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Some Cytological Features of *Rana arvalis* Nilss. Frogs on the Territory of the Eastern Ural Radioactive Trace

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Abstract—The study of *Rana arvalis* Nilss. population inhabiting the area of Lake Berdanish, which is polluted with radionuclides to a density of 1000–500 Ci/km^2, revealed several specific cytological, cytogenetic, and phenotypic features indicating potential genetic risk for populations of this territory. The structure of the population under study differs from that of other natural *R. arvalis* populations, reflecting the unfavorable ecological situation in the area, and provides evidence for the presence of specific adaptive features ensuring the existence and reproduction of species populations under new conditions.

The range of studies on the response of animals to different anthropogenic factors is expanding. In particular, this concerns a complex of problems related to the stability of populations. An important role in these studies belongs to the determination of sensitivity of different systematic groups and species to pollutants that act largely depending on the specific functional features of a certain organ or tissue.

A prominent place in studies on the impact, biogenic migrations, and accumulation of pollutants belongs to radioactive pollution due to the severity of possible distant cytogenetic consequences. The intensive development of the nuclear industry and the expanding sphere of its applications increased the possibility of accidental emergency situations leading to the appearance of land and water areas with high radionuclide contents. When a zoocenosis is exposed to radiation in nature, various effects manifested in the mortality of less tolerant organisms and the survival of radioresistant forms may be observed in it (Sokolov and Il'enko, 1978). Some vertebrate species (mainly rodents) characteristically respond to the chronic impact of ionizing radiation by widening the range of natural variation in most of population parameters and morphological indices (Maslova, 1981).

The dynamics of radionuclide accumulation, biogenic migration, and associated effects were studied in detail with reference to several systematic groups (Bezel' *et al.*, 1994; Gunderina, 1997; Kryukov, 1994). However, amphibians, which are widespread, numerous, and account for a considerable proportion of biomass in biocenoses, have not been adequately studied in this respect. The amphibians in all phases of their life cycle are a convenient object for evaluating the state of terrestrial and aquatic ecosystems. There are data on the radionuclide accumulation in the organism of moor frog *Rana arvalis* Nilss. (Usachev *et al.*, 1993). To date, researchers have already described the cases of genetic adaptations expressed in changes in the frequencies of micronuclei and the accumulation of β-chromosomes in *R. temporaria* and *R. arvalis* populations inhabiting polluted territories for 40 years (Eliseeva *et al.*, 1989). Genetic monitoring of the brown frog population from territories of the Republic of Belarus polluted with radionuclides (^{137}Cs 177–2331 kBq/m^2; ^{90}Sr 3.7–284 kBq/m^2) indicated that the frequency of aberrant metaphases in bone marrow cells of these frogs is three to ten times higher than in the control (Eliseeva *et al.*, 1994).

Of all the amphibian species occurring in the Urals, including anthropogenically transformed areas, *R. arvalis* is the most widespread. This relatively abundant and ecologically adaptable amphibian species is highly resistant to the transformed medium (Vershinin, 1985). For this reason, this species was chosen as an object of our study on evaluating certain cytological parameters of amphibians from populations chronically exposed to ionizing radiation. Specific features of the main morphological, morphophysiological, and other parameters of these population parameters were discussed in detail previously (Pyastolova *et al.*, 1996).

MATERIAL AND METHODS

We studied *R. arvalis* population inhabiting the southwestern shore of Lake Berdanish, which is located at a site of the Eastern Ural Radioactive Trace (EURT) with pollution density (^{90}Sr) of 1000–500 Ci/km^2 (the territory of the Southern Ural Reserve near the Test Research Station of the Mayak Production Association, Chelyabinsk oblast). As a control, we used the population of the same species from a relatively clean region of the Dolgobrodskoe reservoir located 90 km west of the reserve boundary. Material was collected in spring and summer of 1993. Cytological indices of young of

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the year and adult animals were studied. For cytological analysis of the liver, we made preparations of 11 adult frogs and 15 young of the year from the polluted site and of the same number of animals from the control population. Each preparation was analyzed under a microscope at a magnification of 10 × 20, and drawings of 100–150 cells were made using an RA-4 drawing apparatus. To determine the area of the cytoplasm and cell nucleus, two perpendicular diameters were measured. There is no accepted standard for the hepatocyte measurement. Specialists working with tissue sections (Ledyaeva, 1949; Vibe, 1961) calculated the cytoplasm volume. As we studied cells spread over the glass slide, it was more correct to calculate the area of their projection (in mm^2) according to the drawing (Bugaeva, 1983).

