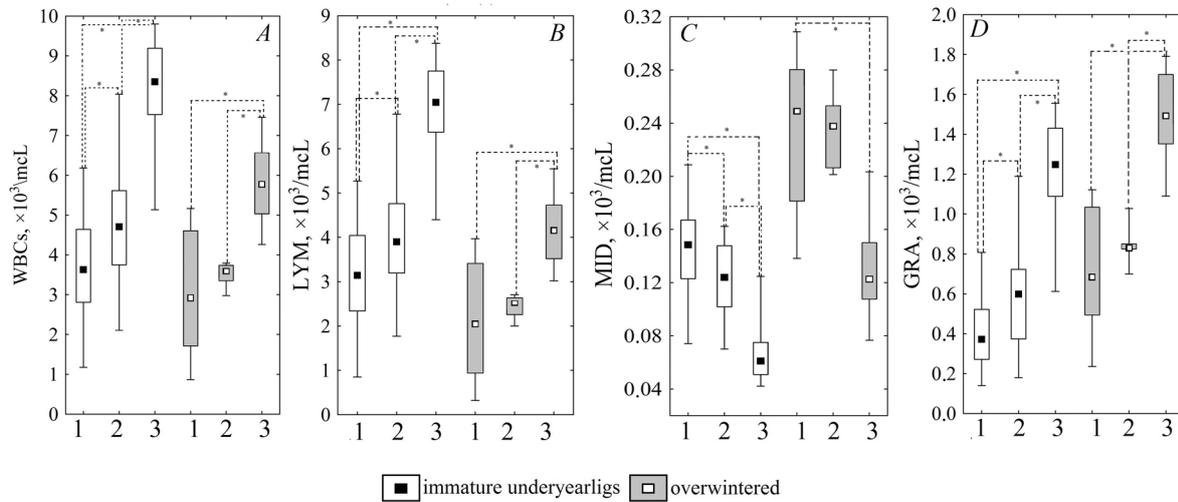


Fig. 2. White blood indices (median and 25%–75% quartiles) of individuals of three species of voles from two reproductive-age groups: 1 — *M. glareolus*; 2 — *M. rutilus*; 3 — *C. rufocanus*; * — The significance level (p) for pairwise comparison of samples is given in accordance with the U -test: * <0.05 . A — white blood cells count, including: B — lymphocytes count; C — monocytes count; D — granulocytes count.

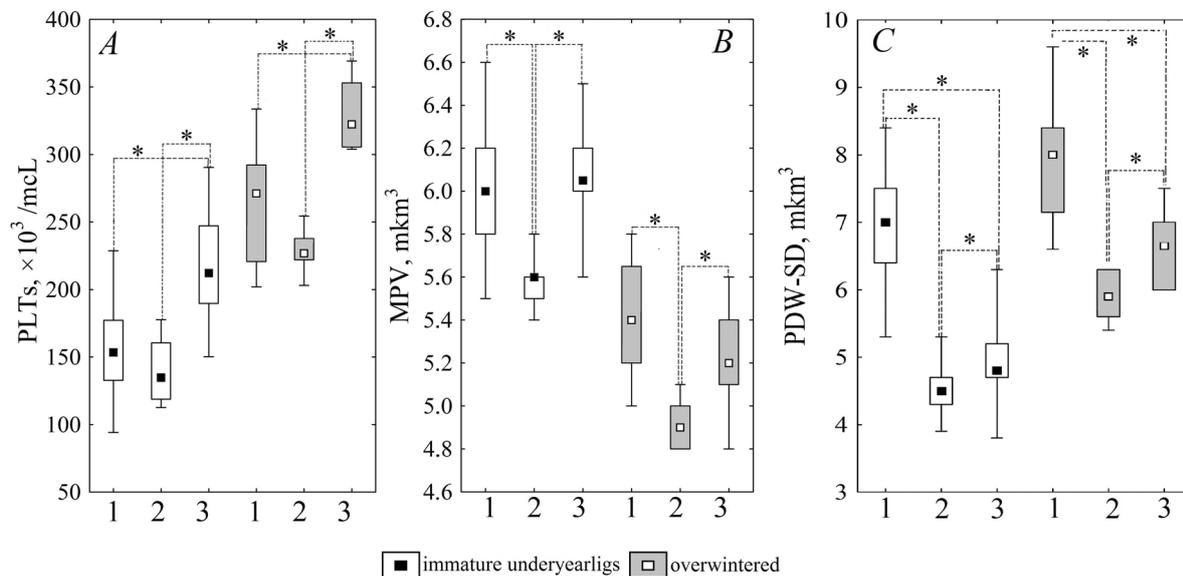


Fig. 3. Indices of hemostasis (median, minimum–maximum, and 25%–75% quartiles) in three species of voles from two reproductive-age groups: 1 — *M. glareolus*; 2 — *M. rutilus*; 3 — *C. rufocanus*; * — The significance level (p) for pairwise comparison of samples is given in accordance with the U -test: * <0.05 . A — platelet (PLTs) count; B — mean volume of PLTs; C — variation of the platelet volume distribution.

the erythrocyte and platelet parameters. The PC-2 axis is based on correlations with other indicators: primarily WBC indicators as well as RDW-SD and platelet distribution width. ANOVA uncovered a significant difference in PC-1 between the reproductive-age status groups and the absence of such a difference among the three species (Fig. 5). PC-2 reflects the species

themselves and does not depend on reproductive-age status. The results showed that the erythrocyte and platelet parameters are subject to substantial ontogenetic changes, which exceed the interspecies differences, whereas the WBC parameters manifested obvious interspecific variation that is greater than their ontogenetic variability.

