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SHORT COMMUNICATIONS

Microsatellite DNA Variation in Ural Bank Vole Populations

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Highly polymorphic microsatellite DNA loci are effective markers of genetic and demographic processes in mammal populations. These processes are studied in terms of the spatial organization of populations and cyclic fluctuations in population sizes (Ehrich et al., 2001; Redeker et al., 2006). The microsatellite DNA variation in mammals and other vertebrates caused by technogenic environment pollution has been studied considerably less. Mutagenic pollution may either increase the genetic diversity (due to de novo mutations) or decrease it as a result of differential elimination of the genotypes noncompetitive in an adverse environment. The complexity of this problem is evidenced by contradictory data on microsatellite DNA variation in different species of vertebrates. An increased mutation rate in microsatellite loci was found in barn swallows exposed to irradiation after the Chernobyl accident (Ellegren, 1997). On the other hand, no such effect has been found in children of Chernobyl liquidators (Slebos et al., 2004; Furitsu et al., 2005). No relationship between heavy metal pollution and microsatellite variation has been found in European eel from Belgium (Maes, 2005); however, such a relationship is very probable in the case of wood mice (Berckmoes et al., 2005).

In this study, we used microsatellite markers to compare genetic diversity in Ural populations of the bank vole (*Clethrionomys glareolus* Schreber, 1780) exposed to different degrees of technogenic impact. All populations studied were located in the southern taiga subzone. Voles were captured in summer of the year 2006 in the area affected by the Middle Ural Cooper–Smelting Plant (MUCP, 56°50′ N, 59°51′ E) and near the village of Shigaevo, Sverdlovsk oblast (57°15′ N, 58°44′ E), where only global pollution has been documented. MUCP has been in operation since 1940. Sulfur dioxide, fluorine-containing compounds, and suspended particles with heavy metals and arsenic adsorbed on them are the main toxic ingredients of its emissions (Vorobeichik et al., 1994). Many of these ingredients are mutagenic.

In the MUCP area, voles were captured in the impact zone (0.5–6.0 km from the emission plume, where the concentrations of pollutants in the forest litter are 3–66 times higher than the regional background levels) and in the peripheral zone (20–30 km from the emission source, where the pollution is, on average, two times higher than the background level). In both cases, the voles were captured in a fir–spruce forest. In the MUCP impact zone, the wood growing stock is drastically decreasing; the herb layer either is absent altogether or consists of only horsetail and grasses. The moss cover is well-developed there (as much as 75%). The soil has been partly eroded. In the peripheral zone, the state of the ecosystem corresponds to the region-specific normal state (Vorobeichik et al., 1994; Mukhacheva and Luk'yanov, 1997). Voles from the spruce–fir–birch forest in the vicinity of the village of Shigaevo, which lived under relatively good conditions (Gileva et al., 2006), were used as a reference group. The distances from Shigaevo to the impact and peripheral zones are 90 and 80 km, respectively.

The chromium, nickel, and arsenic concentrations in the vole liver were measured by means of synchrotron x-ray fluorescence (SXRF); the cadmium, lead, copper, and zinc concentrations, by means of atomic absorption spectroscopy using an AAS-6 Vario spectrometer. Four microsatellite loci consisting of dinucleotide repeats (MSCg4, MSCg9, MSCg15, and MSCg20) served as genetic markers (Gockel et al., 1997). DNA was isolated from the muscle tissue of the voles by salt extraction. Microsatellite DNA was amplified according to Gockel et al. (1997); the sizes of alleles were determined in denaturing polyacrylamide gel by means of an

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Table 1. Arsenic and heavy metal concentrations (μg/g dry weight) in the liver of bank voles from three Ural populations (the numbers of animals are indicated in parentheses)

Element	Zones		
	impact*	peripheral*	reference
Arsenic	2.26 ± 0.49 (5)	0.22 ± 0.02 (5)	–
Chromium	10.80 ± 1.46 (5)	7.25 ± 0.69 (5)	2.45 ± 0.35 (20)
Nickel	0.63 ± 0.14 (5)	0.64 ± 0.07 (5)	0.68 ± 0.22 (20)
Copper	12.12 ± 0.43 (45)	8.12 ± 0.24 (208)	8.91 ± 0.42 (10)
Zinc	99.81 ± 2.30 (45)	102.93 ± 2.25 (208)	69.52 ± 3.32 (10)
Cadmium	11.20 ± 0.62 (45)	1.37 ± 0.10 (208)	0.21 ± 0.05 (8)
Lead	4.64 ± 0.27 (45)	2.55 ± 0.19 (208)	1.55 ± 0.19 (8)

* Sources: Mukhacheva and Bezel', 1995; Bezel' et al., 2007.

Table 2. Genetic diversity parameters in three bank vole populations averaged over four microsatellite loci

Parameter	Zones		
	impact	peripheral	reference
Number of animals	16	35	26
H_E	0.824	0.842	0.826
H_O	0.719	0.829	0.808
F_{IS}	0.132*	0.016	0.022
Mean number of alleles per locus	9.00	11.5	9.00
Allele diversity	9.00	9.17	7.96
Number of private alleles	2	8	2
Garza–Williamson index (±s.e.)	0.395 ± 0.081	0.420 ± 0.089	0.360 ± 0.045

Note: H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; * $P = 0.008$.

