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CYTOGENETIC EFFECTS OF COMPLEX POLLUTION OF WATER BODIES OF THE NIZHNI NOVGOROD REGION

E.B. Romanova, E.S. Ryabinina

National Research Lobachevsky State University of Nizhni Novgorod,
603950, Russia, Nizhni Novgorod, Gagarin Ave., 23

Abstract. The assessment of micronucleus species in erythrocyte of amphibians living in different hydrochemical conditions of water bodies of the Nizhni Novgorod region in the dynamics of four-year monitoring (2016-2019) was carried out. The content of erythrocyte of blood of *Pelophylax ridibundus* decreased, the proportion of erythrocyte with micronuclei increased, the ratio of different types of micronuclei changed, attached micronucleidue prevailed in erythrocytes. In the blood of *Pelophylax ridibundus*, attached micronuclei were found 2.1 times more often than in *P. lessonae*.
A positive relationship was established between the sum of all types of micronuclei ($\rho = 0.61$), the number of erythrocytes with attached micronuclei ($\rho = 0.64$) and the content of sulfates in the water; the number of erythrocytes with loosened micronuclei and the content of nitrites ($\rho = 0.47$). Negative associations were found between: the content of erythrocytes in the blood and the complex pollution of the water ($\rho = -0.47$) and the concentration of manganese ($\rho = -0.54$); the proportion of erythrocytes with rod-shaped micronuclei and a general level of pollution ($\rho = -0.54$), as well as with the content of manganese in the water ($\rho = -0.50$). The results obtained reflect the ecological dysfunction of the habitat and indicate significant violations of the cytogenetic homeostasis of the organism of amphibians in the urbanized territory.

Keywords: amphibians, micronucleus test, cytogenetic homeostasis

Violation of homeostasis, expressed in a change in the hematological and cytogenetic characteristics of animals, is the first reaction to any stressful effect and appears earlier than these deviations can be detected by physicochemical methods (Zakharov, Krysanov, Pronin, 1996, 2007). Amphibians have long been successfully used as zooindicators of environmental quality. Amphibians living in water bodies of the urbanized territory are characterized by high ecological plasticity and tolerance. They can adapt to existence in severely disturbed landscapes and demonstrate reactions to the entire spectrum of factors characteristic of a given environment. The characteristic of cytogenetic homeostasis of indicator species makes it possible to identify initial changes in the state of organisms in an anthropogenic environment pollution. Our earlier studies revealed an increase in the cytogenetic instability of green frogs in urban areas, manifested by an increase in the proportion of attached and disintegrated micronuclei in erythrocyte and bone marrow, and we obtained dimensional characteristics of micronuclei (Romanova & Ryabinina, 2017, 2018a, b, 2019; Romanova & Ryabinina, 2018; Romanova et al., 2019).

The aim of the work was to analyze the relationship between the indicators of cytogenetic stability of indicator species of amphibians of the Nizhni Novgorod region and the hydrochemical characteristics of their environment in the dynamics of four-year monitoring (2016–2019).

Materials and methods

In the work, we used individuals from natural populations of *Pelophylax ridibundus*, Pallas 1771 and *P. lessonae*, Camerano 1882. The collection of material was carried out from 2016 to 2019 in the territory of ten water bodies of the Nizhni Novgorod region. Water samples were taken in the coastal zone of the studied reservoirs. In water, total content of iron, manganese, copper, chromium, nitrate (NO_3), nitrite (NO_2), lead, nickel, zinc, chlorides, sulfates, sulfides, cobalt and pH were determined using spectrophotometry. Based on the analysis results was calculated specific combinatorial index of water pollution (SCIWP), which is a relative ratio of the pollution degree for surface waters (RD 52.24.643-2002). Blood was obtained from each frog for preparation of smears and determination of the total content of erythrocyte (Menshikov et

al., 1987). For each frog 2000 erythrocyte were scanned (Zhuleva & Dubinin, 1994). Given the shape of the micronucleus and its location relative to the main cell nucleus, the following were distinguished type of micronuclei: a) rounded; b) attached; c) rod-shaped; d) disintegrated. For statistical analysis, we calculated the Kruskal – Wallis criteria (H); the Dunn's test (D); Mann-Whitney (U), the Spearman's rank correlation coefficient (ρ), the Wilcoxon's (W), the z criterion when comparing the proportions. The classification of the studied reservoirs was carried out using the principal component method. The value of the level of statistical significance was taken a = 0.05.