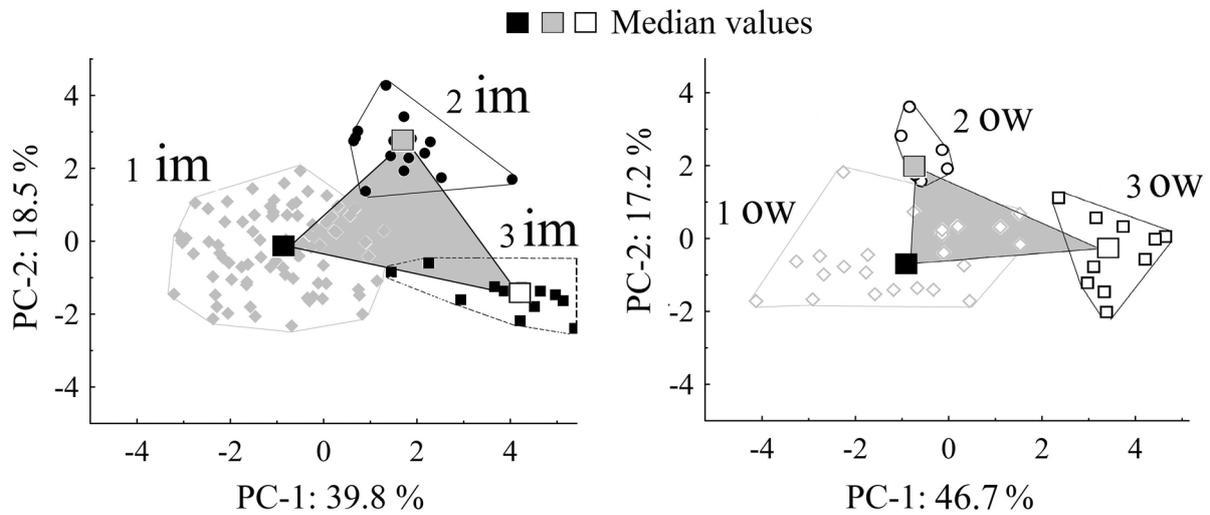


Fig. 4. Arrangement of the three samples in three species of voles (1 — *M. glareolus*; 2 — *M. rutilus*; 3 — *C. rufocanus*) from two reproductive-age groups (im — immature under-yearlings; ow — overwintered individuals) in the plane of the two principal components (PC-1, PC-2) based on the 11 blood parameters.

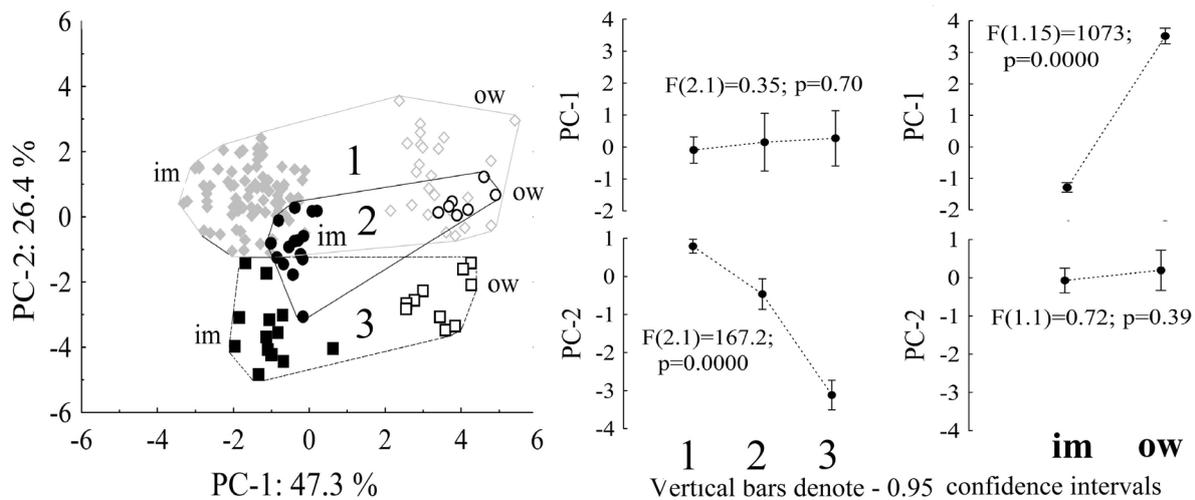


Fig. 5. Arrangement of the six samples in three species of voles (1 — *M. glareolus*; 2 — *M. rutilus*; 3 — *C. rufocanus*) from two reproductive-age groups (im — immature under-yearlings; ow — overwintered individuals) in the plane of the two principal components (PC-1, PC-2) based on the 11 blood parameters.

Discussion

Respiratory capacity of the blood in the studied species: interpretation of erythrocyte indices

In the phylogeny of vertebrates, the transition to a terrestrial lifestyle was associated with an increase in respiratory capacity of the blood. It has been achieved due to 1) an increase in the number of erythrocytes and a reduction in their size, 2) acceleration of blood movement through the vessels, and 3) an increase in intracellular

concentration of haemoglobin and its affinity for oxygen (Snyder, 1973; Hochachka & Somero, 1980; Snyder & Sheafor, 1999; Kostelecka-Myrcha, 1973; Aliko & Gumeni, 2004).

The sample of *C. rufocanus* individuals studied by us here, especially in comparison to the bank vole, was characterised by a higher RBC count with smaller RBC size (MCV; see Fig. 1). This pattern confirms the previously noted inverse correlation between the number of erythrocytes and their size. The increase in the proportion of “small” erythrocytes can be considered a better approach to blood aeration, quite understandable

Table 2. Pearson correlation (R_{xy}) between PC-values (Y) and hematological parameters (X) based on a multivariate analysis of the three vole samples taking account two reproductive-age groups.

Parameter	PC-1	PC-2	PC-1	PC-2
	immature underyearlings		individuals that overwintered	
RBCs	0.77	0.17	0.75	0.11
MCH	0.62	0.67	-0.32	0.69
MCV	-0.03	0.89	-0.33	0.64
RDW-SD	-0.53	0.20	-0.75	0.01
MCHC	0.61	0.06	0.62	0.54
LYM	0.85	-0.28	0.84	0.04
MID	-0.78	0.14	-0.83	0.11
GRA	0.88	-0.29	0.91	-0.01
PLTs	0.50	-0.25	0.80	-0.08
MPV	-0.25	-0.65	-0.40	-0.75
PDW-SD	-0.58	-0.20	-0.20	-0.33

Table 3. Pearson correlation coefficient (R_{xy}) between PC-values (Y) and hematological parameters (X) based on six samples.

Parameter	PC-1	PC-2
RBCs	0.81	-0.39
MCH	0.93	-0.06
MCV	0.75	0.27
RDW-SD	0.51	0.56
MCHC	0.91	-0.18
LYM	-0.20	-0.89
MID	0.52	0.73
GRA	0.48	-0.81
PLTs	0.81	-0.28
MPV	-0.84	0.04
PDW-SD	0.31	0.51

from the standpoint of a general physicochemical law: the finer the medium, the larger is the contact area of the two phases, and the higher is the diffusion rate. In addition, a higher degree of cell saturation with haemoglobin (MCHC) is achieved. This situation usually arises due to an increase in the surface-to-volume ratio, that is, due to the discoid biconcave shape. Even with the same capillarisation, this shape provides greater contact with oxygen in the lungs and is a determinant of functional usefulness of erythrocytes. Besides, the discocyte has high deformability and elasticity: characteristics that allow it to move both through large vessels and through capillaries and are necessary for the functionality of erythrocytes (Zinchuk, 2001; Kostelecka-Myrcha, 2002).

