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# Species-Specific Features of Inter- and Intrapopulation Variation in the Level of Chromosome Instability in Rodents

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

Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences, ul. Vos'mogo Marta 8, Yekaterinburg, 620144 Russia

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**Abstract**—Studies on the common vole *Microtus arvalis* revealed a distinct cytogenetic response to the influence of ionizing radiation, a wide range of variation in the group average indices of chromosome instability beyond the zone of technogenic impact, and the influence of natural factors (viral infections and fluctuations of population size) on the frequency of chromosome aberrations. In the northern mole vole *Ellobius talpinus*, neither the mutagenic effect of ionizing radiation nor interpopulation differences in the level of chromosome aberrations in the absence of anthropogenic impact were detected. Variation of genome instability of the rodents is significantly contributed to by species demography and population cycles.

**Key words:** population, variation, mutation process, chromosome aberrations.

The concept of self-regulation is among the main concepts of population ecology. Its essence is that populations have feedback mechanisms, which mediate the effects of environmental factors on population dynamics; these mechanisms are highly diverse and can be of different nature: behavioral, physiological, and, what is especially important, genetic. The response of a population to such changes depends on the parameters of its external environment (Bol'shakov *et al.*, 1996). In this context, various anthropogenic influences, especially radiation effects, are of primary importance.

The analysis of the mutation process in natural rodent populations under different levels of anthropogenic impact often yields ambiguous results. Studies in the zones of radiation accidents are an illustrative example. Thus, in Byelorussia (1991), mole voles chronically irradiated at a dose rate of up to 0.690 mGy/day had an increased frequency of chromosome aberrations (Goncharova and Ryabokon, 1995); on the other hand, in the 10-km zone around the Chernobyl NPP, where the dose rate reached 86.96 mGy/day (1997–1998), neither cytogenetic nor molecular methods revealed any intensification of mutagenesis in the same species (Wickliffe *et al.*, 2002). Hence, it is necessary to analyze in detail the natural factors modifying the levels of genomic instability in mammals in the environments differing in the level of anthropogenic transformation.

In this work, we studied interpopulation variation in the frequency of structural chromosome aberrations in two rodent species differing in their population organization and ecophysiological characteristics; in particular, attention was focused on their population–ecological features. These were the common vole *Microtus*

*arvalis* Pallas, 1799 and the northern mole vole *Ellobius talpinus* Pallas, 1770 (Rodentia, Microtinae). Among other representatives of the Microtinae, the latter species is distinguished by an underground mode of life, family-based organization of colonies, longer period of generation change, longer life span, etc.

## MATERIAL AND METHODS

Animals from the areas differing in the level of anthropogenic load were studied. Thus, the animals of both species were captured in the Eastern Ural Reserve (EUR) located in the front part of the Eastern Ural Radioactive Trace (EURT), which formed in the 1950s–1960s after several accidents at the Mayak Radiochemical Plant. There, the initial level of soil contamination with  $^{90}\text{Sr}$  was 500–1000 Ci/km<sup>2</sup>. In addition, northern mole voles were captured in the vicinity of the village of Muslyumovo, near the Techa River. This area was also contaminated with radionuclides from the Mayak plant, but to a considerably lesser degree (soil contamination with  $^{90}\text{Sr}$  at the site of animal trapping was 0.2 Ci/km<sup>2</sup>). The territory of the Predural'e Reserve (Kamai railway station, Perm oblast), where common voles were captured, was contaminated with DDT some time ago. In addition, the level of chromosome aberrations was investigated in the animals of both species from several localities of the Middle and Southern Urals and Transural Region with the global level of contamination.

A total of 149 common voles and 228 northern mole voles were karyotyped. Metaphase chromosome preparations of bone marrow cells were made by conventional methods (MacGregor and Varley, 1986). For each

**Table 1.** Chromosome aberrations in the bone marrow of *M. arvalis* from the areas with different levels of the anthropogenic impact