For cytogenetic analysis, we used corneal epithelium, one of few meristematic tissues in the animal organism with a relatively high mitotic activity in the intact state. This activity and cell size in were studied in adult frogs and young of the year captured in nature, as well as in individuals which initially developed on test and control plots but metamorphosed under laboratory conditions. The Carnoy mixture was used as a fixative (Lillie, 1965). Total specimens were made by conventional methods (Epifanova, 1965) and stained with hematoxylin according to Boemer (Roskin and Levinson, 1957).

To analyze tissue growth, we determined cell density by counting the number of cells per microscopic field limited by a rectangular diaphragm (3025 μm^2 in area) and calculated the average size of an epithelial cell in μm^2. The same preparations were used to analyze mitotic activity (the values are shown in parts per thousand) by scanning 100 microscopic fields per preparation and determining the number of dividing cells among a total of 15 000 cells. Mitotic activity and cell size in the corneal epithelium were studied in young of the year that underwent metamorphosis under laboratory and natural conditions and in mature animals from the test and control plots. Samples of skeletal bones were ashed in a muffle furnace at 400°C and analyzed for β-radioactivity (Bq/g dry weight) in a RFT VA-0-120 counter. The latter was performed by Dr. N.M.Lyubashevskii.

RESULTS AND DISCUSSION

The data on β-radioactivity of *R. arvalis* skeleton showed that the highest radionuclide accumulation (here and below, animals from the polluted territory are discussed in this respect) is characteristic of one-year-old frogs. Radionuclide content in two-year-olds was the lowest, and that in three-year-old frogs was slightly higher. We attribute this fact to high mortality in the part of one-year-old population that accumulated the greatest amounts of radionuclides. Four-year-old frogs were only found in the control sample. Females accumulated almost twice more radionuclides than males.

Table 1. Level of β-radioactivity of the initial dry tissue of *R. arvalis*, Bq/g

Groups of frogs	EURT (n = 11)	Control (n = 11)
Striata	323.0 ± 61.8 (n = 7)	39.5 ± 39.5 (n = 2)
Other morphs	1597.3 ± 1341.7 (n = 4)	4.4 ± 2.6 (n = 9)
Females	933.1 ± 671.6 (n = 8)	30.7 ± 24.5 (n = 3)
Males	95.0 ± 17.2 (n = 3)	3.4 ± 2.6 (n = 8)
One year	1112.3 ± 753.1(n = 7)	0 (n = 1)
Two years	170.0 ± 27.4 (n = 3)	0 (n = 1)
Three years	354.0 ± 0 (n = 1)	5.7 ± 3.15 (n = 7)
Four years	No animals	39.5 ± 39.5 (n = 2)

Note: The data on the same animals are grouped with respect to the morph, sex, and age.

The animals of the striata morph, whose skin is three times less permeable for potassium (p varies from 0.0005 to 0.004) (Vershinin and Tereshin, 1996; Vershinin, 1997), accumulate five times less than stripless animals. The presence of the stripe is controlled by the dominant allele of an autosomal gene (Schupak, 1977). It is apparent that this hereditary physiological feature allows frogs of this morph to gain selective advantage in populations inhabiting polluted and transformed territories. It should be noted that the high average level of radionuclide accumulation by females is accounted for by stripless individuals only (Table 1). Because of a small sample size, the significant distinctions were only revealed with respect to radionuclide accumulation by different morphs (F = 6.066; p = 0.05).

The data on the effect of the chemical substances on the size of hepatocytes and their nuclei and on their relationship in amphibians are scarce. Studies on natural *R. arvalis* populations showed that the size of liver cell increases in animals developing in water bodies polluted by industrial discharge. Moreover, the nucleus increases more than the cytoplasm, and this leads to a decrease in their ratio (Pyastolova and Trubetskaya, 1989). In frogs from the zone of Chernobyl Nuclear Power Plant, hepatocytes with signs of destruction were observed, and other cells of the hemopoetic system were hypertrophied (Nosova *et al.*, 1994).