Compared with *C. rufocanus*, *M. rutilus* demonstrates a different strategy for achieving blood oxygen capacity: it has larger erythrocytes with higher haemoglobin content, with fewer of them in peripheral blood. This state of affairs can arise when one or more intermediate mitotic divisions are omitted in nuclear erythroid progenitors, resulting in induction of erythroid differentiation because of an elevated haemoglobin biosynthesis rate. Such an

accelerated transition during the maturation of erythroid cells leads to the formation of larger erythrocytes (macrocytes) (Gavrilov *et al.*, 1985; Wickramasinghe, 1992). Other factors that cause macrocytosis are changes associated with the larger RBC membrane surface area and the permeability of the RBC plasma membrane to cations via passive diffusion (Parshina *et al.*, 2007). The cells are thicker as an adaptation intended for the slow release of oxygen.

For *M. glareolus*, it is assumed that the oxygen capacity of blood is low in comparison with other species due to the low number of peripheral blood erythrocytes and their haemoglobinisation (see Fig. 1). As the literature data show, *M. glareolus* is characterised by a high proportion of erythrocyte precursor cells (> 2% reticulocytes) in the circulatory bed (Tarakhtii & Mukhacheva, 2011). In our study, this has been indirectly confirmed by an increased RDW-SD (see Fig. 1): the heterogeneity of the cell population in size is usually increased with growth of proportion of reticulocytes in the blood having a larger volume. The reticulocytosis is often provoked with erythrodieresis, that is, intravascular

or intra-organic destruction of erythrocytes (Hoehn *et al.*, 2015). In this regard, the increased RDW-SD in *M. glareolus* also could be due to a high proportion of ageing erythrocytes, which have a decreasing size and a lower MCHC. A lower degree of saturation with haemoglobin (MCHC) suggests a reduced surface-to-volume ratio. This specificity of the “morph” is associated with high viscosity of the intracellular contents and, consequently, low deformability of the RBCs, observed during the ageing process of erythrocytes (Hoehn *et al.*, 2015). Based on the reduced number of erythrocytes in the peripheral blood and cellularity of the bone marrow, relative to at least the red vole (0.78 million/hip/g body weight versus 1.31 million/hip/g body weight according to Tarakhtii & Mukhacheva, 2011), increased erythrodieresis in *M. glareolus* is not compensated by a sufficient level of a bone marrow haematopoiesis.

Our findings show that erythrocyte parameters undergo large ontogenetic changes (Fig. 1) that exceed interspecies differences (PC-1, see Table 2 and Fig. 5). In principle, the higher values of erythrocyte parameters in the voles that overwintered — relative to immature individuals of each species — indicate higher functionality of RBCs. This phenomenon is most likely caused by higher energy requirements for growth and reproduction in this group. It should also be borne in mind that in the year of the population size peak, the reproductive “mission” exclusively or mainly falls to the group of individuals that overwintered (Kshnyasev & Davydova, 2021).

Respiratory capacity of the blood and basal metabolic rate (BMR)

The revealed species-specific features of respiratory capacity of the blood should be compared with basal metabolism indicators, which determine, at the organismal level, total mitochondrial respiration of tissues and consequently ATP production under aerobic conditions (Ernster & Schatz, 1981). In warm-blooded animals, 30–80% of daily energy metabolism is represented by BMR (Blaxter, 1989), which is the sum of energy consumption rates of metabolically active organs. In addition to the heart and intestines, which perform mechanical work, internal organs expend energy mainly on the processes of biosynthesis and active transmembrane transport (Hochachka & Somero, 1980). Locomotor muscles at rest consume very little oxygen and ATP, thereby barely contributing to basal metabolism (McArdle *et al.*, 2009).

Naturally, BMR depends on taxonomic affiliation — which determines the species’ ecological and behavioural characteristics — and on a dietary spectrum (Koteja & Weiner, 1993; McNab, 2008). In particular, with adjustment for differences in body weight, it is obvious that animals adapted to living in arid environments (these are usually “hamsters”) tend to have slower metabolism than do animals that live under harsh conditions characterised by low temperatures and long winters (these are usually “voles”) (McNab, 1979; Maloiy *et al.*, 1982; Lovegrove, 2003; Dittmann *et al.*, 2015). Features of diet affect spe-

cific dynamic action of food (energy). The highest energy consumption (and, of course, BMR) is observed after ingestion of protein (up to 40% of basal metabolism) and to a lesser extent after fat ingestion (14%); there is only a slight increase after carbohydrate ingestion (7%). It is quite possible that within even one taxonomic group of Arvicolidae, BMR in the three species will be determined by diet and the temperature regime.

Despite the “innate” adaptation of *C. rufocanus* to the environment at northern latitudes, according to literature data, *C. rufocanus* has relatively low BMR in the thermoneutral range (20°C) and when cooled (0°C). Because *C. rufocanus* possesses the best heat-insulating properties of fur, this species has lower intensity of chemical thermoregulation, that is, smaller basal metabolic reactivity to cooling (Bashenina, 1977). Geographical differences in this parameter are insignificant [2.7 ml O²/(g·h) in the Polar Urals and 2.98 ml O²/(g·h) in the South Urals] (Kovalchuk & Yastrebov, 2003). It is likely that by concentrating on stony placers, where temperature and humidity regimes are relatively constant, *C. rufocanus* avoids the direct influence of extreme abiotic factors; therefore, these voles can survive critical periods while avoiding considerable energy expenditures. The second factor explaining the low BMR may be the consumption of low-calorie food: the prevalence of vegetative parts of plants in the stomach of *C. rufocanus* is more than 50%.

The BMR of *M. rutilus* is higher than that of *C. rufocanus* and is subject to clear-cut latitudinal differences [5.6 ml O²/(g·h) in the Polar Urals, 3.45 ml O²/(g·h) in the Middle Urals and 3.16 ml O²/(g·h) in the South Urals] (Kovalchuk & Yastrebov, 2003). According to Bashenina (1977), *M. rutilus* has high intensity of chemical thermoregulation: metabolic rate under the action of cooling (at 0°C) in this species increases 2.7-fold, which is significantly greater than that in the other two species, especially *C. rufocanus*. The observed differences are related to the fact that *M. rutilus* has higher thermal conductivity of the integument owing to less dense and shorter fur.

