

UNDERSTANDING THE GENETIC CONSEQUENCES OF ENVIRONMENTAL TOXICANT EXPOSURE: CHERNOBYL AS A MODEL SYSTEM

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(Received 12 November 2008; Accepted 15 April 2009)

Abstract—We sampled vole populations in Ukraine with the dual goal of characterizing population diversity and of providing a biogeographic perspective to evaluate experimental designs used for previous studies. Our data indicate that genetic diversity in bank vole populations is widely variable across regions and that diversity estimates in contaminated sites are unremarkable compared to those in uncontaminated areas. Furthermore, the relative frequencies of haplotypes have remained statistically identical throughout multiple sampling periods. Thus, the genetic data from bank vole populations in Ukraine fail to support the hypothesis that mutational changes in contaminated regions are the product of exposure to Chernobyl radiation. Our results suggest that genetic diversity in radioactive regions of Ukraine is probably a function of natural geographic variation rather than increased mutational pressure from radiation exposure and underscore the importance of adequate geographic sampling in studies designed to elucidate the effects of toxicant exposure.

Keywords—Chernobyl Bank vole Population genetics Comparative studies Ionizing radiation

INTRODUCTION

In contemporary toxicological literature, population genetics analyses often are used to detect differences between reference and exposed populations, and differences are presumed to be a biological consequence of exposure to environmental contaminants. The investigative tactic is considered to be particularly valuable in instances when preexposure data are unavailable but the nature, extent, and temporal boundaries of the contamination event are documented. Such methodology has been represented as a practical approach for assessing the causal relationships between multigenerational exposure to polluted environments and biological effects [1–4]. Selecting reference sites and/or populations useful for comparative purposes can be complicated, however, both because of the numerous factors that can influence the demographic characteristics of a species and because the relative impact of these factors differs across taxonomic lines [5,6]. Thus, designing studies that can discriminate between natural inter- and intrapopulation variation and variation consequential to contaminant exposures is challenging.

Studies employing comparative methods can be found in the scientific literature for contaminants ranging from atrazine [7,8] to organochlorine pesticides [1,9,10] as well as for a variety of exposed organisms. Among these are studies examining the biological implications of exposure to environmental radiation [4,11–14]. Several previous studies conducted by our research group [4,15–17] have employed comparative methods to evaluate variation in bank vole populations from radioactively contaminated and reference sites near Chernobyl, Ukraine.

The bank vole (*Myodes glareolus* = *Clethrionomys glareolus* [18]) is an arvicoline rodent with a Palearctic distribution extending south from Scandinavia into the temperate and boreal forests of Europe and western Russia. *Myodes glareolus* has been the focus of much research, both because of the unique dynamics regulating population structure in the species [19,20] and because of its status as a general indicator of ecosystem health [21–23]. Ecological and demographic features of bank voles have been well documented in certain regions (e.g., Scandinavia), but population dynamics in other areas of its distribution remain largely unstudied. Moreover, little of the existent work has sought to characterize population-level phenomena using genetic data. A limited number of population genetics studies have assessed subdivision related to dispersal in *M. glareolus*, but collection sites have been confined to areas of linear, continuous habitat over narrow geographic ranges [24,25] or highly fragmented landscapes in which interpopulational dispersal was restricted by the presence of inhospitable matrix between sites [26]. Such studies provide valuable information in terms of dispersal over small spatial scales or as it relates to anthropogenic barriers, but results may lack applicability to the general patterns and processes that dictate variation in genetic structure over larger, less fragmented land areas. Bilton et al. [27] and Kotlík et al. [28] evaluated the putative existence of European glacial refugia, as inferred from haplotype composition, in bank vole populations across western and central Europe. The studies encompassed a large geographic area, but only two or three specimens were examined for most localities. Other research investigating genetic variability in bank voles has had a similarly limited focus and been concerned primarily with evaluating populations in regions contaminated by chemical, physical, or viral agents [4,15–17,29,30].

Recent studies [4,15–17] have evaluated genetic composition in vole populations from contaminated sites close to the

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Published on the Web 4/23/2009.

