

Differentiating ability of haematological indicators: comparative blood test of sympatric rodent species of the subfamily Arvicolinae (*Clethrionomys glareolus*, *Clethrionomys rutilus*, and *Craseomys rufocanus*)

Natali Orekhova (✉ naorekhova@mail.ru)

Institut of plant and animal ecology <https://orcid.org/0000-0002-8415-3201>

Yu A. Davydova

Institut of plant and animal ecology

G. Yu. Smirnov

Institut of plant and animal ecology

Research Article

Keywords: erythrocyte and leukocyte indices, rodents, *Clethrionomys glareolus*, *Clethrionomys rutilus*, *Craseomys rufocanus*

Posted Date: June 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-552749/v1>

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Abstract

Among the representatives of the subfamily Arvicolinae the interspecific differences (*Clethrionomys glareolus*, *Clethrionomys rutilus*, and *Craseomys rufocanus*) in the respiratory capacity of red blood and the immune status are presented in conjunction with the basal metabolic rate, the level of sociality and eurybionism, and thermoregulatory function. The degree of differentiation of voles in terms of haematological parameters has corresponded to the level of phylogenetic relationships between species. Correct conduct of interspecific comparisons of haematological parameters of voles is possible only within the same reproductive-age groups.

Introduction

The nature of interspecies differentiation according to various systems of characters (morphophysiological, cytogenetic, biochemical, ethological) reflects the ways and mechanisms of the historical transformation of species under the influence of a continuously changing combination of environmental factors. Of particular interest is the assessment of the differentiating ability of hematological indicators. As a functional system, blood ensures the timely delivery of oxygen and nutrients to cells and tissues and removes metabolic products from organs and interstitial spaces. It occupies a special place in the creation of a cooperative exchange, forming communication channels through the synthesis and transport of intercellular communication molecules, such as cytokines and autocoids, leukotrienes, prostaglandins (Kostelecka-Myrcha 1973; Jain 1986; Hoffman et al. 2003).

Experimental data and theoretical concepts (Morrison and Tietz 1957; Morrison 1964; Kovalchuk and Tsvirenko 1997; Kizhina et al. 2020) indicate that as a result of a historically long process of differentiation of species in each region of the range, a phenotype is formed; it is closely related to the original ecological and climatic characteristics of the habitat. Therefore, there is a connection between the adaptive capacity of blood and geographical characteristics (Kovalchuk and Yastrebov 2003; Bottaeva et al. 2019), seasonal variability (Wiger 1979; Wołk 1981; Tarakhtiy et al. 2009; Kusumoto 2015), population dynamics (Lazutkin et al. 2016), technogenic pollution (Topashka-Ancheva et al. 2003; Tarakhtii and Mukhacheva 2011; Tête et al. 2015; Orekhova 2018), hypoxia (due to burrowing, elevation, or diving hypoxia), and temperature (Bolshakov et al. 1989; Frase 2002; Thomas and Ono 2015; Bottaeva et al. 2019).

Based on of these literature data, within the order Rodentia, the species-specificity of the hematological indicators is difficult to analyse due to the heterogeneity of the compared samples for many natural factors (habitat, year, season of capture, and other) and endogenous parameters (sex, reproductive-age 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 blood” interspecific differences. Voles as a biological type can be regarded as ‘specialists’ in maximising biological production, ensuring the sustainable existence of species in the upper trophic levels (McNab 1992). This study aimed to compare three species of voles –

Clethrionomys glareolus, *Clethrionomys rutilus*, and *Craseomys rufocanus* – based on the peripheral red and white blood indices. The samples were calibrated by the catching sites, year of the catching, and reproductive-age status.

Materials And Methods

Sites of the catching. We used materials obtained in 2020 during the monitoring of small mammals in the Visim State Nature Biosphere Reserve, protecting an area of southern taiga in the Middle Ural Mountains (Zapovedniki yevropeyskoy 1988). The entire study area belongs to the temperate continental climatic region. 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 (with fluctuations from 350 to 700 mm). In terms of physical and geographical zoning, the study area belongs to 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, gray alder, dwarf shrub, green moss, and grass). The catching site for the objects ($57^{\circ}22'\text{N}$, $59^{\circ}46'\text{E}$, 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 height of 699 m (Bolshoi Sutuk mountain).

The objects of study. We examined representatives of three species of rodents of the subfamily Arvicolinae: the bank vole (*Clethrionomys glareolus* Schreber, 1780), the red-backed vole (*Clethrionomys rutilus* Pallas, 1779), and the gray red-backed vole (*Craseomys rufocanus* Sundevall, 1846). For more than two decades, *Cl. glareolus* has been dominant in this area, and the captures of *Cl. rufocanus* and *Cl. rutilus* have been rare (Kshnyasev and Davydova 2021).

Cl. rufocanus is distributed in the northern Palaearctic, extending from northern Scandinavia through northern Russia to Kamchatka, northeastern and northern Korea, Mongolia, China, Sakhalin (Russia), and Hokkaido (Japan). In the Ural Mountains, the area of *Cl. rufocanus* in the meridian direction extends far to the south, including mountainous landscapes (Berdyugin 1984). Within northeastern Asia, this species has a sympatric range with *Cl. rutilus*, which has a Holarctic distribution, found throughout northern Europe, Asia, Alaska, and Canada. *Cl. glareolus* has a Palearctic distribution. It is native to Europe, Asia Minor, and parts of Western Siberia. In Europe, it is found from Southern Ireland, the British Isles, and the central and eastern Pyrenees to the Black Sea regions of Turkey; it lives in isolation in southwestern Transcaucasia (Macdonald 2001). The northern border of the area as a whole coincides with the border of the distribution of forests, that is, it runs through the central regions of the Kola Peninsula, along 65°N on the Ural ridge and south with the northern border of the forest-steppe (Shenbrot and Krasnov 2005). *Cl. rutilus* and *Cl. glareolus* have overlapping geographic ranges in Europe and Asia.