The respiration parameters seen in *M. glareolus*, according to Kovalchuk and Yastrebov (2003) are slightly higher than those in *M. rutilus* [3.5 ml O²/(g·h) in the Polar Urals, 3.6 ml O²/(g·h) in the Middle Urals and 4.5 ml O²/(g·h) in the South Urals]; however, the geographical differences in energy metabolism disappear when northern regions are examined. The inadequate resistance of *M. glareolus* to prolonged exposure to the cold in an experiment (progressive hypothermia, body weight loss and decreased oxygen consumption and mitochondrial protein concentration in tissues) limits the successful movement of this species to the Subarctic, in contrast to *M. rutilus* and *C. rufocanus*.

It is known that a prerequisite for high adaptability of a species to its habitat is the greatest biological efficiency at the lowest possible BMR. In this regard, the relatively low BMR in *C. rufocanus* indicates that the organisation of metabolic processes is designed to optimise the body’s expenditure of functional and energy reserves. At the same time, RBCs have lower demand for the processes

of gas exchange. A lighter functional load on these cells leads to long-term preservation of the optimal structure, their longer life expectancy, and as a consequence, a greater number of these cells in the circulatory bed.

In *M. glareolus*, the high organ oxygen demand raises the erythrocyte physiological load. Intensive functioning of organelles of differentiated cells is always accompanied by their quicker decay rate (so-called wear metabolism). The high intensity of destruction of erythrocytes in *M. glareolus* is not balanced adequately by erythropoiesis, apparently owing to low efficiency of compensatory mechanisms in bone marrow.

M. rutilus occupies intermediate positions in the sense of tensions of gas exchange and “well-being” of the erythrocyte population. Most likely, the erythropoiesis system is in a state of “economical” functioning (blockage of cell division with induction of differentiation). Meanwhile, larger erythrocytes are formed with diminished respiratory capacity.

Components of (non)specific immune defence in the species under study: interpretation of leucocyte indices; relations with their sociability, eurybionism and metrics of thermoregulation

For implementation of protective mechanisms underlying recognition and neutralisation of pathogens (from viruses to parasitic worms), there is a set of universal blood cells (leucocytes) that represent the main cellular link between innate and adaptive (acquired) immunity. The cells that embody a nonspecific (innate) immune response include (in addition to tissue macrophages and dendritic cells), peripheral WBCs of the granulocytic series (neutrophils, eosinophils and basophils), which recognise and destroy foreign objects/cells by phagocytosis, or in the case of large foreign bodies (e.g. parasites) via a release of harmful substances upon direct contact. Characteristics of innate immunity, which is also called constitutional immunity, depend on genetic selection, which determines minimal variation of innate immunity within a species (Sheldon & Verhulst, 1996; Litman *et al.*, 2005). Components of innate immune defence are important mediators activating the mechanisms of adaptive immunity, which primarily is realised by T and B lymphocytes (Lydyard & Porakishvili, 2012). The adaptive immune system, as a consequence of appearing later in evolution, provides a more potent immune response as well as immunological memory, due to which each foreign microorganism is “memorised” because of antigens unique to it. This type of immunity is most susceptible to the influence of various environmental factors (Lokhmiller & Moshkin, 1999).

In *C. rufocanus*, the higher immune status is confirmed by the largest number of WBCs — both owing to lymphocytes and granulocytes — as compared with the other two species (see Fig. 2). Unlike *M. glareolus* and *M. rutilus*, *C. rufocanus* demonstrates higher

sociability as indirectly evidenced by specific features of sound signalling in this species, namely, the presence of “chirping” in the vocal repertoire (Rutkovskaya, 1992). In the natural environment of this species, so-called matrix clusters arise when sexually mature female under-yearlings claim their areas on the periphery of a maternal territory and reproduce successfully (Ims, 1989). Thus, the high frequency of intragroup contacts may also result in the highest likelihood of transmission of pathogens within a species, a possibility that requires an activated immune system.

Aside from sociability, the thermoregulatory function can also be named among the factors influencing immune status. An immune response may weaken at a low ambient temperature owing to competition with thermoregulation for limited resources. *C. rufocanus*, with its high intensity of physical thermoregulation, is considered adapted to cool environments and cold stress. Even at temperatures under 23°C, this species of rodents can allocate enough energy to maintain its normal body state and adequate immune function (Kusumoto & Saitoh, 2008). In this respect, *C. rufocanus* differs from *Microtus agrestis* Linnaeus, 1761 and *Apodemus peninsulae* Thomas, 1906, whose immune status declines at temperatures <25°C (McDevitt & Speakman, 1994; Speakman *et al.*, 2003).

Among the examined species, *M. glareolus* is distinguished by the lowest number of WBCs due to reduced numbers of granulocytes and lymphocytes (see Fig. 2); these data are suggestive of less active functioning of this species' immune system relative to *C. rufocanus*. A distinctive characteristic of *M. glareolus* is low mobility and modest exploratory activity in combination with the smallest size of the territory it uses (Wartha & Henriques-Normark, 2008). Thus, this species moves away from interspecies competition and intragroup contacts, and hence there is a lower risk of transmission of pathogens. In *M. rutilus*, high locomotor and exploratory activities (Kravchenko & Moskvitina, 2008) are associated with large sizes of individual plots, explaining this species' intermediate positions on the studied parameters.

Myodes glareolus is characterised by a high relative number of monocytes in peripheral blood (see Fig. 2). Monocytes are a transport form of macrophages that get settled in loose connective tissue and the parenchyma of organs (Kupffer's cells in the liver, microglia in the brain and interstitial cells in the lungs) and along blood vessels (Zimmerman *et al.*, 2010; Lydyard & Porakishvili, 2012). The greater number of monocytes in *M. glareolus* can probably be ascribed to heightened tissue demand for macrophages because of, among other things, greater intracellular and intravascular haemolysis. It is known that haemolysis can occur in the lumen of blood vessels (intravascular haemolysis) and in cells of the reticuloendothelial system (intracellular haemolysis). In response to the enhanced destruction of erythrocytes, the number and functioning of macrophages increase (Grigorovich, 1966).

