

## Interspecies Diversity of the Genome Responses to Chronic Irradiation in Natural Populations of Rodents

E. A. Gileva, Academician V. N. Bol'shakov, and L. E. Yalkovskaya

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Delayed genetic consequences of radiation accidents have been a major focus of interest of radiobiologists for many years. Because of considerable controversy of both experimental and natural data, this problem is still a subject of extensive discussion. This controversy can be illustrated, e.g., by the titles of two articles published almost simultaneously: *Very High Mutation Rate in Offspring of Chernobyl Accident Liquidators* [1] and *Children of Chernobyl Cleanup Workers Do not Show Elevated Rates of Mutations in Minisatellite Alleles* [2]. The problem of stochastic effects associated with chronic exposure to low-dose radiation and, particularly, changes of these effects in a sequence of generations is thought to be especially complex. In general, it may be concluded that the incidence of these effects in the populations of the zones of radioactive accidents is lower than this value predicted from the nonthreshold concept of the action of ionizing radiation [3, 4]. However, because only two or three (very seldom, four) generations of people took their turn in areas of nuclear accidents, the extent of genetic hazard to further generations remains uncertain. The information obtained in the studies using small mammals may be useful for the prognosis of this hazard, because small mammals are very close to humans in terms of physiological characteristics and organization of the genome, but the lifespan of one generation in the former is much shorter than in the latter.

However, it should be emphasized that the studies of rodents in the areas affected by the Chernobyl accident gave rather conflicting results, which implied the existence of interpopulation and interspecies differences in the chromosome sensitivity to radiation. For example, the frequency of chromosome aberrations in bank vole in the Belarus regions with a dose rate of 0.690 mGy/day was increased [5], whereas the micronucleus test did not detect any cytogenetic disorders in animal of the same species living in the 10-km-wide Chernobyl zone, in which the dose rate reaches 86.96 mGy/day [6]. Kostenko *et al.* [7] found that, within the zone affected by the Chernobyl accident,

there were signs of certain cytogenetic difference between the species of the genera *Clethrionomys* and *Microtus*. However, it is uncertain whether this difference is accidental or it is related to the species and population specificity of genome responses to ionizing radiation. To solve this problem, the range of tested species and variants of radioecological situations should be extended. For example, it is of considerable interest to analyze the level of genome instability in rodent populations living in areas of the East Ural Radioactive Track (EURT). In the 1950s and 1960s, these areas were contaminated as a result of the Kyshtym accident. These studies provide a unique opportunity to assess the response of the mammalian genome to chronic low-dose irradiation and to trace this response for many dozens of generations. Comparative analysis of pairs of rodent species differing from each other in population organization, as well as ecological and physiological characteristics, is of particular interest. The common vole (*Microtus arvalis* Pallas 1779) and northern mole vole (*Ellobius talpinus* Pallas 1770) (Rodentia, Microtinae) are a pair of such species. In contrast to other species of Microtinae, northern mole vole is characterized by an entirely underground life, family structure of settlements, long generation time, long lifespan, etc. We studied the genome instability in *M. arvalis* and *E. talpinus* from the East Ural Reserve (EUR) located in the head part EURT, where the initial level of soil contamination with  $^{90}\text{Sr}$  ranged from 500 to 1000 Ci/km<sup>2</sup>, and in northern mole vole from the Techa River banks, where the radioactive contamination was significantly lower than in the EUR. Animals from several areas of the Middle Urals, South Urals, and Trans-Ural region were used as controls.

Preparations of metaphase chromosomes were made from bone marrow. The content of  $^{90}\text{Sr}$  in the bone and muscular tissues was measured using the radiochemical method and  $\beta$ -radiometry at the Department of Radiochemistry, Ural State Technical University. The dose rate of incorporated  $^{90}\text{Sr}$  was calculated by the method of Chesser *et al.* [8].

It follows from Table 1 that the mean frequency of chromosome aberrations in the bone marrow of *M. arvalis* specimens from EURT was five to ten times higher than in control populations (the difference was statistically significant at a high confidence level).

Institute of Plant and Animal Ecology, Ural Division,  
Russian Academy of Sciences, ul. Vos'mogo Marta 202,  
Yekaterinburg, 620144 Russia

**Table 1.** Chromosomal abnormalities in the bone marrow of *Microtus arvalis* from Ural regions with different levels of radioactive contamination

Region	Geographic coordinates	Concentration of <sup>90</sup> Sr in bone and muscular tissues, Bq/g dry weigh	Number of animals	Cell count	Mean percentage of cells, %			Fraction of chromosome aberrations	Fraction of cells with multiple aberrations among damaged cells
					with chromosome aberrations	aneuploid and polyploid	with gaps		
EURT, Chelyabinsk oblast	55°47' N 60°55' E	118.0	7	350	4.57	1.14	2.86	0.24	0.13
Bainy village, Sverdlovsk oblast	56°42' N 62°08' E	0.13	14	700	0.71	0.14	1.14	–	–
Orenburg Reserve, Orenburg oblast	51°08' N 57°38' E	0.10	9	450	0.44	0.67	1.56	–	–
Kristalka village, Orenburg oblast	53°00' N 53°28' E	<0.10	7	350	0.86	0.86	1.43	–	–
	$\chi^2$				31.490	4.644	4.449		
	<i>P</i>				<0.0001	0.200	0.217		