Sampling site	Geographical coordinates	Number of animals	Number of cells	Average frequency of cells with chromosome aberrations, %	Possible environmental mutagens
EURT, Chelyabinsk oblast	55°47'N 60°55'E	7	350	4.57	Technogenic radioactive contamination of the soil (500 Ci/km <sup>2</sup> by <sup>90</sup> Sr)
Group I					
Bainy, Sverdlovsk oblast	56°42'N 62°08'E	14	700	0.71	Global contamination
Arkaim Reserve, Chelyabinsk oblast	52°37'N 59°33'E	5	500	1.00	"
Kristalka, Orenburg oblast	53°00'N 53°28'E	7	350	0.86	"
Orenburg Reserve, Orenburg oblast	51°08'N 57°38'E	9	450	0.44	"
Group II					
Biological Station of Ural State University, Dvurechensk, Sverdlovsk oblast:	56°37'N 61°08'E				
1995		13	1250	3.62	} Viral infections? " } Global contamination
1996		20	1079	3.58	
1997		12	1074	0.84	
Group III					
Predural'e Reserve, Kamai railway station, Perm oblast:	57°20'N 57°09'E				
right bank of the Sylva River		31	1550	0.92	Traces of DDT
left bank of the Sylva River		31	1550	1.85	"
				$\chi^2 = 77.430$ $P < 0.0001$	

animal, 50–100 metaphase cells were studied to reveal chromosome aberrations. True chromosome breaks were distinguished from gaps by generally accepted criteria (Brogger, 1982). The concentrations of <sup>90</sup>Sr in the bone and muscle tissues were determined by the radiochemical method and beta-radiometry at the Department of Radiochemistry, Ural State Technical University. The dose rate accounted for by incorporated <sup>90</sup>Sr was calculated according to Chesser *et al.* (2000). The concentrations of heavy metals in the liver were determined at the Laboratory of Ecotoxicology, Institute of Plant and Animal Ecology, by the method of atomic absorption spectrometry. The concentrations of DDT and its metabolites—DDD and DDE—in the liver were determined at the regional veterinary laboratory of Yekaterinburg by means of gas–liquid chromatography.

## RESULTS AND DISCUSSION

The results of the cytogenetic analysis of common voles (Table 1) indicate that differences in the frequency of chromosome aberrations between *M. arvalis* from different populations are highly significant. In the bone marrow of animals from the front part of the EURT, the average frequency of cells with chromosome aberrations was five to ten times higher than in populations from the areas with the global level of radioactive contamination ( $\chi^2 = 30.167$ ;  $P < 0.0001$ ). Chromosome-type aberrations and cells with multiple injuries, which are commonly regarded as markers of radiation damage, were detected in animals from the EURT (their proportions were 0.24 and 0.13, respectively) and were lacking in other populations. Hence,

the genome of *M. arvalis* exhibited a distinct response to a strong radiation impact.

Averages of indices of chromosome instability were compared for both species at intra- and interpopulation levels. Separate data on *M. arvalis* were united in accordance with the origin and power of the main mutagenic factor to some groups for the comparison (Table 1). The frequency of chromosome aberrations in populations of animals inhabiting territories with the global contamination level (group I) varied between 0.44 and 1.00%. Statistically, differences between these populations are not significant ( $\chi^2 = 1.050$ ;  $P = 0.789$ ), which is possibly due to an insufficient number of observations in some groups. However, it is noteworthy that the extreme values of chromosome aberration frequencies differ by a factor of approximately 2.5. This problem undoubtedly deserves further study.

In common voles of group II (Biological Station of the Ural State University) karyotyped in 1995 and 1996, the frequencies of chromosome aberrations, being almost equal and fairly high, exceed those recorded in 1997 by a factor of more than 4.5 ( $\chi^2 = 21.198$ ,  $P < 0.0001$ ). Specific multiple injuries in their cells, with the prevalence of chromatid-type aberrations (numerous point fragments, chromosome pulverization, etc.), suggested that the increased genomic instability in those years might be of a viral nature. Viral clastogenesis in wild rodents was described in detail in our previous study on the bank vole *Clethrionomys glareolus* (Gileva *et al.*, 2001).

Differences in the level of chromosome aberrations between the voles from the Predural'e Reserve (group III) captured on the right and left banks of the Sylva River ( $\chi^2 = 5.31$ ;  $P = 0.021$ ) are also significant. In the late 1990s, the peripheral area of the reserve on the right bank was treated with DDT. This pesticide and its metabolites are mutagenic and can increase the frequency of chromosome aberrations in animal cells (Clark, 1974; Larsen and Jalal, 1974); therefore, we could expect a specific response of the vole genome to this treatment. However, the right bank was the area where the lowest frequency of chromosome aberrations was observed. No connection between DDT content and cytogenetic damage was revealed; the livers of animals from both banks contained only traces of this pesticide and its metabolites (<0.001 mg/kg fresh weight). Likewise, our colleagues S.B. Rakitin and O.V. Polyavina revealed no increase in chromosome aberration frequency in bank voles with similar DDT, DDE, and DDD concentrations in the liver.