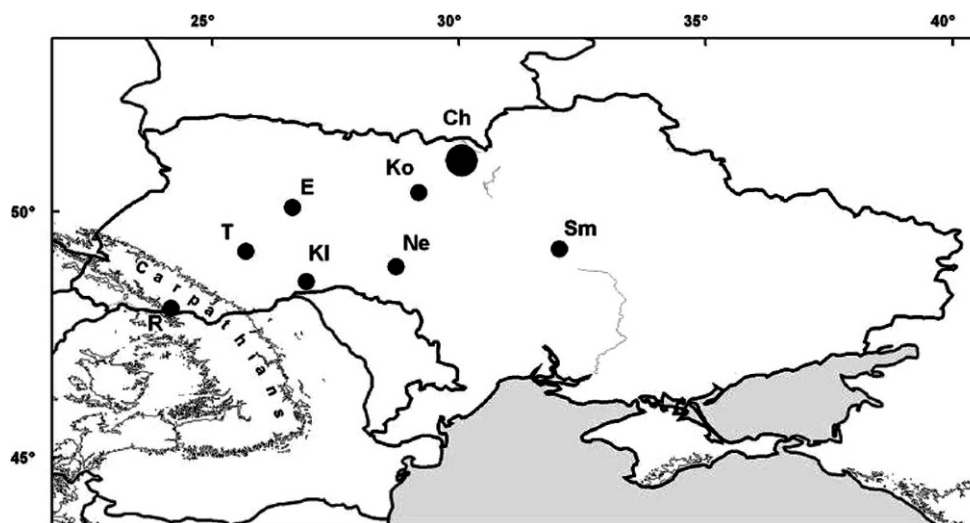


Fig. 1. Map of Ukraine, with collection sites for bank voles (*Myodes glareolus*) assessed for the present study. Shown are Chernobyl (Ch) sites evaluated in previous studies [4,15–17], including radioactively contaminated sites Glyboke Lake and Red Forest, as well as reference sites Nezamozhnyia, Nedanchichy, Oranoe, Krasnoye, Paryshev, and Stupnikovo. Sites added for the present study include sites in northern Ukraine (Ko = Korostyshev), western Ukraine (E = Ezyaslav; Kl = Klamentsy; R = Rakhiv; T = Terebovlya), and central Ukraine (Ne = Nemirov; Sm = Smela).

Chernobyl Nuclear Power Plant near Chernobyl, Ukraine, to understand whether multigenerational exposure to radiation has resulted in genetic alteration. As with the aforementioned research on patterns of dispersal, the Chernobyl studies were conducted in a geographically restricted area. Their results indicated that genetic diversity in the highly contaminated Red Forest area was elevated with respect to other localities. The authors noted that several alternative explanations, including those related to typical demographic processes, could account equally well for observed differences, thus making it difficult to draw definitive conclusions [4,15–17]. Other Chernobyl studies [11,13,31,32] have implicated radiation exposure as the causal agent of deleterious morphological and genetic effects in rodent populations. The spatial breadth of these studies was unclear, however, because detailed collection site data were not provided.

Expanding sampling efforts to encompass a larger geographic range in Ukraine is of benefit in at least two respects. The first is the addition of genetic information on Ukrainian bank vole populations to the existing archives maintained in public repositories such as GenBank® (National Center for Biotechnology Information). Currently, knowledge regarding rodent biodiversity in Ukraine is limited primarily to that gathered as a consequence of conducting studies in radioactively contaminated regions of northern Ukraine. Data that can resolve questions about composition and diversity in vole populations also can be used to address concerns about the general vitality of the ecosystems in which they reside, including those defined by the presence of environmental toxicants. Additionally, the inclusion of an expanded sampling regime will provide a baseline perspective for evaluating whether the variation in contaminated and uncontaminated sites is unusually elevated compared to that observed across similar scales in other regions of Ukraine.

Applying such knowledge to the Chernobyl studies will be a useful means of assessing the apparently increased mutational pressure associated with environmental radiation exposure so that its magnitude and importance relative to typical demographic processes may be evaluated. The present study

seeks to characterize genetic structure in bank vole populations at greater magnitudes of spatial resolution and to understand the extent to which population dynamics contribute to the observed differences in contaminated regions.

MATERIALS AND METHODS

Study specimens

From 1997 to 2004, our team collected 545 *M. glareolus* specimens in Ukraine at 15 collection localities encompassing northern ($n = 9$), western ($n = 4$), and central ($n = 2$) portions of the country (Fig. 1). We sampled most localities once, although at least two reference localities (Oranoe and Stupnikovo) and one radioactive site (Red Forest) were sampled multiple times. Localities, with associated sample sizes and collection years, are detailed in Table 1 and Appendix 1. The locality identified as Nezamozhnyia was called Chista in previous studies [4,15–17] but was renamed for the present study to reflect its proper topographic designation. Averaged body burdens of radiocesium also are listed for specimens from each site. We designated the Red Forest and Glyboke Lake sites as contaminated sites, whereas all other collection localities were considered to be reference sites. Variation in background radiation levels and the mosaic pattern of deposition resulting from the Chernobyl accident, however, made the task of clearly defining reference and contaminated sites somewhat complex. For example, absorbed doses in the animals from Stupnikovo and Krasnoye exceed dose levels in specimens from other reference localities. If, however, it is assumed that the effects of Chernobyl radiation are linearly related to dose [33], then any genetic effect resulting from exposure at the levels experienced in Stupnikovo and Krasnoye is expected to result in patterns of variation more closely resembling those of reference sites than of contaminated sites. As such, both regions are considered to be reference sites for the purpose of subsequent analyses.

We collected liver tissue from specimens immediately following euthanization and preserved the tissues in 5 ml of lysis buffer [34] or 95% ethanol. Tissues, as well as skin and skull or skull-only preparations for select specimens, are

Table 1. Haplotype (*h*) and nucleotide diversity (ND) with associated standard error (SE) for Ukrainian bank vole (*Myodes glareolus*) populations^a