These species are closely associated with the forest and forest-steppe zones; they inhabit several mountain systems, rising to the mountains' upper belts, but there are some habitat differences. *Cl. rutilus*

are more typical of the indigenous boreal taiga of the lower sub-belt; they go far to the north up to the coasts of the Polar Basin, where they are colonising the tundra biotopes (Chernyavsky et al. 2003). *Cl. glareolus* is more typical of the mixed forests of the upper sub-belt. It penetrates the tundra and steppe only through the floodplain forests of river valleys. It is not found on bare soil, and ample ground cover seems necessary (König 1973). *Cr. 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 only inhabit other biotopes when population is highly abundant (Berdyugin 1984).

All three species have a similar dietary spectrum but differ from each other in the proportion of different types of feed in the diet. Thus, *Cr. rufocanus* is characterised by a predominance of the vegetative parts of plants rather than seeds in the diet. The seeds of herbaceous and woody plants are dominant in the diets of *Cl. glareolus* and *Cl. rutilus* for all seasons. At the same time, *Cl. rutilus* is considered to be a plant polyphage: it supplements its diet with lichens and berries (Hansson 1985).

Research methods. The capture of animals was carried out in the spring and autumn period in 2020 (the year of the peak of the population) using wooden live traps. The rodents were brought to the laboratory and after 3 days of habituation, the animals were sacrificed by decapitation. Trunk blood from each animal was collected immediately in tubes with sodium citrate as the anticoagulant (5.0% [w/v], 0.2 ml citrate/ml blood). Using an Abacus junior vet (Austria) haematology analyser, the following parameters of erythrocytes, leucocytes, and platelets were measured and calculated:

1. A) Red blood cells – haemoglobin concentration (HGB), red blood cell (RBC) count, mean corpuscular haemoglobin (MCH = $HGB / RBC \text{ count}$), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCHC = $HGB / RBC \text{ count} \times MCV$), and the degree of variability in the volume of erythrocytes (RDW-SD).
2. B) White blood cells – total leucocyte (WBC) count, and the absolute and relative numbers of lymphocytes (LYM), monocytes (MID), and granulocytes (GRA);
3. C) Components of haemostasis – platelet (PLT) count, mean corpuscular volume (MPV), and the degree of variability in the volume of platelets (PDW-SD).

The species is attributed by the exterior, craniological, and odontological signs). Segregation into reproductive-age groups (overwintered individuals, immature and sexually mature underyearlings) was carried out by analysing the reproductive and dental systems (Olenev 2002). In this work, the analysis included immature (im) and overwintered animals (ow) [females and males]: *Cl. glareolus* (im [31 and 52]), (ow [10 and 14]); *Cl. rutilus* (im [3 and 12]), (ow [2 and 5]); and *Cr. rufocanus* (im [7 and 7]), (ow [5 and 5]).

The calculations were performed using STATISTICA version 8.0 and STATGRAPHICS version 8.0 software (StatSoft 2012). For the pairwise comparison of samples, the nonparametric Mann–Whitney *U* test was used; it does not require normally distributed data. Multivariate analysis of the haematological similarity of the samples was performed by principal components and classification analysis (PCCA). This

technique transforms multivariate data of intercorrelated variables into a new mutually uncorrelated data set by linear variables called principal components (PCs). Thus, PCCA provides reduces the entire data set's dimension and clusters analysed samples in the PC scale range (Mather 1986). The interpretation of PCs was carried out in the module of one-way analysis of variance (ANOVA) with two factors, namely species and the reproductive-age groups. Interconnection between PC values and haematological parameters is based on correlation analysis.

Results

The graphic illustration of the interspecies variability of blood parameters, taking into account the reproductive-age status, is shown in Figures 1–3.

The highest RBC count was found for *Cr. rufocanus*, while *Cl. glareolus* had the lowest number of erythrocytes in peripheral blood and a lower saturation of these cells with haemoglobin (MCHC). *Cl. rutilus* exhibited an increased erythrocyte volume (MCV) and haemoglobin content (MCH) (Fig. 1).

Differences in white blood are associated primarily with the total number of lymphocytes and granulocytes (*Cl. glareolus* < *Cl. rutilus* < *Cr. rufocanus*) and monocytes (*Cr. rufocanus* < *Cl. rutilus* ≤ *Cl. glareolus*) (Fig. 2). *Cr. rufocanus* had the highest total WBC count.

Of the three indicators of haemostasis (Fig. 3), the gray red-backed vole had the highest number of platelets (*Cl. rutilus* ≤ *Cl. glareolus* < *Cr. rufocanus*). The red-backed vole had fewer platelets and the smallest platelet volume (*Cl. rutilus* < *Cl. glareolus* = *Cr. rufocanus*) and the smallest width of their volume distribution (*Cl. rutilus* < *Cl. glareolus* < *Cr. rufocanus*).

The interspecific variation of voles from two reproductive-age groups can be represented graphically using a multivariate comparison of data by PCCA (Fig. 4).

Based on the close relationship between the PC values and haematological data (Table 1), the main contribution to the variability of PC-2 is made by the MCH, MCV, and MPV parameters.

The PC-1 axis is based on correlation with other indicators. All three species, taking into account the reproductive-age status, were quite well isolated in the plane of two components (Fig. 4). The similarity of *Cr. rufocanus* with *Cl. glareolus* is obvious in PC-2, and *Cl. rutilus* forms the most distant cluster, a phenomenon that is explained by the three indicators. The distance between *Cl. glareolus* and *Cl. rutilus* in PC-2 is smaller than between these species and *Cr. rufocanus*. The level of interspecies differences is slightly higher for immature under-yearlings.