ALFexpress-II automatic gel sequencer (Amersham Biosciences) with the use of the Alfwin Fragment Analyser 1.03 software.

The results were treated using the Arlequin 2.00 and 3.11, Fstat 2.9.3.2, Micro-Checker 2.2.2., Genepop on the Web, GenAlEx 6, Popgene 1.31, and Microsatellite Tools for Excel software packages. Most parameters were estimated by different methods using two or three software packages. Below, we present only the estimates for which equal values were obtained by at least two methods. Probabilities were calculated on the basis of 10^7 randomizations (Khromov-Borisov et al., 2004). Statistical analysis did not show linkage disequilibrium or the presence of null alleles.

The concentrations of the studied elements, most of which (arsenic, cadmium, lead, chromium, and nickel) are toxic (Hartwig, 1995), were substantially increased in the liver of bank voles from the area affected by MUCP, especially impact sites (Table 1), compared to those in voles from the reference zone (Shigaevo), where these concentrations were close to the global average levels (Bezel' et al., 2007).

All the loci studied proved to be highly polymorphic, which is typical of rodents (Li et al., 2002), the number of alleles varying from 11 in MSCg9 to 16 in MSCg20. In western European *C. glareolus*, the numbers of alleles in these loci are the same or somewhat smaller (Barker et al., 2005; Redeker et al., 2006). The allele sizes varied from 112 to 138 bp in the MSCg4 locus, from 160 to 180 bp in the MSCg9 locus, from 111 to 137 bp in the MSCg15 locus, and from 122 to 164 bp in the MSCg20 locus. As can be seen in Table 2, the mean heterozygosity in all populations was high and close to the values observed in other rodents (e.g., Ehrich et al., 2001; Berckmoes et al., 2005).

The allelic richness index taking into account the sample size was somewhat higher in voles from the MUCP area. The number of private alleles (those that were found in only one population) was the largest in the MUCP peripheral zone. In general, the genotype frequencies for individual loci fit the Hardy–Weinberg equilibrium; significant heterozygote deficits were found only for the MSCg4 locus in the impact zone ($P = 0.045$) and for the MSCg20 locus in the Shigaevo population ($P = 0.018$) (after the use of the sequential

Table 3. Estimates of the genetic differentiation of bank vole populations based on the data on four microsatellite loci (the probabilities are indicated below the diagonal)

Locality	Zones		
	impact	peripheral	reference
F_{ST} statistic			
Impact zone	–	0.0170	0.0718
Peripheral zone	0.026	–	0.0419
Reference zone	0.000	0.000	–
R_{ST} statistic			
Impact zone	–	–0.0133	0.0201
Peripheral zone	0.719	–	0.0537
Reference zone	0.147	0.022	–

Bonferroni procedure, $P > 0.05$ in both cases). The inbreeding index (F_{IS}) calculated for all loci was significant only in the impact population. Apparently, consanguineous crosses were more probable there because of a decreased population density near MUCP. The values of the Garza–Williamson index (the ratio of the number of alleles to the range of their sizes) were similar in all populations and were far from both 0 and 1. These values indicated that the populations studied had retained genetic diversity, despite the bottlenecking (Garza and Williamson, 2001) that could have been expected after the population depression phase that preceded the capture of the voles.

The interpopulation genetic differentiation was estimated in two ways–allele lengths, i.e., the numbers of repeats, not taken (AMOVA, F_{ST}) or taken into account (AMOVA, R_{ST}). The interpopulation differentiation was low: it accounted for 4.14% ($P = 0.000$) and 2.57% ($P = 0.079$) of the total variance in the former and latter cases, respectively. The populations studied significantly differed from one another in the genetic structure (in all variants of comparison, with the allele sizes not taken into account) (Table 3), all loci considerably contributing to these differences. The samples from the area affected by MUCP were substantially closer to each other than to the voles from the Shigaevo population. When estimated using R_{ST}, significant differentiation was found only between the peripheral and reference zones and was mainly accounted for by differentiation with respect to the MSCg20 locus.

Thus, the populations studied exhibited a moderate degree of genetic differentiation. The largest differences were observed between animals from the reference zone, which were subjected only to the global technogenic exposure, and voles from the MUCP area with increased concentrations of mutagenic pollutants in their bodies, which could increase intrapopulation genetic diversity. However, a tendency towards this increase was the most distinct in the peripheral zone, where the mutagenic pollution was lower than in the impact zone. This contradiction was probably related to insufficient sizes of the samples studied and calls for further investigation. The small number of populations and limited sizes of samples in this study prevent us from drawing an unambiguous conclusion on the role of technogenic factors (including mutagenic contaminants) in the formation of the genetic diversity of Middle-Ural bank voles. The differences in the genetic structure between the compared populations may have resulted not only from the technogenic stress, but also from isolation by distance: remember that the distances between the Shigaevo population and both zones studied in the MUCP area were several tens of kilometers.

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