Results and discussion

The species identification of *Pelophylax ridibundus* and *Pelophylax lessonae* was carried out according to the external morphological features generally accepted in determining green frogs (Bannikov et al., 1977). Together with graduate student Elena Kondakova were prepared slides of epithelial cells of the intestines of lake frogs and studied chromosome sets. The work was carried out at the Department of General and Medical Genetics, IBBM Lobachevsky university. The karyotype of *Pelophylax ridibundus* has a diploid number of chromosomes (2n = 26) and includes 5 pairs of large and 8 pairs of small chromosomes (Fig. 1), which corresponds to the standard karyotypes described in the literature (Suryadnaya, 2003).

In the all the studied reservoirs, the concentration of polluting chemicals was high and significantly exceeded the threshold limit value for fishery reservoirs (TLV), which we selected as the most stringent of the existing regulatory and technical documentation. The class of water quality in water bodies in the period from 2016 to 2019 varied and for most bodies of water in 2019 corresponded to IV-V class, according to the calculations of the specific combinatorial index of water pollution (SCWIP). In the subsequent analysis, we used private estimation coefficients as the initial data, which take into account the multiplicity of exceeding the TLV of a specific pollutant at a water body.

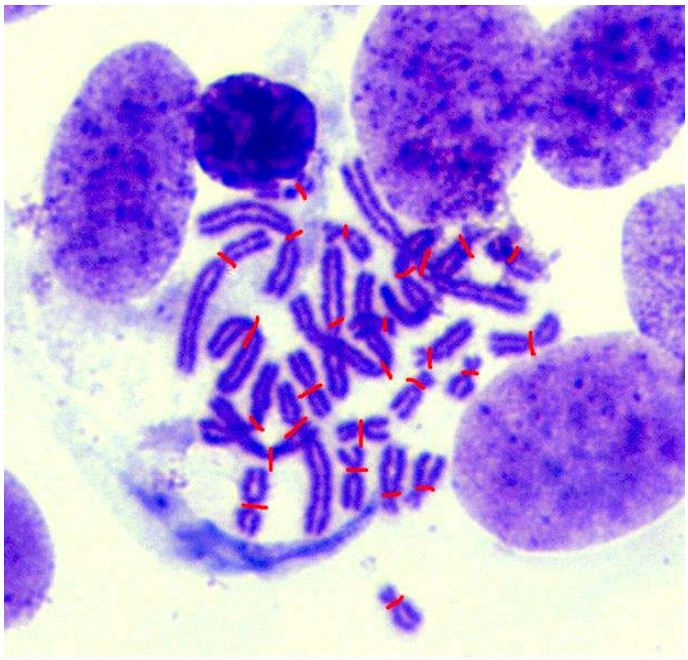

Fig. 1. The chromosomes of *Pelophylax ridibundus* during metaphase
Image of the preparation of intestinal epithelial cells was made by Elena Kondakova & Elena Ryabinina on a direct microscope for studies in a bright field Carl Zeiss Primo Star (Carl Zeiss Miscroscopy GmbH, Germany) with a color digital camera AxioCam105

In order to classify water bodies, a cluster analysis based on the euclidean distance using the full communication method and ordination by the principal component method was carried out. Four groups of water bodies that form separate clusters were identified, with close indicators of chemical pollution of the environment and the dominance of different species of frogs. The I cluster, dominated by *P. ridibundus*, combined bodies of water with the highest copper content (Me 3.10; IQR 1.67) and a high SCWIP value (Me 28.63; IQR 46.7). The II cluster combines the least polluted water bodies with the dominance of *P. ridibundus.* The content of iron and manganese in them did not exceed 2 TLV. The value of the SCWIP for reservoirs of the II cluster: Me- 2.06; IQR 8.37. The III cluster combined samples of water bodies (mainly with the dominance of *P. lessonae*), in which a relatively high content of manganese, iron, copper, and chromium was noted. The value of the SCWIP of the III cluster: Me 12.30, IQR 3.10. The IV cluster – included water samples from the lake Vtorchermet (2016-2018), which was dominated by *P. lessonae* and five water bodies dominated by *P. ridibundus.* This cluster was characterized by a lower iron content compared to the III cluster, the value of the SCWIP: Me 6.27, IQR 2.33.