Comparison of interspecific and ontogenetic variations is presented in Figure 5 in a joint analysis of two reproductive-age groups. Based on the close relationship between the PC values and haematological data (Table 2), the main contribution to the variability of PC-1 is made by the erythrocyte and platelet parameters. The PC-2 axis is based on correlation with indicators, first of all, white blood indicators and values of RDW-SD and PDW-SD. ANOVA shows the significant difference of PC-1 values between

reproductive-age status and absence of such difference between species (Fig. 5). The PC-2 values reflect the species themselves and do not depend on the reproductive-age status. The results show that the erythrocyte and platelet parameters have a high degree of ontogenetic changes, which exceeds the level of interspecies differences, while the white blood parameters have a clear interspecific variation that is higher than the ontogenetic variability.

Discussion

Red blood. In the phylogeny of vertebrates, the transition to a terrestrial lifestyle was associated with the deepest rearrangements in the system of external respiration. Life in terrestrial conditions required a large expenditure of energy and, consequently, the oxygen demand of tissues increased. An increase in blood aeration has been achieved due to: 1) an increase in the oxygen-carrying function of the blood due to an increase in the number of erythrocytes; 2) a reduction in the size of erythrocytes; 3) an acceleration in the movement of blood through the vessels; and 4) an increase in the intracellular concentration of haemoglobin and its affinity for oxygen (Snyder 1973; Hochachka and Somero 1980; Snyder and Sheafor 1999; Kostelecka-Myrcha 2002; Aliko 2008).

In this regard, researchers believe that during the evolution of vertebrates, the increase in the concentration of erythrocytes in the peripheral blood was inversely related to their size. According to the literature, mammals have the greatest number of erythrocytes: $9 \times 10^6/\mu\text{l}$, compared with $3 \times 10^6/\mu\text{l}$ in birds, $0.90 \times 10^6/\mu\text{l}$ in reptiles, $0.46 \times 10^6/\mu\text{l}$ in tailless amphibians, and $0.08 \times 10^6/\mu\text{l}$ in tailless amphibians (Hawkey et al. 1991). The volume of RBCs varies widely between mammalian species, from $\sim 15 \text{ mkm}^3$ to $\sim 90 \text{ mkm}^3$ and in reptiles it is up to 300 mkm^3 (Wintrobe 1933; Hawkey et al. 1991).

The sample of the *Cr. rufocanus* studied by us, especially in relation to the bank vole, was characterized by a higher RBC count with smaller cell size (MCV) (see Fig. 1). Such a pattern confirms the previously noted inverse relationship between the number of erythrocytes and their size. An increase in the proportion of 'small' erythrocytes can be considered a better option of blood aeration, which is quite understandable from the general physicochemical law standpoint: the finer the medium, the larger the contact area of the two phases, and the higher the diffusion rate. In addition, takes place a higher degree of cell saturation with haemoglobin (MCHC). This situation usually arises due to an increase in the surface-to-volume ratio, that is, due to discoid biconcave shape. Even with the same capillarisation, it provides greater contact with oxygen in the lungs, and it is a criterion for the functional usefulness of erythrocytes. Besides, the discocyte has high deformability and elasticity, features that allow it to move both in large vessels and small capillaries and are necessary for the functional viability of erythrocytes (Zinchuk 2001; Kostelecka-Myrcha 2002).

Compared with *Cr. rufocanus*, *Cl. rutilus* demonstrates a different strategy for achieving blood oxygen capacity: there are larger erythrocytes with higher haemoglobin content, with fewer of them in the peripheral blood. This situation can arise when excluding one or more intermediate mitotic divisions in nuclear erythroid progenitors, induction of erythroid differentiation due to activation of haemoglobin

biosynthetic 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 increased RBC membrane surface area and the permeability of their plasma membrane for passive diffusion of cations (Parshina et al. 2007). The cells are thicker as an adaptation for the slow release of oxygen.

For *Cl. glareolus*, due to the low number of peripheral blood erythrocytes and their haemoglobinisation (see Fig. 1), it is assumed that the oxygen capacity of their blood is low in comparison with other species. As the literature data show, *Cl. glareolus* is characterised by a high proportion of erythrocyte precursor cells (> 2% reticulocytes) in the circulatory bed (Tarakhtiy and 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 *Cl. 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 Tarakhtiy and Mukhacheva 2011), increased erythrodieresis in *Cl. glareolus* is not compensated by a sufficient level of a bone marrow haematopoiesis.

The revealed interspecific features of respiratory capacity of red blood should be compared with the basal metabolism indicators, which determine, at the organismal level, the total value of mitochondrial respiration of tissues and consequently ATP production under aerobic conditions (Ernster and Schatz 1981).

In warm-blooded animals, 30%–80% of the daily energy exchange is constituted by the basal metabolic rate (BMR) (Blaxter 1989), which is the sum of the metabolically active organ energy consumption. In addition to the heart and intestines, which perform mechanical work, the internal organs expend energy mainly on the processes of biosynthesis and active transmembrane transport (Hochachka and Somero 1980). Locomotor muscles at rest have very low oxygen and ATP consumption, contributing very little to basal metabolism (McArdle et al. 2014).