Our results show that *C. rufocanus* and *M. rutilus* individuals that overwintered have low immune status

as evidenced by the smaller number of WBCs (Fig. 2). Immune function may compete for available resources with other processes, including growth and reproduction (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000; Zuk & Stoehr, 2002). In this regard, owing to the high cost of reproduction, immune function is reduced in sexually mature voles, in agreement with research by others (Feore *et al.*, 1997; Saino *et al.*, 2000).

The vascular-platelet mechanism

It ensures the arrest of bleeding in the smallest vessels because of the adhesive-aggregation function of platelets (White & Clawson, 1980). A specific feature of *M. rutilus* is thrombocytopeny relative to the other two species, that is, fewer platelets in peripheral blood with smaller volume and lower heterogeneity of these cell populations in the circulatory bed. As a rule, smaller platelets are typical in *M. rutilus* with a predominance of young (immature) cells.

Phylogenetic relationships among the species

There is a shorter distance between *M. glareolus* and *M. rutilus*, both with and without the stage of ontogenesis taken into account (Figs. 4 and 5). The phylogenetic affinity of these species, aside from cytogenetic (Abramson *et al.*, 2009) and paleontological (Smirnov *et al.*, 1986; Tesakov, 1996) data, is confirmed by the absence of differences between these species in the number and morphology of chromosomes (Rausch & Rausch, 1975), according to hybridisation assays performed on both laboratory animals and wild ones (Dekonenko *et al.*, 2003; Osipova & Sokin, 2008). In the sympatry zone of these species, including the Urals, thanks to molecular genetic methods, bank voles with the mitochondrial genome borrowed from the red-backed vole have been identified (Abramson *et al.*, 2009; Borodin *et al.*, 2011). Introgression of mitochondrial genomes often leads to the integration (into the genome of the recipient species) of a set of genes that increase the adaptability of organisms to new environmental conditions. This ability allows the species to occupy new ecological niches, habitats and territories quickly. An analysis of the scale, geographical distribution and prevalence of this introgression in populations of *M. glareolus* made it possible to put forward a hypothesis that this trait is adaptive and associated with the dispersal of this species to the north under more extreme conditions of hypothermia (Potapov *et al.*, 2007).

Conclusion

Of the three representatives of the subfamily Arvicolinae, *M. glareolus* can be regarded as a species with limited homeostatic capabilities of RBCs (fewer RBCs with lower density of haemoglobin distribution within the cell and more pronounced anisocytosis). By contrast, in *C. rufocanus*, tissue oxygen demand can be successfully satisfied due to a greater number of “smaller” erythrocytes with higher density of haemoglobin within

the cells, which are more uniform in size. Respiratory capacity of the blood in *M. rutilus* is maintained at the level of *C. rufocanus*, but in a different manner, i.e. with fewer RBCs in the circulatory bed but with higher haemoglobin content in them.

The analysed parameters of WBCs are clearly subject to interspecific variation, which is higher than ontogenetic variability. *C. rufocanus*, relative to the other two species, has higher immune status, which is supported by granulocytes and lymphocytes. This finding is mostly explained by the desire for social contacts inherent in this species and therefore a higher risk of infection with pathogens. The large number of monocytes in the peripheral blood of *M. glareolus* is probably related to stronger tissue demand for macrophages, e.g. due to increased erythrodiuresis.

Interspecies differences in erythrocyte and leucocyte indices are seen only in each of the two reproductive-status groups that were analysed. The higher functional capacity of RBCs in voles that overwintered is caused by the higher energy requirements for growth and reproduction in this group. Owing to the high cost of reproduction, immune function of sexually mature individuals is low in all three species.

ACKNOWLEDGMENTS. The experiments described in this manuscript were supported by the State Contract of the Institute of Plant and Animal Ecology, UB RAS.

References

- Abramson N.I. & Lissovsky A.A. 2012. [Subfamily Arvicolinae] // Pavlinov I. & Lissovsky A.A. (eds.). [The Mammals of Russia: A Taxonomic and Geographic Reference. (Archives of Zoological Museum of MSU. Vol.52)]. Moscow: KMK Scientific Press. P.127–141. [in Russian].
- Abramson N.I., Lebedev V.S., Tesakov A.S., & Banikova A.A. 2009. Supraspecies relationships in the subfamily Arvicolinae (Rodentia, Cricetidae): an unexpected result of nuclear gene analysis // Molecular biology. Vol.43. No.5. P.34–846.
- Aliko V. & Gumeni D. 2004. Evolution of hematological parameters of peripheral blood obtained from representative individuals of Amphibia, Aves and Mammalia classes // Biological Studies. Vol.8. P.112–118.
- Bashenina V. 1977. [Ways of adaptation of murine rodents]. Moscow: Nauka. 355 p. [in Russian].
- Berdugin K.I. 1996. [Small mammals in the upper belts of the Ural Mountains]. Abstract of PhD Dissertation. Yekaterinburg: IPAE UB RAS. 14 p. [in Russian].
- Blaxter K. 1989. Energy metabolism in animals and man. New York: Cambridge University Press. 336 p.
- Borodin A.V., Davydova Yu.A., & Fominykh M.A. 2011. [A natural hybrid between the red (*Clethrionomys rutilus*) and the bank (*Clethrionomys glareolus*) vole (Arvicolinae, Rodentia) in the Middle Urals] // Zoologicheskyy zhurnal. Vol.90. No.5. P.634–640. [in Russian, with English summary].