Site	¹³⁷ Cs (Bq/g)	<i>n</i>	<i>uhlth</i>	<i>h</i> ± SE ^b	ND ± SE
Northern Ukraine					
Nezamozhnyia (1999)	~0.44	22	0/3	0.48 ± 0.09	0.0019 ± 0.0018
Nedanchichy (1999)	~0.44	30	1/4	0.19 ± 0.10	0.0011 ± 0.0013
Oranoe (1998, 1999)	~0.44	65	0/4	0.63 ± 0.04	0.0027 ± 0.0022
Glyboke Lake (1996)	2,403	32	2/6	0.73 ± 0.05	0.0043 ± 0.0031
Red Forest (1997–2001)	24,720	231	0/7	0.73 ± 0.02	0.0047 ± 0.0034
Krasnoye (2004)	7.01	13	0/4	0.69 ± 0.12	0.0051 ± 0.0037
Paryshev (2004)	4.46	48	2/8	0.61 ± 0.07	0.0039 ± 0.0028
Stupnikovo (2004)	44.7	46	0/5	0.68 ± 0.04	0.0034 ± 0.0026
Korostyshev (2004)	~0	10	3/5	0.82 ± 0.10	0.0096 ± 0.0063
Western Ukraine					
Rakhiv (2004)	~0	16	3/4	0.64 ± 0.08	0.0060 ± 0.0041
Terebovlya (2004)	~0	4	1/2	0.50 ± 0.27	0.0017 ± 0.0021
Klementsyy (2004)	~0	1	0/1	1.00 ± 0.00	0.0000 ± 0.0000
Ezyaslav (2004)	~0	14	2/2	0.44 ± 0.11	0.0045 ± 0.0034
Central Ukraine					
Smela (2004)	~0	9	1/3	0.56 ± 0.16	0.0021 ± 0.0021
Nemirov (2004)	~0.44	4	1/4	1.00 ± 0.18	0.0052 ± 0.0046

^a Also shown is *uhlth*, which denotes the ratio of unique haplotypes (*uh*) to total haplotypes (*th*) detected in a particular collection site. Sites, collection years, and sample sizes (*n*) are included as well. Internal activities of radiocesium were averaged for each site [46,47] (S. Gaschak, International Radioecology Laboratory, Ukraine, personal communication) and are indicated as ¹³⁷Cs (Bq/g).

^b Diversity estimates were pooled across all sampling periods for sites from which specimens were collected multiple times (i.e., Oranoe and Red Forest).

housed in the International Radioecology Laboratory in Slavutych, Ukraine, or in the Museum of Texas Tech University in Lubbock, Texas, USA. Additionally, the database of the Museum of Texas Tech University contains information for specimens included in the present study.

We supplemented our sequence data with archived sequences from GenBank to evaluate nucleotide differences among *Myodes* sp., to verify specimen identification, and to provide appropriate outgroup sequences for phylogenetic analyses. Representatives of the species *Myodes glareolus*, *californicus*, *gapperi*, *rutilus*, and *rufocanus* are included in our analyses and archived under GenBank accession numbers AJ236833, AF367179 to AF367201, and EF421408 to EF421411.

Molecular methods

We extracted genomic DNA from the preserved liver tissues using standard phenol chloroform and alcohol precipitation methods. We amplified and sequenced a 291-bp segment of the mitochondrial control region and associated tRNA (265-bp control region, 26-bp tRNA) following the methods described by Matson et al. [15], although we used BigDye[®] Terminator version 3.1 [35] in our sequencing reactions. We used Sequencing Analysis software version 3.4.1 (PE Applied Biosystems) and AssemblyLIGN[™] software version 1.09b [36] to verify and assemble sequences.

Data analysis

We evaluated individual sequences and assigned them to haplotypes using MacClade software version 4.05 [37]. We verified unique haplotypes (i.e., those detected in a single specimen) by repeating the processes of DNA amplification and sequencing of individuals for which such haplotypes were identified. Because the presence of numts (nuclear sequences of mitochondrial origin) may confound molecular analyses of mtDNA [38], sequence data were evaluated for transition to transversion ratios, presence of indels, and codon position bias. Because none of the phenomena normally associated with

numt contamination were evident in our data set, we presume that the actual mitochondrial genome is represented.

We constructed an unrooted haplotype network using the 95% parsimony criterion as implemented in TCS version 1.18 [39] to evaluate geographic structuring in Ukrainian bank vole populations. To assess evolutionary relationships among the haplotypes, we constructed a minimum evolution tree, based on the Tamura–Nei model of nucleotide substitution, using MEGA version 3.1 [40]. We also used MEGA to construct a genetic distance matrix for all possible pairwise population comparisons. We conducted a correlation analysis in which we assessed the relationship between genetic distance and linear geographic distance using a Mantel test performed with 1,000 permutations [41]. We used Arlequin version 3.0 [42] to estimate Nei's gene diversity (*h*) and nucleotide diversity for each of the localities. Additionally, we evaluated possible demographic expansion using Arlequin to generate mismatch distributions for each population, with Rogers' Expansion Model [43] used as the basis for predicted distributions. We evaluated the degree of genetic structuring, based on hierarchical *F*-statistics, among Ukrainian vole populations using the nested analysis of molecular variance (AMOVA) function in Arlequin. Significance of variance estimates for *F*-statistics and AMOVA variance components as compared to estimates generated at random were evaluated using a randomization procedure with 10,100 permutations.