Naturally, the BMR depends on the taxonomic affiliation, which determines the species' ecological and behavioural characteristics, and also dietary spectrum (Koteja and Weiner 1993; McNab 2008). In particular, when leveling differences in body weight, animals adapted to living in arid environments (these are usually 'hamsters') tend to have a slower metabolism than those that live in harsh conditions characterised by low temperatures, long winters (these are usually 'vole') (McNab 1979; Maloiy et al. 1982; Lovegrove 2003; Dittmann et al. 2015). Features of the diet affect the specific dynamic action of

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

Despite the 'innate' adaptation of the *Cr. rufocanus* to the conditions of northern latitudes, according to the literature data, *Cr. rufocanus* has a relatively low BMR in the thermoneutral range (20°C) and when cooled (0°C). The best heat-insulating properties of fur in *Cr. rufocanus* determine the lower intensity of chemical thermoregulation, that is, basal metabolic reactivity on cooling (Bashenina 1977). The geographical differences are insignificant (2.7 ml O²/g-hour in Polar Urals and 2.98 ml O²/g-hour in South Urals) (Kovalchuk and Yastrebov 2003). It is likely that concentrating in stony placers, where the temperature and humidity regimes are relatively constant, *Cr. rufocanus* avoids the direct influence of extreme abiotic factors; therefore, they can survive critical periods without significant energy expenditures. The second explanatory factor for a low BMR may be the consumption of low-calorie food: the occurrence of vegetative parts of plants in the stomachs of *Cr. rufocanus* is more than 50%.

The BMR of *Cl. rutilus* is higher than that of *Cr. rufocanus*, and it is sharply subject to latitudinal zoning (5.6 ml O²/g-hour in the Polar Urals, 3.45 ml O²/g-hour in the Middle Urals, and 3.16 ml O²/g-hour in the South Urals) (Kovalchuk and Yastrebov 2003). According to Bashenina (1977), *Cl. rutilus* has a high intensity of chemical thermoregulation: the metabolic rate under the action of cooling (at 0°C) in this species increases 2.7 times, which is significantly more than in the other two species, especially *Cr. rufocanus*. The observed differences are related to the fact that *Cl. rutilus* has a higher thermal conductivity of the integument due to the less dense and shorter fur.

The respiration parameters found in *Cl. glareolus*, according to Kovalchuk and Yastrebov (2003) are slightly higher than in *Cl. rutilus* (3.5 ml O²/g-hour in the Polar Urals, 3.6 ml O²/g-hour in the Middle Urals, and 4.5 ml O²/g-hour in the South Urals); however, geographical differences in energy metabolism do not appear when moving to the northern regions. The decreased resistance of *Cl. glareolus* to prolonged exposure to cold in the experiment (progressive hypothermia, decrease in animal weight, oxygen consumption, mitochondrial protein in tissues) limits the successful movement of this species into the Subarctic, in comparison with *Cl. rutilus* and *Cr. rufocanus*.

It is known that the criterion for the high adaptability of a species to its habitat is the greatest biological efficiency at the lowest possible level of basal metabolism. In this regard, the comparatively low BMR in *Cr. rufocanus* indicates that the organisation of metabolic processes aims to optimise the body's expenditure of functional and energy reserves. At the same time, RBCs have less demand in the processes of gas exchange. A decrease in the functional load on these cells leads to the long-term preservation of the optimal structure, an increase in their life expectancy, and, as a consequence, the number of the population in the circulatory bed.

In *Cl. glareolus*, the high organ oxygen demand leads to an increase in the erythrocyte physiological load. The intensive functioning of the structures of differentiated cells is always accompanied by an increase in their decay rate (so-called 'wear metabolism'). The high intensity of destruction of erythrocytes in *Cl. glareolus* is not balanced in the proper volume by erythropoiesis, apparently due to the low efficiency of compensatory mechanisms of the bone marrow.

The *Cl. rutilus* occupies intermediate positions in terms of the tensions of energy exchange and the state of 'well-being' of the erythrocyte population. Most likely, the erythropoiesis system is in a state of 'economical' functioning (blocking of cell division with induction of differentiation). At the same time, there is a production of larger erythrocytes with reduced respiratory capacity.

The results show that erythrocyte parameters have a high degree of ontogenetic changes (Fig. 1) that exceed the level of interspecies differences (PC-1, see Table 2 and Fig. 2). In principle, the higher values of erythrocytes in overwintered animals, relative to immature individuals of each species, indicate a higher functionality of red blood. This phenomenon is most likely provoked by the higher energy requirements for growth and reproduction in this group. It should also be borne in mind, in the year of the population size peak, the 'mission' of reproductive function is assigned exclusively or mainly on the overwintered group of individuals (Kshnyasev and Davydova 2021).

White blood. To implement protective mechanisms for pathogen (from viruses to parasitic worms) recognition and neutralisation, there are a set of universal cells (leucocytes) that often behave like independent unicellular organisms. They together represent the main cellular link of innate and acquired immunity. The cells that embody a nonspecific ('innate') immune response include (in addition to tissue macrophages and dendritic cells), cells of the peripheral white blood of the granulocytic series (neutrophils, eosinophils, and basophils), which recognise and destroy foreign particles by phagocytosis, or, in the case of large foreign bodies (e.g. parasites) due to the release of harmful particles upon direct contact. The features of nonspecific immunity, which is otherwise called constitutional, are genetic selection, which determines its minimal variability within the species (Sheldon and Verhulst 1996; Litman et al. 2005). The components of the nonspecific immune defence are important mediators in activating the mechanisms of acquired immunity, which, first of all, is realised by T and B lymphocytes (Lydyard and Porakishvili 2012). The acquired immune system, as a result of a later stage of evolution, provides a more intense immune response, as well as immunological memory, due to which each foreign microorganism is 'memorised' by antigens unique to it. This type of immunity is most susceptible to the modifying influence of various environmental factors (Lokhmiller and Moshkin 1999).

In *Cr. rufocanus*, the increased immune status is confirmed by the highest content of white blood corpuscles, both due to lymphocytes and granulocytes, compared with the other two species (see Fig. 2). Unlike the *Cl. glareolus* and *Cl. rutilus*, *Cr. rufocanus* demonstrates a higher level of sociality that is indirectly evidenced by the features of the sound signaling of this species, namely, the presence of 'chirping' in the vocal repertoire (Rutkovskaya 1992). In the natural environment for this species, the formation of the so-called 'matrix clusters' occurs when sexually mature female underyearlings create

their areas on the periphery of the maternal territory and reproduce successfully (Ims 1989). Thus, the high frequency of intragroup contacts may also result in the maximum likelihood of transmission of pathogens within a species, a possibility that requires an activated immune system.