- Bottaeva Z.Kh., Tembotova F.A., Emkuzheva M.M., Bersekova Z.A. & Chapaev A.K. 2019. Effect of ecogeographic factors along latitudinal-longitudinal gradient on the red blood system of the Caucasian snow vole (*Chionomys gud*), a species autochthonous to the Caucasus // Russian Journal of Ecology. Vol.50. No.1. P.34–42.
- Chernyavsky F.B., Lazutkin A.N. & Mosin A.F. 2003. Variations of some physiological and biochemical indices in the population of the red-backed vole (*Clethrionomys rutilus*) // Biology Bulletin. Vol.3. No.30. P.291–298.
- Dekonenko A., Yakimenko V., Ivanov A., Morozov V., Nikitin P., Khasanova S., Dzagurova T., Tkachenko E. & Schmaljohn C. 2003. Genetic similarity of Puumala viruses found in Finland and western Siberia and of the mitochondrial DNA of their rodent hosts suggests a common evolutionary origin // Infection, Genetics and Evolution. Vol.3. No.4. P.245–247.
- Dittmann M.T., Hebel C., Arif A., Kreuzer M. & Clauss M. 2015. Metabolic rates of three gazelle species (*Nanger soemmerringii*, *Gazella gazella*, *Gazella spekei*) adapted to arid habitats // Mammalian Biology. Vol.80. No.5. P.390–394.
- Ernster L. & Schatz G. 1981. Mitochondria: a historical review // Journal of Cell Biology. Vol.91. No.3. P.227s–255s.
- Feore S.M., Bennett M., Chantrey J., Jones T, Baxby D & Begon M 1997. The effect of cowpox virus infection on fecundity in bank voles and wood mice // Proceedings of the Royal Society of London. Series B. Vol.264. No.1387. P.1457–1461.
- Frase B.A. 2002. Hematological parameters of high-elevation bushy-tailed woodrats // The Southwestern Naturalist. Vol.47. No.3. P.508–510.
- Grigorovich N.A. 1966. Pathogenesis of hemolytic anemia caused by phenylhydrazine (experimental data) // Bulletin of Experimental Biology and Medicine Vol.61. No.2. P.29–32.
- Hansson L. 1985. *Clethrionomys* food: generic, specific and regional characteristic // Annales Zoologici Fennici. Vol.22. No.3. P.315–318.
- Hoehn R.S., Jernigan P.L., Chang A.L., Edwards M.J. & Pritts T.A. 2015. Molecular mechanisms of erythrocyte aging // Biological Chemistry. Vol.396. No.6–7. P.621–631.
- Hoffman R., Benz E.J., Shattil S.J., Furie B., Cohen H.J., Silberstein L.E. & McGlave P. 1999. Hematology: basic principles and practice. New York: Churchill Livingstone. 2584 p.
- Hochachka P.W. & Somero G.N. 1980. Biochemical adaptation. Princeton: Princeton University Press. 563 p.
- Jain N.C. 1986. Schalm's veterinary haematology 4th edition. Philadelphia: Lea and Febiger. 1221 p.
- Ims R.A. 1989. Kinship and origin effects on dispersal and space sharing in *Clethrionomys rufocanus*. // Ecology. Vol.70. No.3. P.607–616.
- Kizhina A.G., Kalinina S.N., Uzenbaeva L.B., Panchenko D.V., Łapiński S., Ilyukha V.A., Pechorina E.F. & Fokina V.O. 2020. Comparative study of erythrocyte morphology and size in relation to ecophysiological adaptations in Rodentia species. // Russian Journal of Theriology. Vol.19. No.2. P.161–171.
- Konig & Claus. 1973. Mammals. London: Collins & Co. 256 p.
- Koteja P. & Weiner J. 1993. Mice, voles and hamsters: metabolic rates and adaptive strategies in muroid rodents. // Oikos. Vol.66. No.3. P.505–514.
- Kostelecka-Myrcha A. 1973. Regularities of variations of the haematological values characterizing the respiratory function of blood in mammals. // Acta Theriologica. Vol.18. No.1. P.1–56.
- Kostelecka-Myrcha A. 2002. The ratio of amount of haemoglobin to total surface area of erythrocytes in mammals. // Acta Theriologica. Vol.47. No.1. P.209–220.
- Kovalchuk L.A. & Tsvirenko S.V. 1997. Energetic metabolism and the blood system of small mammals living under different climatic and geographical conditions // Russian Journal of Ecology. Vol.28. No.1. P.51–56.
- Kovalchuk L.A. & Yastrebov A.P. 2003. [Ecological Physiology of Small Mammals in the Urals] Ekaterinburg: Izdatel'stvo NISO. 184 p. [in Russian, with English summary].
- Kravchenko L.B. & Moskvitina N.S. 2008. Behavioral and physiological peculiarities of three species of forest voles (*Clethrionomys*, Rodentia, Cricetidae) related to their spatial population structure // Zoologicheskyy zhurnal. Vol.87. No.2. P.1509–1517 [in Russian, with English summary].
- Kshnyasev I.A. & Davydova Yu.A. 2021. Population cycles and the Chitty syndrome // Russian Journal of Ecology. Vol.52. No.1. P.70–75.
- Kusumoto K. & Saitoh T. 2008. Effects of cold stress on immune function in the grey-sided vole, *Clethrionomys rufocanus*. // Mammal Study. Vol.33. P.11–18.
- Kusumoto K. 2015. Humoral immune response of overwintered gray red-backed voles (*Myodes rufocanus bedfordiae*) under cold stress in spring. // Bulletin of the Faculty of Agriculture, Saga University. Vol.100. P.15–26.
- Lazutkin A.N., Yamborko A.V. & Kiselev S.V. 2016. Energy and immune parameters of northern red-backed voles (*Clethrionomys rutilus*) at different population densities in the Kolyma river basin // Russian Journal of Ecology. Vol.47. No.6. P.562–567.
- Litman G.W., Cannon J.P. & Dishaw L.J. 2005. Reconstructing immune phylogeny: new perspectives // Nature Reviews Immunology. Vol.5. No.11. P.866–879.
- Lokhmiller R.L. & Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? // Oikos. Vol.88. No.1. P.87–98.
- Lokhmiller R.L. & Moshkin M.P. 1999. [The adaptive significance of the variability of immunocompetence in population of small mammals] // Sibirskiy ekologicheskii zhurnal. Vol.6. No.1. P.37–58. [in Russian, with English summary].
- Lydyard P.M. & Porakishvili N. 2012. Cells, tissues and organs of the immune system // Immunology (8th edition). Male D., Brostoff J., Roth D.B. & Roitt I.M. (eds.) Elsevier. P.17–50.
- Lovegrove B.G. 2003. The influence of climate on the basal metabolic rate of small mammals: a slow-fast metabolic continuum // Journal of Comparative Physiology. Part B. Vol.173. No.2. P.87–112.
- Malojy G.M.O., Kamau J.M.Z., Shkolnik A., Meir M., Arieli R. 1982. Thermoregulation and metabolism in a small desert carnivore: the Fennec fox (*Fennecus zerda*) (Mammalia) // Journal of Zoology. Vol.198. No.3. P.279–291.