We grouped the populations according to the natural assemblages suggested by the aforementioned data analyses, with one group containing 13 locations (Nezamozhnyia, Nedanchichy, Oranoe, Glyboke Lake, Red Forest, Krasnoye, Paryshev, Stupnikovo, Korostyshev, Terebovlya, Klementsyy, Smela, and Vinnitsa) and two other groups containing only a single locality (Ezyaslav or Rakhiv, respectively). We further examined genetic partitioning among Ukrainian populations by randomly assigning collection sites to groups (e.g., regional groupings) and performing an AMOVA, in a manner similar to that outlined above, for each grouping strategy. Additionally, we conducted an AMOVA in which we assessed genetic

Table 2. Polymorphic sites and nucleotide substitutions identified in haplotypes from the bank vole (*Myodes glareolus*) and in other species from the genus *Myodes* (n = 545)

Haplotype ^a	11	40	50	56	69	139	143	151	152	153	156	157	170	196	197	240	247	267	270
1	C	C	A	T	A	A	A	C	T	C	T	C	C	A	T	C	T	T	C
2	.	.	G	T	T
3	C	.
4	T
5	.	.	.	C
6	T
7	T	.	T
8	C
9	.	.	.	C	T	.	T
10	T
11	.	T	T	.	.	C
12	T	C	.
13	G	.	C	.	C	T
14	T
15	.	.	.	C	T	T	C	.
16	T
17	C
18	C	.	.	T	C	.	.
19	A	C	.
20	T	G
21	A
22	T	T	T
23	T	T	T	C
24	T	.	.	C	T	T
25	G
26	G
27	T	.	.	.
AJ236833	-	.	.	C	T	T	.	C	.	.	C	.
AF367195	-	.	.	C	T	T	C	.
AF367196	-	T	T	C	.
AF367199	-	A	T
<i>M. californicus</i>	-	T	.	.	.	T	T	.	C
<i>M. gapperi</i>	-	.	G	C	.	.	.	T	C	A/T	C	A	T	.	T	T	C	C	A
<i>M. rufocanus</i>	T	A	.	C	.	.	.	T	.	.	.	T	T	.	A	.	.	C	A
<i>M. rutilus</i>	-	.	G	T	.	.	C	A/T	T	.	C	.	C	.	.

^a Haplotype 1 is provided as a point of reference for other haplotypes. Detailed information about sequence position is provided by Matson et al. [18]. *Myodes glareolus* haplotypes (1–27, AJ236833, AF367195, AF367196, and AF367199) are listed individually; haplotypes for other *Myodes* sp. are represented by consensus sequences.

structure in northern populations only. We assigned populations to one of three groups as follows: Group 1, containing Oranoe, Glyboke Lake, Red Forest, Krasnoye, Paryshev, and Stupnikovo; group 2, containing Nezamozhnyia and Nedanchichy; and group 3, containing Korostyshev. We assessed genetic differentiation between populations by computing F_{ST} (variation partitioned in the subpopulation relative to the total population) values based on pairwise differences, and we subsequently used the F_{ST} values to generate estimates of gene flow (Nm) between populations as $Nm = 0.5(1/F_{ST} - 1)$, where Nm represents the effective population size of females (N) and the female migration rate (m) (after Slatkin [44]). Both procedures were implemented in Arlequin. We approximated times of divergence for Rakhiv and Ezyaslav, as well as for the remaining Ukrainian populations, based on data from Matson and Baker [45], in which the rate of evolution for the extended terminal associated sequences domain of the mitochondrial control region was calibrated at 3.6 to 4.2% per million years in arvicoline rodents. Comparisons of genetic distances to the proposed rate provide a rough estimate of divergence times among populations.

Radioactive body burden

Internal radiocesium burdens for specimens collected from the respective localities were estimated following the methods

described by Chesser et al. [46,47]. Detailed discussion of methodology may be found therein. Averaged internal radiocesium values for each collection site are listed in Table 1.

RESULTS

We detected 27 haplotypes in the *M. glareolus* specimens collected from northern, central, and western Ukraine (Fig. 1 and Table 1). We observed 19 variable nucleotide positions (Table 2), with transitions representing the majority of substitutions (17 of 19) in the combined data set. Pyrimidine transitions (13 events) occurred at greater frequencies than purine transitions (four events). Two transversion substitutions occurred at positions 196 and 270.

Haplotype diversity

Of the 27 haplotypes observed in Ukrainian bank voles, 16 of them were unique to a specific locality. All newly identified and unique haplotypes were verified by two investigators through independent amplification and sequencing. Two of the haplotypes were unique to the radioactively contaminated site Glyboke Lake, whereas other unique haplotypes were identified in reference sites (Tables 1 and 3). The remaining haplotypes identified in northern Ukraine were shared between at least two localities. Haplotype compositions varied among sites, although either haplotype 1 or 4 appeared at the highest

Table 3. Averaged haplotype frequencies for bank voles (*Myodes glareolus*) from collection sites in Ukraine

Haplotype	Locality ^a														
	Nz	N	O	GL	RF	K	P	S	Ko	R	T	Kl	E	Sm	Ne
1	0.27	0.03	0.49	0.41	0.44	0.54	0.23	0.46	0	0	0.75	0	0	0.67	0.25
2	0	0	0	0	0.20	0	0	0.04	0	0	0	0	0	0	0
3	0	0	0.06	0.03	0	0.15	0.02	0	0	0	0	0	0	0	0.25
4	0.68	0.90	0.35	0	0.16	0	0.04	0.02	0.10	0	0	1.0	0	0.22	0
5	0	0	0	0.13	0.11	0	0	0.30	0	0	0	0	0	0	0
6	0	0	0	0.31	0.01	0.15	0.58	0.17	0	0	0	0	0	0	0
7	0	0	0.09	0	0.01	0	0	0	0	0	0	0	0	0	0
8	0.05	0.03	0	0	0.07	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0
11	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0.02	0	0.20	0	0	0	0	0	0
13	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0.15	0.04	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0.20	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0.10	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0.40	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0.29	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0.71	0	0
21	0	0	0	0	0	0	0	0	0	0.38	0	0	0	0	0.25
22	0	0	0	0	0	0	0	0	0	0.50	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0.06	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0.06	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0