In addition to sociality, to the factors influencing the immune status can also be attributed the thermoregulatory function. The reduction of the immune response under low temperature may occur due to competition with thermoregulation for limited resources. *Cl. rufocanus*, with a high intensity of physical thermoregulation, is considered adapted to cool environment and cold stress. Even at temperatures under 23°C this type of rodents can allocate enough energy to maintain their body condition and exercise immune function (Kusumoto and Saitoh 2008). With this *Cl. rufocanus* differs from *Microtus agrestis* and *Apodemus peninsulae*, which reduce the immune status at temperatures less than 25°C (McDevitt and Speakman 1994; Speakman et al. 2003; Masaki et al. 2005).

Among the evaluated species, *Cl. glareolus* is distinguished by the lowest number of WBCs due to the reduced number of granulocytes and lymphocytes (see Fig. 2); this suggests a lower level of functioning of this species' immune system relative to *Cr. rufocanus*. A distinctive feature of *Cl. glareolus* is low mobility and exploratory activity; combined with the minimum size of the territory it uses (Wartha and Henriques-Normark 2008). Thus, this species moves away from interspecies competition and intragroup contacts, and hence there is a lower probability of transmission of pathogens. In *Cl. rutilus*, a high level of motor and exploratory activity (Kravchenko and Moskvitina 2008) is associated with large sizes of individual plots, explaining this species' intermediate positions in our research.

The immature individuals of the *Cl. glareolus* and *Cl. rutilus* have a higher lymphocyte/granulocyte ratio (*Cr. rufocanus* [5.7] < *Cl. rutilus* [7.1] < *Cl. glareolus* [7.8]), which indicates the predominance of specific immunity over nonspecific. The immature individuals of eurytopic species with high migration activity (compared with 'stenotopic' *Cr. rufocanus*) interact with a wider and less predictable range of pathogens, which only can be resisted if a functional reactivity of the induced immunity is prevailed (Lokhmiller and Moshkin 1999).

Cl. glareolus is characterised by a high level of monocytes (MID) in the peripheral blood (see Fig. 2). Monocytes are a transport form of macrophages that are localised in loose connective tissue and the parenchyma of organs (Kupffer's cells in the liver, microglia in the brain, interstitial cells in the lungs) and along the blood vessels. (Zimmerman et al. 2010; Lydyard and Porakishvili 2012). The increase in the number of monocytes in *Cl. glareolus* is probably associated with an increased tissue demand for macrophages due to, among other things, increased intracellular and intravascular haemolysis. It is known that haemolysis can occur in the lumen of blood vessels (intravascular haemolysis) and the cells of the reticuloendothelial system (intracellular haemolysis). In response to the increased destruction of erythrocytes, an increase in the number and function of macrophages occurs (Grigorovich 1966).

The results show that overwintered individuals of *Cl. rufocanus* and *Cl. rutilus* have a decreased immune status; this is confirmed by the lower content of white blood corpuscles (Fig. 2). Immune function may compete for available resources with other functions, including growth and reproduction (Sheldon and

Verhulst 1996; Lochmiller and Deerenberg 2000; Zuk and Stoehr 2002). In this regard, owing to the high cost of reproduction, immune function is reduced in reproductive voles, which confirms others research (Feore et al. 1997; Saino et al. 2000).

Vascular-platelet mechanism. It ensures the arrest of bleeding in the smallest vessels due to adhesive-aggregation function of platelets (White and Clawson 1980). A characteristic feature of *Cl. rutilus* is the manifestation, relative to other species, of thrombocytopeny, that is, fewer platelets in the peripheral blood with a smaller volume and weak heterogeneity of these cell populations in the circulatory bed. As a rule, smaller platelets are typical with a predominance of young (immature) forms.

Phylogenetic relationships between species. There is a closer distance between *Cl. glareolus* and *Cl. rutilus*, represented both with and without the stage of ontogenesis (Fig. 4,5). The phylogenetic affinity of these species, in addition to cytogenetic (Amramson et al. 2009) and paleontological (Evropeiskaya rzhaya 1981; Smirnov et al. 1986; Tesakov 1996) data, is also confirmed by the absence of differences between species in the number and morphology of chromosomes (Rausch and Rausch 1975), by the fact of hybridisation in the experiment, as well as in the natural environment (Dekonenko et al. 2003; Osipova and Sokin 2008). In the sympatry zone of these species, including in the Urals, thanks to molecular genetic methods, bank vole individuals with the borrowed mitochondrial genome of the red-backed vole were identified (Amramson 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 complex 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 frequency of occurrence of this introgression in populations of *Cl. glareolus* made it possible to put forward a hypothesis about the adaptive nature of this phenomenon 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, *Cl. glareolus* can be called a species with limited homeostatic capabilities of the red blood (fewer RBCs with a lower density of haemoglobin distribution in the cell, more pronounced anisocytosis) to disperse to the north under more extreme hypothermia conditions.

By contrast, in *Cr. rufocanus*, the tissue oxygen demand can be successfully realised due to the increased number of 'smaller' erythrocytes with a high density of haemoglobin distribution in the cell more uniform in size.

The respiratory blood capacity of *Cl. rutilus* is maintained at the level of *Cr. rufocanus*, but differently, that is, with fewer RBCs in the circulatory bed but with higher haemoglobin content in them.