The analysis of the environment of green frogs in the Nizhni Novgorod region allowed us to proceed to a comparative analysis of the samples to identify quantitative and/or qualitative changes in the cytogenetic parameters of different types of green frogs living in similar and significantly different hydrochemical conditions. First, we note the differences in the number of erythrocytes of peripheral blood of amphibians of different clusters. The suppression of the intensity of erythropoiesis was found in *P. ridibundus* living in conditions of extreme pollution of the aquatic environment (clusters I and III), compared with frogs living in less polluted environmental conditions (clusters II and IV) (Fig. 2). In the blood of *P. lessonae*, a lower content of red blood cells was found compared with *P. ridibundus* ($u = 3.35$, $p < 0.01$), when living in similar environmental conditions (cluster IV).

The erythrocyte with micronuclei of different species were found in the blood of individuals of all populations. In *P. ridibundus* living in the most favorable hydrochemical environmental conditions (cluster II), the proportion of erythrocyte with micronuclei was the smallest (2.9 ± 0.3) ‰ compared to other samples. With an increase in the level of contamination determined by SCWIP, the proportion of erythrocyte

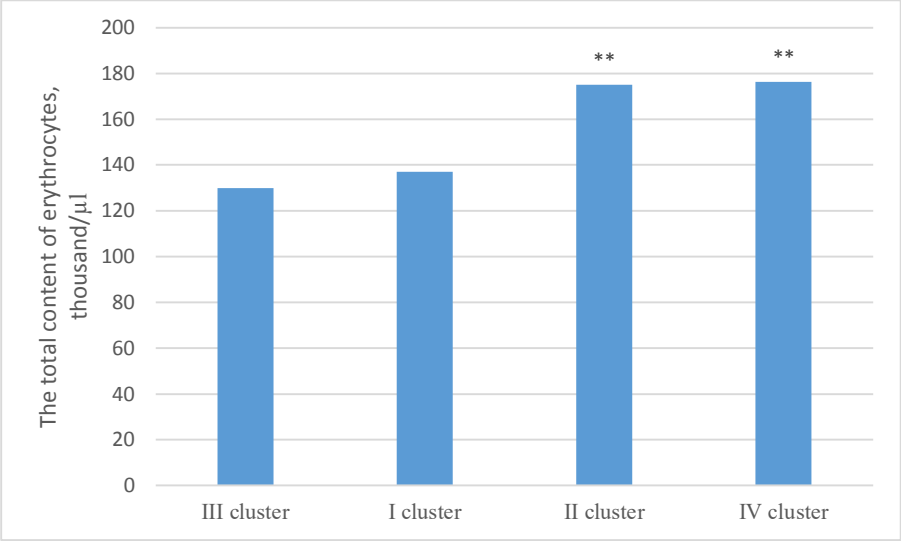

Fig. 2. The total number of the erythrocytes in the blood of *P. ridibundus*, living of the water bodies of different hydrochemical composition
** – statistically significant differences with I cluster are indicated

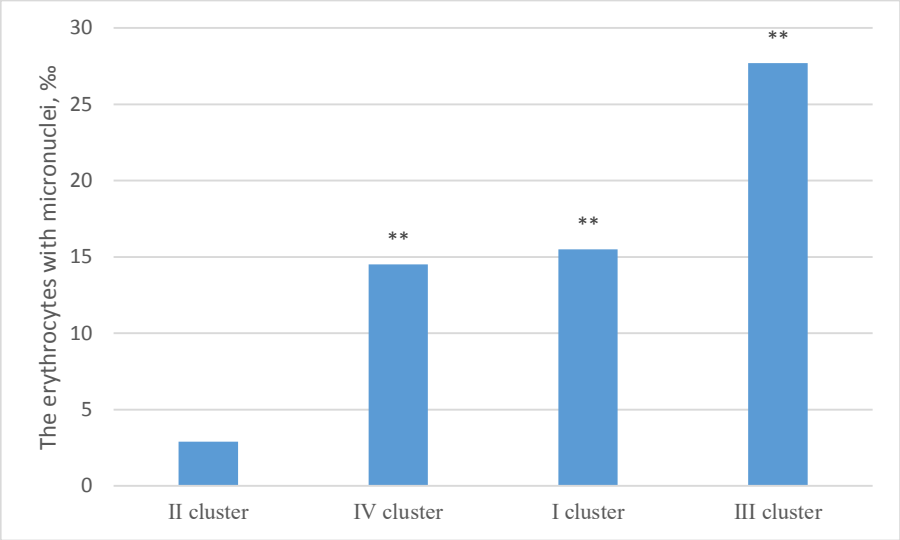

Fig. 3. The proportion of red blood cells with micronuclei (‰) in the blood of *P. ridibundus,* living of the water bodies of different hydrochemical composition
** – statistically significant differences with II cluster are indicated

with micronuclei in the blood of lake frogs increased (Fig. 3).