^aNezamozhnya (Nz), Nedanchichy (N), Oranoe (O), Glyboke Lake (GL), Red Forest (RF), Krasnoye (K), Paryshev (P), Stupnikovo (S), Korostyshev (Ko) are in northern Ukraine. Rakhiv (R), Terebovlya (T) Klementsy (Kl), and Ezyaslav (E) are in western Ukraine. Smela (Sm) and Nemirov (Ne) are in central Ukraine.

frequencies for the majority of the collection areas. Exceptions were the localities Korostyshev, Rakhiv, and Ezyaslav. Haplotypes 1 and 4 were either absent or present at low frequencies in the three collections sites, and the majority of haplotypes identified in these sites were unique to their respective localities (Tables 1 and 3). Table 2 provides detailed information on nucleotide substitutions characterizing bank vole haplotypes from specimens collected in Ukraine. Thirteen of the variable nucleotide positions contained polymorphisms that were represented in at least one other haplotype, whereas six of the nucleotide polymorphisms were novel (Table 2).

We also compared haplotype polymorphisms in *M. glareolus* from Ukraine to those identified in *M. glareolus* from areas outside of Ukraine and other species of *Myodes*. Five nucleotide polymorphisms and three polymorphic sites were unique to Ukrainian *M. glareolus* haplotypes. Other unique haplotypes were comprised of novel combinations of nucleotides but did not contain polymorphisms specific to bank voles from Ukraine.

Haplotype (*h*) and nucleotide diversity estimates, with associated standard errors, are shown for all collection sites in Table 1. Diversity estimates were highly variable among sites, with values for *h* ranging from 0.19 ± 0.09 (mean \pm standard error) in Nedanchichy to 1.00 ± 0.18 (mean \pm standard error) in Nemirov (Fig. 2). Diversity estimates for the Klementsy locality were disregarded, because only a single specimen was available from that site. Nucleotide diversity estimates were roughly concordant with the patterns observed for *h*, with values ranging from 0.011 ± 0.0013 (mean \pm standard error) in Nedanchichy to 0.0096 ± 0.0063 (mean \pm standard error) in Korostyshev (Fig. 3). In general, both *h* and nucleotide diversity tended to be higher in northern collection sites than in either western or central collection sites, with the exception

of the Nezamozhnya and Nedanchichy localities (the easternmost areas of northern Ukraine).

Haplotype network and phylogram analyses

The parsimony network for all haplotypes from Ukraine revealed the star-like topology typical of a recently radiated group [48,49]. The most parsimonious explanation for the

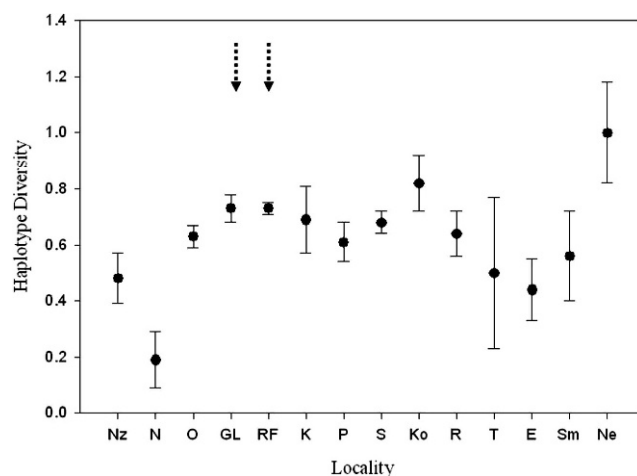


Fig. 2. Haplotype diversity estimates (*h*) for bank vole (*Myodes glareolus*) populations from northern, western, and central Ukraine. Diversity estimates in radioactively contaminated sites are indicated by dashed arrows. Standard error estimates are represented by vertical lines. Northern collection sites: GL = Glyboke Lake; K = Krasnoye; Ko = Korostyshev; N = Nedanchichy; Nz = Nezamozhnya; O = Oranoe; P = Paryshev; RF = Red Forest; S = Stupnikovo. Western collection sites: E = Ezyaslav; R = Rakhiv; T = Terebovlya. Central collection sites: Sm = Smela; Ne = Nemirov.

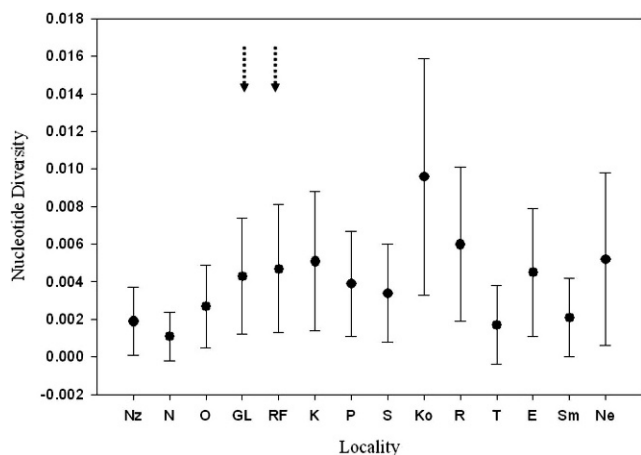


Fig. 3. Nucleotide diversity (ND) estimates for bank vole (*Myodes glareolus*) populations from northern, western, and central Ukraine. Diversity estimates in radioactively contaminated sites are indicated by dashed arrows. Standard error estimates are represented by vertical lines. Northern collection sites: GL = Glyboke Lake; K = Krasnoye; Ko = Korostyshev; N = Nedanchichy; Nz = Nezamozhnyia; O = Oranoe; P = Paryshev; RF = Red Forest; S = Stupnikovo. Western collection sites: E = Ezyaslav; R = Rakhiv; T = Terebovlya. Central collection sites: Sm = Smela; Ne = Nemirov.