The parameters of white blood have a clear interspecific variation, higher than the ontogenetic variability. *Cr. rufocanus*, relative to the other two species, has an even higher immune status that is supported by granulocytes and lymphocytes. This is primarily due to the desire for social contacts inherent in this species and, therefore, the likelihood of infection with pathogenic agents. A high number of monocytes in the peripheral blood in *Cl. glareolus* is probably associated with an increased tissue demand for macrophages, including due to increased erythrodiuresis.

Interspecies differences in erythrocyte and leukocyte indices are manifested exclusively in each of two reproductive-age groups. The higher functional capacity of red blood in overwintered individuals is provoked by the higher energy requirements for growth and reproduction in this group. Owing to the high cost of reproduction, for each species, the immune function of reproductive individuals is low.

Declarations

Funding The experiments described in this manuscript were supported by the State Contract (№ 0400-2019-0006) of the Institute of Plant and Animal Ecology, UB RAS.

Compliance with ethical standards

Ethical approval 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).

Conflict of interest/disclaimer The authors declare no financial, consulting or personal conflicts of interest in relation to the work presented.

Consent to Publish Not applicable

Consent to Participate Not applicable

Availability of data and material All data generated or analysed during this study are included in this published article [supplementary information file].

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Figures

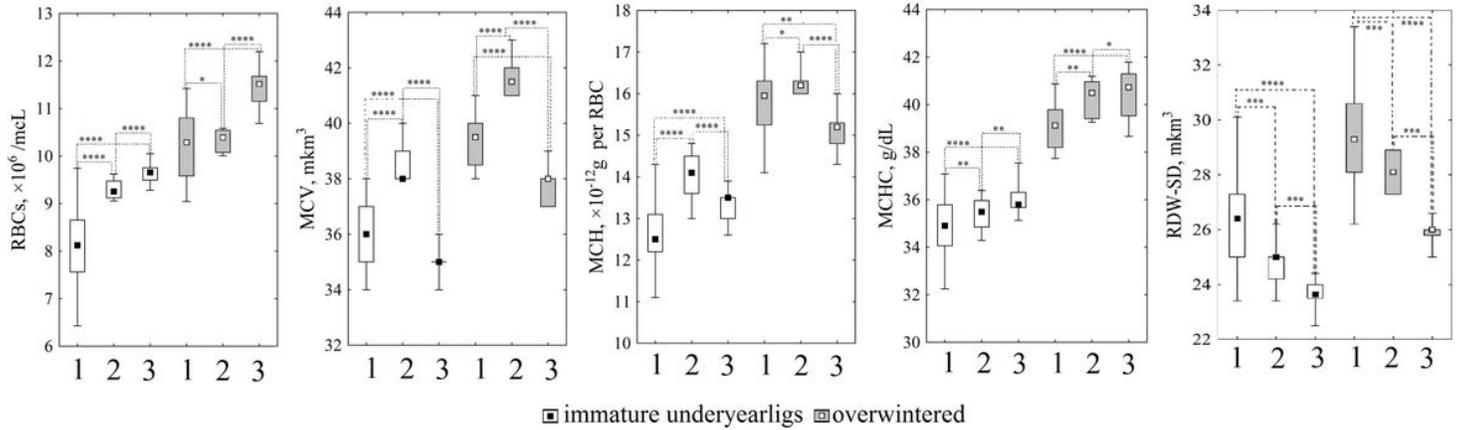


Figure 1

Erythrocyte indices (median, minimum–maximum, and 25%–75% quartiles) of individuals of three species of voles from two reproductive-age groups: 1 – *Cl. glareolus*; 2 – *Cl. rutilus*; 3 – *Cr. rufocanus* *– The significance level (p) for pairwise comparison of samples is given in accordance with the U-test: * > 0.1; ** < 0.1; *** < 0.05; **** < 0.0001