Although neither chromosome aberrations nor cells with multiple damage, which are usually regarded as markers of radiation-induced effects, were observed in three control populations, they were found in animals from EURT. On the other hand, the mean frequency of aberrant cells in the northern mole vole specimens collected in EUR was 2.48%, and there was no statistically significant difference between the values of this parameter in these animals and control animals from the Techa River banks (Table 2). Control and exposed populations of northern mole voles were characterized by similar values of the frequency of chromosome aberrations and the number of cells with multiple damage. In the two species of rodents studied in this work, the frequencies of gaps and cells with numerical chromosome mutations did not exceed the control level.

Thus, no cytogenetic responses to chronic exposure to radiation were found in northern mole vole specimens collected in areas with heavy radioactive contamination. The mean dose rate induced by incorporated <sup>90</sup>Sr in northern mole voles and common voles was 0.985 and 0.413 mGy/day, respectively. According to the results of calculations performed by O.V. Tarasov [9], the total radiation dose absorbed by the skeleton of small mammals from external and internal sources in EUR is 0.4–0.5 Gy/yr. According to V.A. Shevchenko *et al.* [10], these estimates fall within the range of doses capable of inducing distinct cytogenetic reaction in small mammals. In our experiments, this was observed in the case of *M. arvalis* but not *E. talpinus*, although

the radioactive strontium concentration in tissues of northern mole voles was almost 2.5 times higher than in tissues of common voles.

The resistance of the northern mole vole genome to the clastogenic effect of ionizing radiation can be attributed to several factors, which are not mutually exclusive. First of all, this can be attributed to the protective effect of melanin, because this effect was repeatedly observed in mammalian tissue cultures [11]. Although coat-color polymorphism is a typical feature of the northern mole vole for over most part of the geographic range of this species, all northern mole voles subjected to karyotyping in this work were black. It may also be suggested that the enhanced radioresistance of northern mole vole chromosomes results from the high efficiency of repair systems inherent in this species. However, this suggestion is inconsistent with the fact that the frequency of spontaneous structural chromosome mutations in northern mole voles is higher than in other Ural rodents. This frequency was about 2% in four control populations (Table 2), whereas the background level of this parameter in the other wild species of Rodentia studied in our experiments was usually below 1% [12]. It seems more probable that the enhanced radioresistance of *E. talpinus* chromosomes in the EURT area is due to certain mutations, the frequency of the mutations increasing upon chronic exposure to ionizing radiation. Probably, the wide distribution of these mutations could be facilitated not only by the selective advantage of radioresistant forms, but also

**Table 2.** Chromosomal abnormalities in the bone marrow of *E. talpinu* from Ural regions with different levels of radioactive contamination

Region	Geographic coordinates	Concentration of $^{90}\text{Sr}$ in bone and muscular tissues, Bq/g dry weight	Number of animals	Cell count	Mean percentage of cells, %			Fraction of chromosome aberrations	Fraction of cells with multiple aberrations among damaged cells
					with chromosome aberrations	aneuploid and polyploid	with gaps		
EURT	55°45' N 60°54' E	291.3	23	2300	2.48	0.83	3.87	0.20	0.19
Muslyumovo village (Techa River bank), Chelyabinsk oblast	55°36' N 61°29' E	0.8–1.2	29	1450	2.21	0.48	2.69	0.17	0.13
Nizhnee village, Chelyabinsk oblast	55°45' N 61°39' E	<0.07	11	1100	2.18	0.45	2.82	0.18	0.04
Klyuchiki village, Kurgan oblast	55°01' N 63°43' E	<0.01	70	5050	1.64	0.48	3.01	0.27	0.02
Yulduz village, Kurgan oblast	56°15' N 63°53' E	<0.02	34	1700	2.24	0.24	3.12	0.24	0.05
Burangulovo village, Bashkortostan	54°35' N 59°28' E	<0.01	36	1800	1.89	0.72	2.28	0.09	0.15
	$\chi^2$				7.053	9.034	9.796		
	<i>P</i>				0.217	0.108	0.081		

by genetic drift, the efficiency of random processes being significantly promoted by isolation of small populations of northern mole voles in the EUR, which is located in the northernmost part of the species range.

It follows from the results discussed above that the irregular radiation-induced response of the mammalian genome is observed within a slightly broader range of radiation doses than it was suggested by Shevchenko *et al.* [10]. Probably, the absence of response in some cases of heavy radioactive contamination of soil was due to radioadaptation of animals. The efficiency of formation of radioadaptation depends on a combination of both selection and stochastic factors. These findings should be taken into account in ecological and genetic monitoring using small mammals. Based on these findings, sets of species with possibly different biotopic preference and demographic population characteristics should be used as test objects.

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