relationships among haplotypes is that all are derived from haplotype 1 or 4, one of which is considered to be ancestral. The basal nature of either haplotype 1 or 4 is equally likely to be based on the distribution and frequency of the haplotypes (after Templeton et al. [50]). The majority of haplotypes were separated from haplotype 1 or 4 by only one to three mutational steps. Five missing (unsampled) haplotypes were present within the haplotype network, indicating that sampling efforts provide a relatively complete picture of the overall haplotype composition present in bank vole populations from Ukraine. Little geographic structure was evident in the network, with the exception of the Rakhiv population. Haplotypes 22, 23, and 24 (identified only at Rakhiv) were clustered, providing some evidence for genetic subdivision related to geographic structure in this population.

Similarly, the topography of the minimum evolution phylogram (Fig. 4) suggested that Ukrainian populations resulted from a recent radiation, as indicated by the shallow levels of divergence among haplotypes. According to the phylogram, haplotype 16, identified only in Korostyshev, represents the most basal haplotype. Given, however, that the haplotypes are separated by few mutational steps, determination of the most basal haplotype using maximum likelihood, Bayesian, or similar analyses was precluded. The phylogram provided little evidence for spatial clustering, although as in the parsimony network, haplotypes unique to the Rakhiv locality were closely grouped within the tree. Mantel test results indicated no significant correlation ($R = 0.19346$, $p = 0.86800$) between genetic and linear geographic distances when all study populations were considered.

Population history

Mismatch distribution results were mixed, with indications of departure from the expected stability in some populations but not in others (data not shown). The observed mismatch distributions for northern populations Nezamozhnyia, Nedanchichy, Oranoe, Glyboke Lake, Red Forest, Krasnoye, Pary-

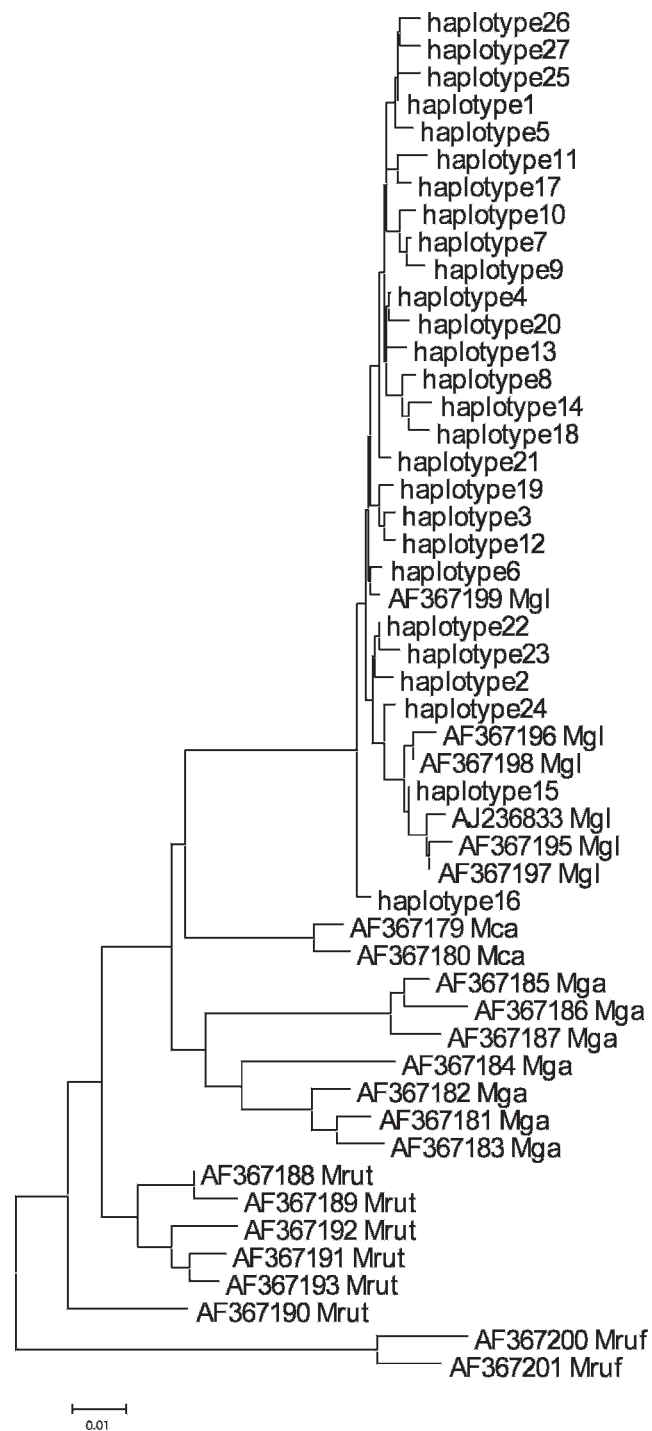


Fig. 4. Minimum evolution phylogeny for all Ukrainian bank vole (*Myodes glareolus*) haplotypes based on the Tamura–Nei model of nucleotide substitution. Haplotypes 1 to 27 are Ukrainian *M. glareolus* haplotypes. Other haplotypes, representing non-Ukrainian *M. glareolus* as well as other *Myodes* sp., were accession on GenBank (National Center for Biotechnology Information); accession numbers for those sequences are provided within the text.