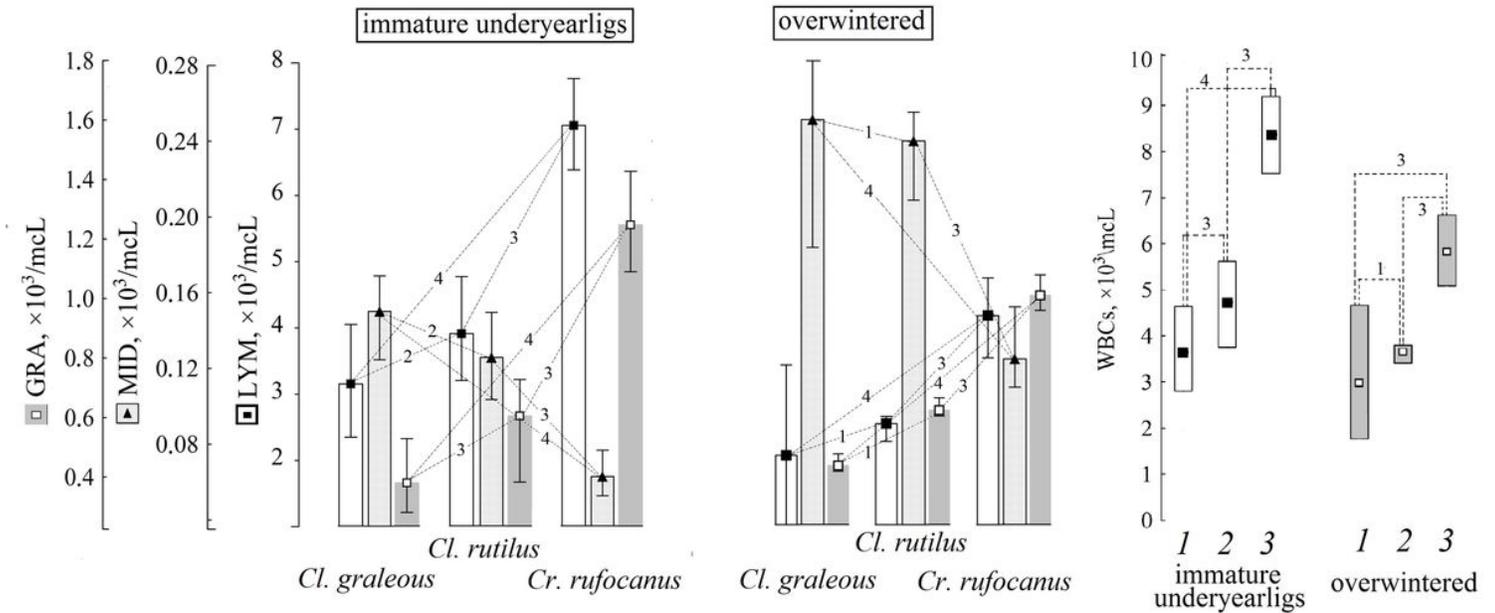


Figure 2

White blood indices (median and 25%–75% quartiles) of individuals of three species of voles from two reproductive-age groups 1 – *Cl. glareolus*; 2 – *Cl. rutilus*; 3 – *Cr. rufocanus* 1,2,3,4– The significance level (p) for pairwise comparison of samples is given in accordance with the U-test: 1 > 0.1; 2 < 0.1; 3 < 0.05; 4 < 0.0001

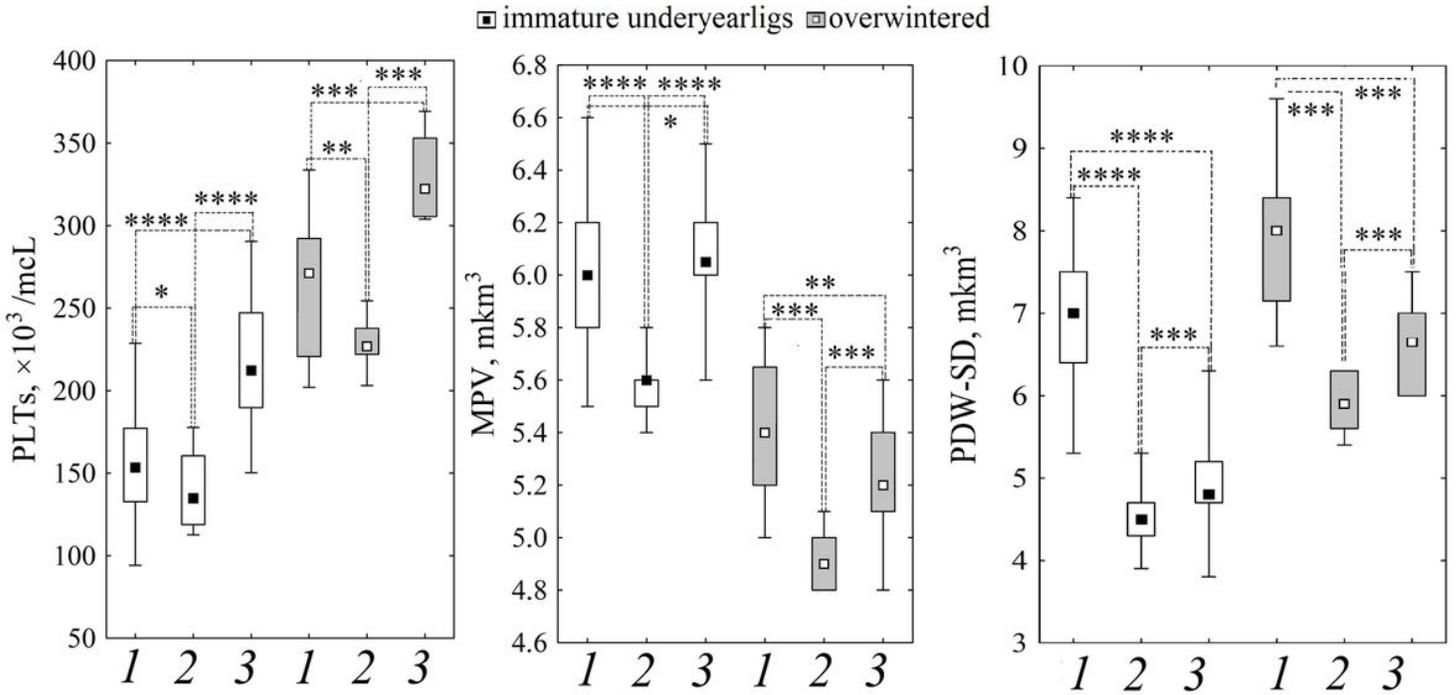


Figure 3

Indices of hemostasis (median, minimum–maximum, and 25% –75% quartiles) in three species of voles from two reproductive-age groups: 1 – *Cl. glareolus*; 2 – *Cl. rutilus*; 3 – *Cr. rufocanus* *–The significance level (p) for pairwise comparison of samples is given in accordance with the U-test: * >0.1 ; ** <0.1 ; *** <0.05 ; **** <0.0001