The ratio of red blood cells with different types of micronuclei also changed in the blood. Determined that, large loosened micronuclei (6.99 ± 0.32 μm^2) prevailed *P. ridibundus* of cluster II. On the contrary, attached micronuclei (1.48 ± 0.03 μm^2) prevailed in individuals of the remaining clusters (Fig. 4).

It is known that disintegrated type of micronuclei belongs to the smallest forms and contains no more than 2–5% DNA of the main nucleus, in contrast to large forms containing 5–12% DNA (Nusse & Kramer, 1984). Micronuclei are form from chromosomal fragments or the whole chromosome, which do not enter the main nucleus during cell division, this leads a change in the mitotic apparatus (Mateuca et al., 2006 Sedelnikova et al., 2007; Fenech et al., 2011; Luzhna et al., 2013).

In this case, small micronuclei arise mainly from acentric fragments of chromosomes, while large ones appear from dicentrics or whole chromosomes (Nusse & Kramer, 1984). It is known that increase in forming the frequency of micronuclei occurs upon exposure to environmental pollutants and mutagens (Kamiguchi & Tateno, 2002; Neri et al., 2003; Kirsch-Volders et al., 2003). It was of particular interest to evaluate the intensity of micronuclei formation in frogs of different species living in similar environmental conditions. There were no statistically significant interspecific differences between the *P. lessonae* and *P. ridibundus* of cluster IV in the total content of red blood cells with micronuclei in the blood. However, in the blood of *P. lessonae*, an increased proportion of red blood cells with micronuclei of the rounded type ($u = 2.97$, $p = 0.003$) and the disintegrated type ($u = 2.00$, $p = 0.04$) was established in comparison with *P. ridibundus*. Under conditions of more intense pollution of the aquatic environment (cluster III) between *P. lessonae* and *P. ridibundus*, differences were found in the total content of micronuclei ($u = 5.84$; $p < 0.01$), as well as in the content of erythrocyte with disintegrated ($u = 2.32$, $p = 0.01$) and attached micronuclei ($u = 6.19$, $p < 0.01$). Cytogenetic anomalies in the form of induction of attached micronuclei in *P. ridibundus* were found 2.1 times more often than in *P. lessonae* (Fig. 5).

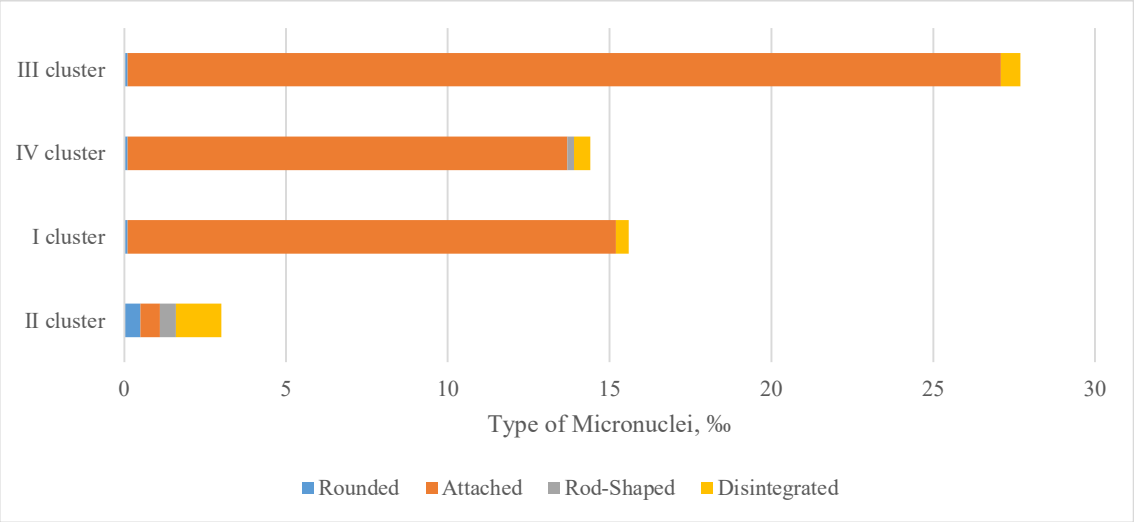

Fig. 4. Change in the ratio of micronucleus types in erythrocytes of *P. ridibundus,* living of water bodies of different hydrochemical composition