shev, Stupnikovo, and Korostyshev, as well as the central population, Smela, tracked closely to the frequencies predicted by Rogers' Expansion Model [43]. Such results, taken together with haplotype network and phylogram analyses, indicate conformity to the expectations of demographic expansion (or departures from neutrality, or some combination of the two) in these populations, as predicted by Rogers and Harpending

Table 4. Analysis of molecular variance (AMOVA) with *Myodes glareolus* populations from northern, western, and central Ukraine^a

Source of variation ^b	df ^c	Sum of squares	Variance components	Variation (%)
Among regions				
<i>All except Rakhiv and Ezyaslav; Ezyaslav; Rakhiv</i>	2	26.719	0.36537	33.31
Among populations/regions	8	55.687	0.12437	11.34
Within populations	516	321.794	0.60716	55.35
Total	526	404.200	1.09689	

^a Populations were grouped according to the following design: Collection sites designated as *all except Rakhiv and Ezyaslav* include Nezamozhnyia, Nedanchichy, Oranoe, Krasnoye, Paryshev, Stupnikovo, Glyboke Lake, Red Forest, Korostyshev, Terebovlya, Klementy, Smela, and Nemirov. *Rakhiv* and *Ezyaslav* are designated as individual groups.

^b Variation among regions refers to the amount of variation partitioned among populations across regions (*all except Rakhiv and Ezyaslav; Rakhiv; Ezyaslav*). Variation among populations within regions (populations/regions) refers to the amount of variation partitioned among collection localities within a region. Variation within populations defines the amount of variation attributable to individuals across all collection localities without regard to the population or region from which they originated.

^c df = degrees of freedom.

[51]. Conversely, mismatch distribution analyses for the western populations Rakhiv and Ezyaslav differed from the expectations outlined by Rogers and Harpending [51] and were distinctly bimodal, suggesting population stability (i.e., equilibrium) in those localities.

Genetic structure

Analysis of molecular variance indicated that when localities were grouped in accordance with the strategy outlined previously (all except Rakhiv and Ezyaslav, Rakhiv, or Ezyaslav), the among-group variation accounted for a significant ($p < 0.001$) 33.31% of the total variation (Table 4). By contrast, AMOVAs conducted on a variety of random groupings yielded among-group variation ranging from 0.29 to 10.98% of total variation. Although a significant portion of total variation was accounted for by among-group variation, in some cases (i.e., at values >6%) values were substantially lower for random groupings compared to the grouping criterion noted above. Separate analysis of the northern sites revealed that among-group variation accounted for 17.63% ($p < 0.001$) of total variation in northern populations, providing evidence for pronounced genetic structure in the easternmost and southernmost regions (Table 5). Among-population-within-region and within-population variation accounted for 10.23 and 72.14%, respectively, of the total variation.

Pairwise comparisons of Ukrainian populations yielded F_{ST} values ranging from 0.0059 to 0.56 (Table 6), indicating that genetic differentiation is variable among sites. High levels of

differentiation occurred between the northern sites Nezamozhnyia and Nedanchichy and the majority of other sites, and moderate degrees of differentiation were evident in comparisons of Korostyshev to other northern sites. Similarly, pairwise F_{ST} values for the western sites Rakhiv and Ezyaslav revealed high levels of differentiation in comparisons with other populations, as well as with each other.

The gene flow estimator, Nm , suggested that gene exchange between most populations and those from the two western sites, Rakhiv and Ezyaslav, was limited (Table 6). The majority of pairwise comparisons resulted in Nm less than one, indicating that genetic drift is more likely than gene flow to have shaped patterns of haplotype variation in the two localities [44]. Conversely, Nm was greater than one for most other population comparisons (although evidence exists for reduced gene flow among some northern populations), implying that the exchange of genetic information has been an important factor in influencing observed haplotype frequencies within nearly all localities examined.

Divergence times

Assuming a mutation rate of 3.6 to 4.2% per million years, a rough estimation for the time of divergence is 298,000 to 255,000 years before present for the Rakhiv population and 149,000 to 128,000 years before present for the Ezyaslav population. Average divergence times for the remaining study populations correspond to approximately 171,000 to 147,000 years before present.

Table 5. Analysis of molecular variance (AMOVA) with *Myodes glareolus* populations from northern Ukraine^a

Source of variation ^b	df ^c	Sum of squares	Variance components	Variation (%)
Among regions				
<i>group 1, group 2, group 3</i>	2	21.986	0.14773	17.63
Among populations/regions	6	30.629	0.08570	10.23
Within populations	488	294.903	0.60431	72.14
Total	496	347.517	0.83773	

^a Populations are grouped according to the following design: *Group 1* includes Glyboke Lake, Red Forest, Oranoe, Krasnoye, Paryshev, and Stupnikovo; *group 2* includes Nezamozhnyia and Nedanchichy; and *group 3* includes Korostyshev.

^b Variation among regions refers to the amount of variation partitioned among populations across regions (*group 1, group 2, group 3*). Variation among populations within regions (populations/regions) refers to the amount of variation partitioned among collection localities within a region. Variation within populations defines the amount of variation attributable to individuals across all collection localities without regard to the population or region from which they originated.