- Mather P.M. 1987. Computer processing of remotely sensed images, an introduction. Chichester: John Wiley and Sons. 360 p.
- McArdle W.D., Katch F.I. & Katch V.L. 2009. Exercise physiology: nutrition, energy, and human performance. 7th edition. Philadelphia: Lippincott Williams and Wilkins. 1038 p.
- McDevitt R.M. & Speakman J.R. 1994. Central limits to sustainable metabolic rate have no role in cold acclimation of the short-tailed field vole (*Microtus agrestis*) // *Physiological Zoology*. Vol.67. No.5. P.1117–1139.
- McNab B.K. 1979. Climatic adaptation in the energetics of heteromyid rodents // *Comparative Biochemistry and Physiology Part A*. Vol.62. No.4. P.813–820.
- McNab B.K. 1992. A statistical analysis of mammalian rates of metabolism // *Functional Ecology*. Vol.6. No.6. P.672–679.
- McNab B.K. 2008. An analysis of the factors that influence the level and scaling of mammalian BMR // *Comparative Biochemistry and Physiology. Part A*. Vol.151. No.1. P.5–28.
- Morrison P.R. & Tietz W.J. 1957. Cooling and thermal conductivity in three small Alaskan mammals // *Journal of Mammalogy*. Vol.38. No.1. P.78–86.
- Morrison P.R. 1964. Adaptation of small mammals to the arctic // *Federation proceedings*. Vol.23. No.6. P.1202–1206.
- Olenev G.V. 2002. Alternative types of ontogeny in cyclomorphic rodents and their role in population dynamics: an ecological analysis // *Russian Journal of Ecology*. Vol.33. P.321–330.
- Orekhova N.A. 2018. Hematological indicators in pygmy wood mouse *Apodemus uralensis* (Muridae, Rodentia) populations as markers of the environmental radiation exposure: East Urals radioactive trace (Russia) // *Environmental Science and Pollution Research*. Vol.25. P.16144–16166.
- Osipova O.V. & Sokin A.A. 2008. [Experimental simulation of ancient hybridization between bank and red voles] // *Doklady Akademii Nauk*. Vol.420. No.1. P.169–171. [in Russian, with English summary]
- Potapov S.G., Illarionova N.A., Andreeva T.A., Baskevich M.I., Okulova N.M., Lavrenchenko L.A., Orlov V.N. 2007. [Transfer of mitochondrial genome of the northern redbacked vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*) in northwestern Europe] // *Doklady Akademii Nauk*. Vol.417. No.1. P.435–438. [in Russian, with English summary]
- Rausch R.L. & Rausch V.R. 1975. Relationships of the red-backed vole, *Clethrionomys rutilus* (Pallas), in North America: karyotypes of the subspecies *Dawsoni* and *Albiventer* // *Systematic Biology*. Vol.24. No.2. P.163–170.
- Rutkovskaya M.V. 1992. [Factors affecting the sound activity of forest voles] // *Izvestiya of the Academy of Sciences of the Union of Soviet Socialist Republics*. Vol.5. P.753–760 [in Russian].
- Saino N., Canova L., Fasola M. & Martinelli R. 2000. Reproduction and population density affect humoral immunity in bank voles under field experimental conditions // *Oecologia*. Vol.124. P.358–366.
- Sheldon B.C. & Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology // *Trends in Ecology and Evolution*. Vol.11. No.8. P.317–321.
- Smirnov N.G., Bolshakov V.N. & Borodin A.V. 1986. [Pleistocene rodents of the north of western Siberia]. Moscow: Nauka. 145 p. [in Russian].
- Snyder G.K. 1973. Erythrocyte evolution: the significance of the Fåhræus-Lindqvist phenomenon // *Respiratory Physiology*. Vol.19. No.3. P.271–278.
- Snyder G.K. & Sheafor B.A. 1999. Red blood cells: centerpiece in the evolution of the vertebrate circulatory system // *Integrative and Comparative Biology*. Vol.39. No.2. P.189–198.
- Sokolov V.E. & Syroechkovsky E.E. (eds). 1988. [Reserves of the European part of the RSFSR. Part I.]. Moscow: Mysl' Part. I. P.61–89. [in Russian].
- Speakman J.R., Ergon T., Cavanagh R., Reid K., Scantlebury D.M. & Lambin X. 2003. Resting and daily energy expenditures of free-living field voles are positively correlated but reflect extrinsic rather than intrinsic effects // *PNAS of the United States of America*. Vol.100. No.24. P.14057–14062.
- StatSoft Inc. 2012. Electronic Textbook on Statistics. Available from <http://www.statsoft.ru/home/textbook/default.htm> (accessed February 2021).
- Tarakhtii E.A. & Mukhacheva S.V. 2011. Blood system peculiarities in the bank vole (*Clethrionomys glareolus*) under chronic environmental pollution // *Biological Bulletin*. Vol.38. No.5. P.518–527.
- Tarakhtii E.A., Sumin M.N. & Davydova Yu.A. 2009. [Variability of red blood characteristics in bank vole (*Clethrionomys glareolus*) related to season and reproductive status of its individuals] // *Uspekhi sovremennoy biologii*. Vol.129. No.2. P.191–197 [in Russian, with English summary]
- Tesakov A.S. 1996. Evolution of bank voles in the late Pliocene and early Pleistocene of eastern Europe (*Clethrionomys*, Arvicolidae) // *Acta Zoologica Cracoviensia*. Vol.39. No.1. P.541–547.
- Tête N., Afonso E., Bouguerra G. & Scheiffler R. 2015. Blood parameters as biomarkers of cadmium and lead exposure and effects in wild wood mice (*Apodemus sylvaticus*) living along a pollution gradient // *Chemosphere*. Vol.138. P.940–946.
- Thomas A. & Ono K. 2015. Diving related changes in the blood oxygen stores of rehabilitating harbor seal pups (*Phoca vitulina*) // *PloS ONE*. Vol.10. No.6. P.e0128930.
- Topashka-Ancheva M., Metcheva R. & Teodorova S. 2003. A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria II: chromosomal aberrations and blood pathology // *Ecotoxicology and Environmental Safety*. Vol.54. No.2. P.188–193.
- Wartha F. & Henriques-Normark B. 2008. ETosis: a novel cell death pathway // *Science Signaling*. Vol.1. No.21. P.25.
- White J.G. & Clawson C.C. 1980. Overview article: Biostructure of blood platelets // *Ultrastructural Pathology*. Vol.1. No.4. P.533–558.
- Wiger R. 1979. Seasonal and annual variations in the prevalence of blood parasites in cyclic species of small rodents in Norway with special reference to *Clethrionomys glareolus* // *Ecography*. Vol.2. No.3. P.169–175.
- Wilson D.E., Reeder D.M. (eds.). 2005. *Mammal Species of the World: A Taxonomic and Geographic Reference*. 3rd edition. Baltimore: Johns Hopkins University Press. 2142 p.