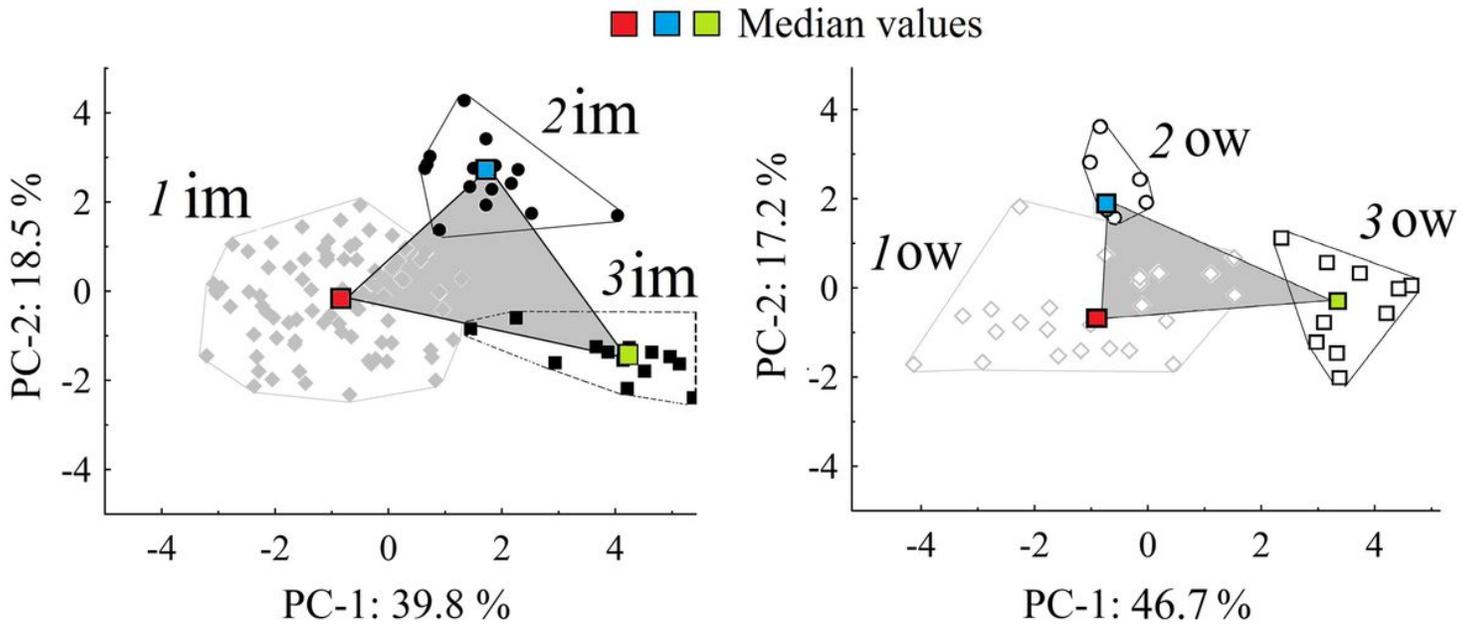


Figure 4

Arrangement of the three samples in three species of voles (1 – *Cl. glareolus*; 2 – *Cl. rutilus*; 3 – *Cr. rufocanus*) from two reproductive-age groups (im– immature underyearlings; ow – overwintered)

individuals) in the plane of the two principal components (PC-1, PC-2) based on the 11 blood parameters

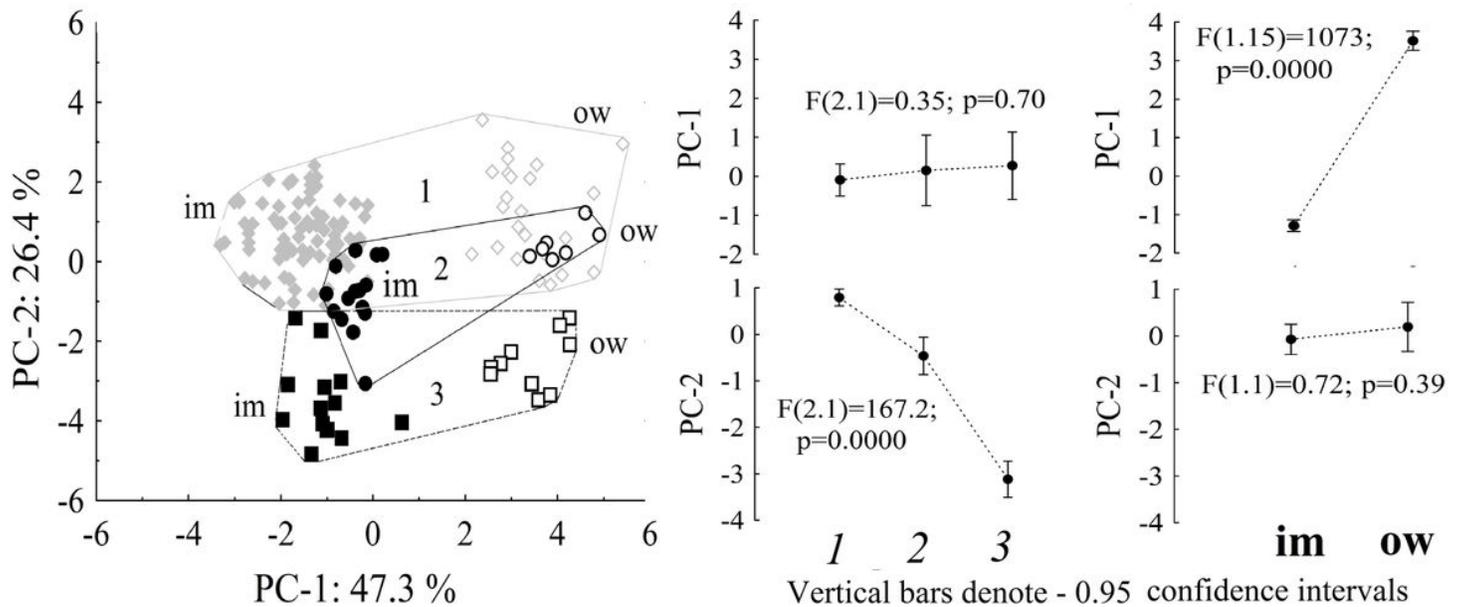


Figure 5

Arrangement of the six samples in three species of voles (– Cl. glareolus; 2 – Cl. rutilus; 3 – Cr. rufocanus) from two reproductive-age groups (im– immature underyearlings; ow – overwintered individuals) in the plane of the two principal components (PC-1, PC-2) based on the 11 blood parameters.

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