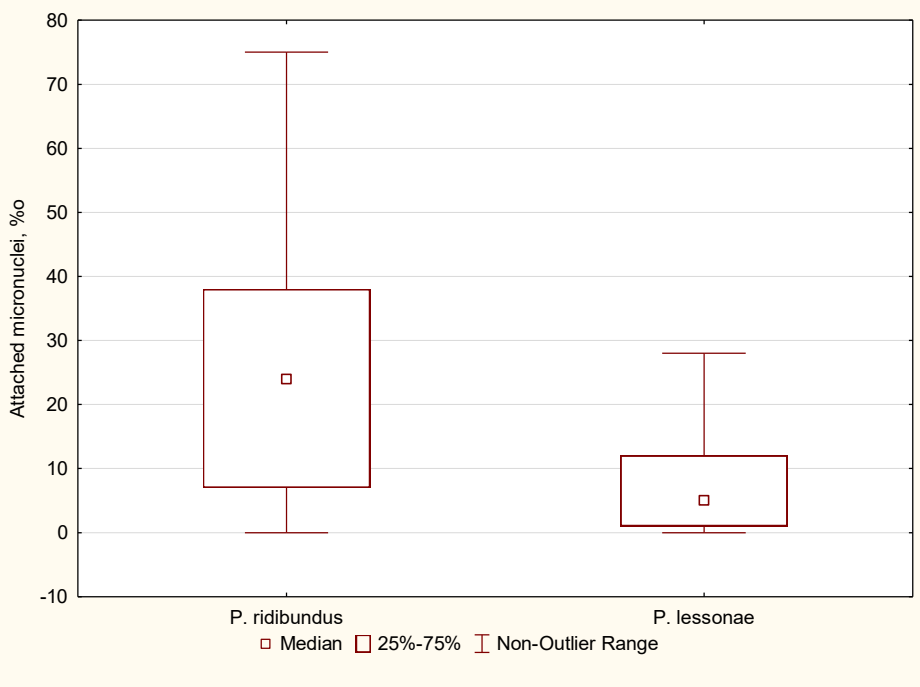

Fig. 5. The proportion of red blood cells with attached micronuclei in the blood of different types of green frogs

Spearman's ranking coefficient revealed a positive relationship between the sum of erythrocytes with all types of micronuclei (ρ = 0.61), the number of erythrocytes with attached micronuclei (ρ = 0.64) and the sulfate content in water; between the number of erythrocytes with disintegrated micronuclei and the content of nitrites (ρ = 0.47). Negative associations were found between: the content of erythrocytes in the blood and the complex water pollution, SCWIP (ρ = –0.47) and manganese concentration (ρ = –0.54); between the proportion of

erythrocytes with rod-shaped micronuclei and a general level of contamination (ρ = –0.54), and manganese concentration (ρ = –0.50).

Conclusion

Analyzing the literature data and our own results, it can be assumed that, under the conditions of complex pollution of the habitat, amphibians had disturbances in mitosis processes, which prevent the inclusion of part of the chromosomes in the main nucleus. The acentric fragments of the chromosomes remained directly in the cytoplasm, subsequently forming the attached type micronuclei. Interspecific differences in the accumulation of micronuclei in amphibian blood erythrocytes can be explained by different biotopic associations (*P. lessonae*, to a greater extent than *P. ridibundus*, is associated with terrestrial biotopes), specific features of nutrition, growth, and lifestyle. Under extreme hydrochemical contamination in species of one ecological group, the processes of erythropoiesis and the parameters of the immune system (responsible for elimination of cells with chromosomal abnormalities) differ significantly more, indicating different ways of adaptation to environmental stress and interspecific features of cytogenetic homeostasis.

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