Concentrations of radionuclides and heavy metals in the tissues of common voles from the right and left banks did not differ and were similar to those in animals inhabiting territories with the global contamination level. However, the numbers of voles on the right and left banks differed markedly. According to the data obtained at the Biological Station of Perm University, the abundance of common voles in 2000 was much

higher on the left than on the right bank: 8.5 vs. 1.0 animals per 100 trap-days, respectively. An increase in population density leads to the increasing load of stress-inducing factors (Shilov, 1977), and stress can cause destabilization of the genetic system in animals (Borodin and Belyaev, 1980; Borodin, 1987). The mutagenic effect of stress has also been demonstrated; in particular, this concerns an increase in the frequency of chromosome aberrations in the bone marrow cells of mice exposed to emotional stress (Seredinin *et al.*, 1980). Stress-inducing factors affect primarily the adrenocortical system (Meerson, 1981), and the effects of stress on the genome are apparently realized through this system (Dyuzhikova *et al.*, 1997). Studying the water vole, Evsikov *et al.* (1999) have found that the change of phases in the population cycle causes changes in the concentrations of corticosteroids in the blood of animals, and these hormones have a mutagenic effect and can increase the frequency of chromosome aberrations (Skorova *et al.*, 1986). Thus, the increased chromosome instability in common voles from the left bank of the Sylva River could be due to their high population density and the resulting stress load corresponding in 2000.

Thus, the common vole beyond the zone of technogenic impact exhibits a broad range of the group average indices of chromosome instability: differences between all populations and intrapopulation groups remain highly significant even when the animals from the front part of the EURT are not taken into account ( $\chi^2 = 69.534$ ,  $P < 0.0001$ ). It should be emphasized that both viral infections (Biological Station of Ural University) and population dynamics (Predural'e Reserve) are natural factors that permanently affect the voles. The question arises as to whether the common vole can be used in ecological-genetic monitoring as an indicator of clastogenic pollutants. This is possible only if the phase of the population cycle and demographic structure is taken into account and kinds of chromosome aberrations are tightly analysed, etc.

A different picture is observed in the northern mole vole (Table 2). We studied animals from six localities distributed over the area extending from 54°35' to 56°15' N and from 59°28' to 63°53' E and considerably differing in landscape conditions. Sampling sites in Chelyabinsk oblast were at the boundary between the forest zone (the subzone of pine-birch forests) and the forest-steppe zone (the subzone of northern forest steppes); in Kurgan oblast, in the forest-steppe zone (the subzone of southern forest steppes); and in Bashkortostan, in the steppe zone (the subzone of grass-herbage steppes). However, the average population frequencies of genomic aberrations in *E. talpinus* proved to be similar in all localities, varying around 2%. As shown in Table 2, interpopulation differences and differences for separate years for animals from Klyuchiki ( $\chi^2 = 3.283$ ,  $P = 0.194$ ) were not significant. Remarkably, the frequency of aberrant cells in the mole voles

**Table 2.** Chromosome aberrations in the bone marrow of *Ellobius talpinus* from the areas with different levels of the anthropogenic impact

Sampling site	Geographical coordinates	Number of animals	Number of cells	Average frequency of cells with chromosome aberrations, %	Possible environmental mutagens
EURT, Chelyabinsk oblast	55°45'N 60°54'E	23	2300	2.48	Contamination of the soil (500–1000 Ci/km <sup>2</sup> by <sup>90</sup> Sr)
Techa River bank, Muslyumovo, Chelyabinsk oblast	55°36'N 61°29'E	29	1450	2.21	Contamination of the soil (0.2–0.5 Ci/km <sup>2</sup> by <sup>90</sup> Sr)
Nizhnee, Chelyabinsk oblast	55°45'N 61°39'E	11	1100	2.18	Global contaminations
Juldus, Kurgan oblast	56°15'N 63°53'E	34	1700	2.24	"
Klyuchiki, Kurgan oblast:	55°01'N 63°43'E				
1998		15	1500	1.47	"
1999		31	3100	1.42	"
2000		39	1950	2.05	"
Burangulovo, Bashkortostan	54°35'N 59°28'E	46	2300	1.91	"
				$\chi^2 = 11.477$ $P = 0.119$	

from the Eastern Ural Reserve, where the radiation impact was fairly strong, did not differ significantly from that in the mole voles from the areas with the global level of contamination and from the Techa River bank. The frequencies of chromatid-type aberrations and cells with multiple injuries in the control and irradiated populations were also similar. Thus, no cytogenetic responses to chronic irradiation were observed in the northern mole voles inhabiting heavily contaminated areas.