Correlation analysis (Table 2) revealed no direct relationship between the size of hepatocyte nuclei and hepatic index in young of the year and mature frogs. In adult animals from the polluted site, hepatic index, the size of hepatocytes, and the karyoplasmatic ratio proved to correlate with each other. A significant correlation between sizes of the liver and its cells was revealed for young of the year and adult frogs (correlation coefficient was two times higher). This is evidence that liver cells of animals from the polluted site contain

Table 2. Coefficient of correlation between hepatic index, sizes of hepatocytes and their nuclei, and karyoplasmatic ratio

Site	Adult frogs			Young of the year		
	hepatocytes	nuclei	karyoplasmatic ratio	hepatocytes	nuclei	karyoplasmatic ratio
Lake Berdanish	0.361	0.198	0.292	0.558*	0.04	0.490
Dolgobrodskoe reservoir	0.691*	0.115	0.658*	0.820**	–0.314	0.781**

* $p < 0.05$, ** $p < 0.01$.

Table 3. Average values of cell and nuclear size and karyoplasmatic ratio in the liver (arbitrary units)

Age group	Hepatocyte size		Nucleus size		Karyoplasmatic ratio	
	1	2	1	2	1	2
Adults	147.3 ± 4.5 (n = 9)	153.1 ± 4.85 (n = 10)	29.9 ± 0.54 (n = 9)	26.3 ± 0.73 (n = 10)	5.04 ± 0.41 (n = 9)	6.02 ± 0.18 (n = 10)
Young of the year	144.0 ± 2.73 (n = 14)	168.8 ± 4.52 (n = 13)	25.1 ± 0.43 (n = 14)	24.3 ± 0.49 (n = 13)	5.83 ± 0.17 (n = 14)	7.25 ± 0.27 (n = 13)

Note: 1, Lake Berdanish, 2, Dolgobrodskoe reservoir.

smaller amounts of nutrients, and their size mainly reflects individual variation of cell size in the sample under study.

Adult *R. arvalis* of test and control groups demonstrated no significant differences in the size of hepatocytes (Table 3). However, the size of hepatocyte nuclei (average for the sample) in the animals from the Berdanish population was 12% greater ($t = 3.96$, $p < 0.01$), and the karyoplasmatic ratio was 16% smaller ($t = 4.3$, $p < 0.01$), i.e., at similar hepatic indices, their nuclei were larger. This may be associated with the disturbance of cell division under the effect of pollution (Bugaeva, 1983) and intensification of hepatocyte polyploidization (Nepomnyashchikh *et al.*, 1996) because polyploidization of the nuclei interferes with the process of cell division (Litoshenko and Paramonova, 1982), and, hence, the karyoplasmatic ratio decreases.

Sizes of hepatocytes in young of the year from test and control populations differed by 14.7% ($t = 4.69$; $p = 0.001$), whereas their nuclei were virtually equal (Table 3). The karyoplasmatic ratio in animals of the polluted zone also changed, compared to that in the control. Thus, both adult frogs and young of the year inhabiting the polluted territory have a smaller energy reserve than animals from the control population. This is apparently explained by the inhibition of metabolic processes and increased energy expenditures for adaption under conditions of radionuclide pollution. According to the results of studes on oxygen consumption by *R. arvalis* frogs from the population inhabiting the territory of the EURT, animals from the polluted territory differed from the control group by a significantly lower level of oxygen consumption (Vershinin and Tereshin, 1996). As anthropogenic impact increases, oxygen consumption by amphibians usually also increases but subsequently decreases again (Vershinin and Tereshin, 1992), which may be evidence for adaptive changes at the level higher than physiological (Shvarts, 1980). Oxygen consumption by animals from the territory of the EURT was more than two times lower than in animals from urban territories, and this, in our opinion, is evidence for the actual inhibition of metabolism in the former.

The increased size of hepatocyte nuclei in adult animals from the polluted area apparently reflect the chronic load on the protective mechanisms of the liver to which young of the year has not yet been sufficiently exposed. In amphibians, cytological alterations in the liver indicate the intense functioning of cell mechanisms providing for natural resistance to environmental effects (Nosova *et al.*, 1994).