- Wolk E. 1981. Seasonal and age changes in leukocyte indices in shrews // *Acta Theriologica*. Vol.26. No.12. P.219–229.
- Zimmerman L.M., Vogel L.A. & Bowden R.M. 2010. Understanding the vertebrate immune system: insights from the reptilian perspective // *Journal of Experimental Biology*. Vol.213. No.5. P.661–671.
- Zinchuk V.V. 2001. [Erythrocyte deformability: physiological aspects] // *Uspekhi fiziologicheskikh nauk*. Vol.32. No.3. P.66–78. [in Russian, with English summary].
- Zuk M. & Stoehr A.M. 2002. Immune defense and host life history // *The American Naturalist*. Vol.160. P.S9–S22.

Appendix 1. Mann-Whitney U test of significant differences for the hematological parameters in females (F) and males (M) in three species of voles from two reproductive-age groups (im — immature underyearlings; ow — individuals that overwintered). Critical values for the Mann-Whitney Test (U) at $p=0.05$ are marked with asterisk.

Hematological parameters	<i>Myodes glareolus</i>				<i>Myodes rutilus</i>				<i>Craseomys rufocanus</i>			
	im F:n=30; M:n=50		ow F:n=10; M:n=14		im F:n=9; M:n=6		ow F:n=2; M:n=5		im F:n=3; M:n=11		ow F:n=6; M:n=4	
	$U(550^*)$	p	$U(36^*)$	p	$U(10^*)$	p	$U(0^*)$	p	$U(3^*)$	p	$U(15^*)$	p
RBCs	671	0.468	50	0.241	24	0.724	4	0.698	16	0.938	9	0.522
MCV	664	0.395	55	0.395	26	0.953	2	0.245	9	0.276	9	0.522
MCHC	745	0.960	58	0.482	18	0.289	4	0.698	16	0.938	8	0.394
RDW-SD	555	0.053	67	0.860	26	0.953	3	0.561	13	0.640	7	0.335
MCH	732	0.862	64	0.747	20	0.443	4	0.698	10	0.350	10	0.749
WBCs	741	0.929	32	0.026	24	0.723	1	0.121	11	0.392	4	0.088
LYM	735	0.881	33	0.030	24	0.723	1	0.121	10	0.311	3	0.055
MID	587	0.106	38	0.060	21	0.479	0	0.052	8	0.186	2	0.033
GRA	701	0.626	37	0.053	25	0.814	2	0.245	8	0.186	8	0.394
PLTs	687	0.534	32	0.028	24	0.723	2	0.245	16	0.938	9	0.522
MPV	698	0.605	58	0.500	22	0.555	4	0.698	15	0.876	10	0.749
PDW-SD	704	0.647	49	0.218	20	0.443	1	0.121	16	0.938	11	0.831

Appendix 2. Comparison of p values (2-tailed) in immature under-yearlings of *Myodes glareolus* and *Myodes rutilus*: independent (grouping) variable is catching season (summer and spring).

Hematological parameters	<i>M. glareolus</i>	<i>M. rutilus</i>
RBC	0.15	0.41
MCV	0.10	0.64
MCHC	0.10	0.64
RDW-SD	0.17	0.44
MCH	0.03	0.59
WBC	0.01	1.0
LYM	0.01	1.0
MID	0.16	0.64
GRA	0.01	0.72
PLT	0.78	0.16
MPV	0.47	0.77
PDW-SD	0.23	0.37

Supplement 3. Multiple comparison of p values (2-tailed) in individuals that overwintered of *Myodes glareolus* and immature under-yearlings of *Craseomys rufocanus*: independent (grouping) variable is catching season.

Hematological parameters	Catching season	Catching season					
		<i>M. glareolus</i> (ow)			<i>C. rufocanus</i> (im)		
		Spring (1)	Summer (2)	Autumn (3)	Spring (1)	Summer (2)	Autumn (3)
RBC	(1)	—	1.0	0.86	—	1.0	1.0
	(2)	1.0	—	0.62	1.0	—	1.0
	(3)	0.86	0.62	—	1.0	1.0	—
MCV	(1)	—	1.0	1.0	—	0.87	1.0
	(2)	1.0	—	1.0	0.87	—	1.0
	(3)	1.0	1.0	—	1.0	1.0	—
MCHC	(1)	—	1.0	1.0	—	0.82	1.0
	(2)	1.0	—	1.0	0.82	—	1.0
	(3)	1.0	1.0	—	1.0	1.0	—
RDW-SD	(1)	—	1.0	1.0	—	0.08	0.31
	(2)	1.0	—	1.0	0.08	—	1.0
	(3)	1.0	1.0	—	0.31	1.0	—
MCH	(1)	—	1.0	0.04	—	0.66	1.0
	(2)	1.0	—	1.0	0.66	—	0.99
	(3)	0.04	1.0	—	1.0	0.99	—
WBC	(1)	—	1.0	0.15	—	1.0	0.92
	(2)	1.0	—	1.0	1.0	—	1.0
	(3)	0.15	0.42	—	0.92	1.0	—
LYM	(1)	—	1.0	0.12	—	1.0	1.0
	(2)	1.0	—	0.39	1.0	—	1.0
	(3)	0.12	0.39	—	1.0	1.0	—
MID	(1)	—	1.0	0.13	—	1.0	0.99
	(2)	1.0	—	0.86	1.0	—	0.49
	(3)	0.13	0.86	—	0.99	0.49	—
GRA	(1)	—	1.0	0.35	—	1.0	1.0
	(2)	1.0	—	0.62	1.0	—	1.0
	(3)	0.35	0.62	—	1.0	1.0	—
PLT	(1)	—	1.0	0.87	—	1.0	1.0
	(2)	1.0	—	0.62	1.0	—	1.0
	(3)	0.87	0.62	—	1.0	1.0	—
MPV	(1)	—	1.0	0.99	—	0.85	0.72
	(2)	1.0	—	0.62	0.85	—	1.0
	(3)	0.99	0.62	—	0.72	1.0	—
PDW-SD	(1)	—	1.0	0.84	—	1.0	0.92
	(2)	1.0	—	0.47	1.0	—	1.0
	(3)	0.84	0.47	—	0.92	1.0	—