Meanwhile, the average dose rates accounted for by incorporated <sup>90</sup>Sr were 0.985 mGy/day in the northern mole vole and 0.413 mGy/day in the common vole. As calculated by Tarasov (2000), the integral dose of radiation (from the external and internal sources) absorbed by the skeleton of small mammals in the Eastern Ural Reserve was 0.4–0.5 Gy per year. This estimation is in the range of doses evoking a distinct cytogenetic response in small mammals (Shevchenko *et al.*, 1993). We observed such a response in *M. arvalis*, rather than in *E. talpinus*, although the radiostrontium concentration in the tissues of the northern mole vole was almost 2.5 times higher than in the common vole (291.3 Bq/g vs. 118.0 Bq/g dry weight).

The resistance of the northern mole vole genome to the clastogenic influence of ionizing radiation may be accounted for by at least three factors that are not mutually exclusive.

(1) The protective action of eumelanin or its metabolites, which has been repeatedly demonstrated in the

tissue culture of mammals (Mosse *et al.*, 2000). The EURT area is inhabited by only black northern mole voles, whereas this species in other parts of the range exhibits polymorphism in coat color.

(2) Specific features of genome organization and functioning in representatives of the genus *Ellobius*. Vorontsov and Lyapunova discovered a unique Robertsonian fan in *E. talpinus* from the Pamir-Alai (extreme variants from  $2n = 54$  to  $2n = 32$ ) and corroborated its subdivision into three chromosome species *E. talpinus* ( $2n = 54$ , NF = 54), *E. tancrei* ( $2n = 54$ , NF = 56), and (*E. alaicus* ( $2n = 52$ , NF = 56) (Vorontsov *et al.*, 1969; Lyapunova and Vorontsov, 1979; Vorontsov and Lyapunova, 1984; Lyapunova *et al.*, 1984). In *E. talpinus* and *E. tancrei*, neither microscopic nor molecular methods can reveal differences between the X and Y chromosomes. Males lack the sequences of *Sry* and *Zfy* genes, which normally are among the important components of the genetic system determining the male sex in mammals (Just *et al.*, 1995). According to our data, northern mole voles have the highest background frequencies of chromosome aberrations (about 2%), compared to other wild Rodentia species from the Urals and Transural Region (as a rule, below 1%) (Polyavina and Yalkovskaya, 1998).

(3) The development of high radioresistance of chromosomes in *E. talpinus* on the EURT territory on the basis of certain inherited mutations, whose frequency increased under the effects of chronic irradiation. The spread of such mutations could be promoted

by both selection for radioresistant forms and random genetic drift, with the efficiency of random processes being enhanced due to a considerable degree of isolation of a small mole vole colony in the Eastern Ural Reserve, in the extreme north of the species range.

Let us return to the absence of interpopulation and intraspecies differences with respect to the frequency of chromosome aberrations in *E. talpinus*, as opposed to *M. arvalis*. Trying to explain this phenomenon, attention must be paid to species-specific features of population-demographic processes. *E. talpinus* is characterized by a considerably smaller range of population fluctuations than most other Microtinae, including *M. arvalis*. Long-term observations by Evdokimov (2001) have shown that, on average, the numbers of *E. talpinus* at the depression and peak phases differ by a factor of only 2–3; therefore, stress factors related to the population cycle and genomic aberrations induced by them are less probable in the northern mole vole than, for instance, in voles from the Predural'e Reserve. The clastogenic effect of viral infections is also less probable: northern mole voles live in more or less isolated colonies, and these factors must interfere with the spread of viruses.

The results of our study show that variation in the levels of genomic instability in rodents largely depends on specific features of their population-demographic structure and population cycles. This dependence must be taken into account while developing methodological approaches to the analysis of the mutation process in natural populations, including those exposed to anthropogenic.

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