The most important criteria for identifying radiation damage of the genetic systems are the intensity of cell proliferation and the induction of chromosomal aberrations (Li, 1963). As could be expected, young of the year from the Berdanish population that underwent metamorphosis in the laboratory demonstrated a pronounced decrease in mitotic activity (Table 4). Cell growth in this group was also retarded, and the size of frogs was significantly smaller than in the control. It is known that pollutants can inhibit the thyroid function, thus inhibiting cell proliferation and morphogenesis (Syuzyumova, 1985) and affecting the rate of metabolic processes (Tokar' *et al.*, 1991). A drastic metabolic inhibition under conditions of radioactive pollution—hypooxygenia—was observed both in mammals (Testov, 1993) and in amphibians (*R. arvalis*) (Vershinin and Tereshin, 1996).

A large average size of epithelial cells in combination with a high mitotic index in frogs from the control

Table 4. Mitotic activity and average size of corneal epithelial cells in young of the year that developed in laboratory

Habitat	*n*	Mitotic activity, %	*t*	Area, μm²	*t*	Average body weight, mg
Lake Berdanish	31	10.75 ± 0.41	4.94	90.4 ± 3	3.99	194.5
Dolgobrodskoe reservoir	27	13.87 ± 0.48		100.9 ± 3		207.8

population is apparently explained by the fact that the intensity of cell proliferation and growth under favorable conditions may be high. Experimental studies showed that these indices are physiologically interrelated (Gatiyatullina, 1978).

Analysis of cell proliferation in young of the year captured in nature on the polluted and control plots (Table 5) indicated that the mitotic activity and cell size in both groups are similar. No significant change in proliferation rate was revealed in young of the year from the polluted territory ($t = 1.06$), whereas their body size and weight proved to be significantly greater. As a rule, body size of young of the year increases with an increase in anthropogenic impact and pollution (Vershinin, 1997).

In adult amphibians captured together with young of the year, mitotic index was lower than in the young (see Table 5), which is possibly accounted for by the difference in rate of metabolic processes associated with differences in body size and age (in the sample from the polluted territory, only four frogs were more than one year of age). A low proliferative activity observed in animals from the control population ($t = 2.36$) is apparently associated with the prevalence of older individuals in this sample.

Thus, differences in proliferative activity in young of the year were only revealed between the groups kept in the laboratory, whereas samples taken from nature did not differ in this parameter. We explain it by the elimination of individuals with a low metabolic rate and, hence, reduced mitotic activity from natural populations. In addition, four out of 31 young of the year from the test group demonstrated the presence of extensive chromosome damage in anaphase cells, such as chromosome bridges and nondisjunction (in 0.08% of the total number of dividing cells). This is markedly higher than in *R. arvalis* young of the year from the vicinity of the Karabash copper-smelting plant (0.0063% of the total number of the scanned dividing cells) where the level of environmental pollution with products of the copper industry is almost catastrophic (Gatiyatullina, 1990).

Table 5. Mitotic activity and average size of corneal epithelial cells in young of the year captured in nature (above the line) and mature individuals (below the line) of *R. arvalis*

Habitat	*n*	Mitotic activity	Area, μm²	Average body weight, mg
Lake Berdanish	10	4.7 ± 0.97	87.9	537.2
	5	4.2 ± 1.72	101.5	4.8
Dolgobrodskoe reservoir	10	6.9 ± 1.84	91.4	207.8
	5	1.6 ± 1.29	98.8	13.9

Previously, we have never detected chromosome aberrations in *R. arvalis* young of the year from natural forest water bodies. The appearance of individuals with structural chromosome rearrangements in animal populations is evidence for a high mutagenic activity of the environment in corresponding habitats. As frogs of the test and control groups were kept in the laboratory under the same conditions, it is apparent that differences in the intensity of proliferation in the young of the year are associated with the effect of radionuclide pollution on early embryonic and larval stages, which resulted in the development of differences in the level of physiological processes.

Thus, the study on the *R. arvalis* population from the coastal zone of Lake Berdanish polluted with radionuclides revealed an increase in the freqiencies of anomalies (Vershinin and Trubetskaya, 1993) at the morphological, cytological (large sizes of hepatocyte nuclei), and cytogenetic levels (an increased proportion of aberrant mitoses), which confirms that conditions existing in this territory present a potential genetic hazard. Structural and functional features of frogs from this population (Pyastolova *et al.*, 1996) demonstrate the prevalence in it of animals physiologically and genetically different from those found in other natural populations. This reflects the effect of unfavorable ecological situation resulting from heavy radionuclide pollution, on the one hand, and provides evidence for certain adaptive changes that provide for the survival and reproduction of this population under new conditions, on the other.

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