^c df = degrees of freedom.

Table 6. Pairwise population variation partitioned in the subpopulation relative to the total population (F_{ST}) and estimates of gene flow (Nm) among populations ($n \geq 4$) of Ukrainian bank voles (*Myodes glareolus*)^a

	Nz	N	O	GL	RF	K	P	S	Ko	R	T	E	Sm	Ne
Nz	—	4.3	7.7	1.0	4.4	1.0	0.69	0.85	1.9	0.61	0.61	0.66	1.58	2.8
N	0.10	—	1.4	0.5	1.7	0.42	0.39	0.41	1.1	0.46	0.19	0.50	0.39	0.59
O	0.061	0.27	—	1.9	6.3	2	0.93	1.6	1.2	0.48	2.3	0.59	14.7	21
GL	0.33	0.51	0.21	—	5.5	23	4.3	84	1.1	0.66	9.1	0.48	5.1	3.7
RF	0.10	0.23	0.073	0.083	—	5.4	2.4	5.0	1.8	0.85	6.6	0.78	14	31
K	0.33	0.54	0.20	0.021	0.085	—	4.9	6.7	1.9	0.80	7.3	0.65	4.6	5.5
P	0.42	0.56	0.35	0.10	0.17	0.092	—	2.1	0.89	0.72	1.4	0.39	1.4	1.3
S	0.37	0.55	0.24	0.0059	0.091	0.069	0.19	—	0.78	0.48	6.2	0.39	4.0	2.4
Ko	0.21	0.31	0.30	0.31	0.22	0.21	0.36	0.39	—	1.2	1.8	1.1	1.8	5.1
R	0.45	0.52	0.51	0.43	0.37	0.39	0.41	0.51	0.30	—	0.56	0.61	0.59	1.1
T	0.45	0.72	0.18	0.052	0.07	0.064	0.25	0.075	0.22	0.47	—	0.46	15	5.5
E	0.43	0.50	0.46	0.51	0.39	0.44	0.56	0.56	0.31	0.45	0.52	—	0.54	0.97
Sm	0.24	0.56	0.033	0.089	0.034	0.098	0.26	0.11	0.22	0.46	0.032	0.48	—	Inf
Ne	0.15	0.46	0.023	0.12	0.016	0.084	0.28	0.17	0.089	0.32	0.083	0.34	0.0010	—

^a Values below the diagonal denote F_{ST} values, and corresponding Nm values are represented above the diagonal. Nezamozhnyia (Nz), Nedanchichy (N), Oranoe (O), Glyboke Lake (GL), Red Forest (RF), Krasnoye (K), Paryshev (P), Stupnikovo (S), Korostyshev (Ko) are in northern Ukraine. Rakhiv (R), Terebovlya (T), and Ezyaslav (E) are in western Ukraine. Smela (Sm) and Nemirov (Ne) are in central Ukraine.

DISCUSSION

The objectives of the present study were to understand the genetic variation present in bank vole populations across an expanded geographic range, to better interpret existing knowledge of biodiversity in the Ukraine, and to provide a foundation for assessing the veracity of ecotoxicological studies previously conducted in Chernobyl [4,15–17].

Structure and diversity in bank vole populations

Herein, mitochondrial sequence data were reported from northern, central, and western Ukraine. Fourteen collection sites ($N = 545$) are currently represented and are separated from each other by distances ranging from as little as 2 to more than 600 km (Fig. 1). Despite the extensive geographic range of the populations studied and the limited vagility of bank voles [25,26], genetic structure in bank vole populations is not significantly correlated with linear distance. Rather, demographic structure likely is regulated by other, less apparent mechanisms.

In specimens from Ukraine, 27 haplotypes were identified, with 16 of these being unique to the populations sampled for the present study (Table 3). Genetic composition was similar for the majority of sites (Table 3). Low levels of genetic differentiation, mediated by high levels of gene flow (Table 6), implies a strong interconnection among geographic areas, a conclusion further reinforced by the fact that the majority of populations share two or more haplotypes with at least one other population. The collective data suggest that bank vole populations in Ukraine consist of recently evolved lineages derived from one or two common ancestors. Both haplotype network and phylogram analyses reveal star-like topologies and short branch lengths that are indicative of a recent radiation (Fig. 4) [48,49]. Mismatch distribution analysis provides additional support for sudden demographic expansion (data not shown) [43]. The results conform to the expectations for population growth, although the data also may be interpreted as being indicative of a departure from strict neutrality in the mitochondrial genome. Based on the consistency of results across all analyses, the former explanation of recent population expansion for patterns of diversity in studied populations is favored. Rapid and repeated evolutionary pulses leading to almost simultaneous divergence of lineages have been well documented at generic, familial, and population levels in arvicoline rodents [52], indicating that the phenomenon

observed in Ukraine is common for this species even in areas where environmental pollutants are not known to be present.

Geographic structure in outlier populations

Although haplotype composition in nearly all collection areas suggests geographic cohesion, genetic subdivision is evident in some northern and western populations. The easternmost sites, Nezamozhnyia and Nedanchichy, and the southernmost site, Korostyshev, in northern Ukraine (Fig. 1) are genetically differentiated from most other sites (Table 6). Furthermore, AMOVA results reveal that among-group structure accounts for a significant portion of total variation when the populations are grouped separately from other northern collection localities (Table 5). Reasons for the observed differences in the sites are unclear, although physical boundaries to dispersal (e.g., the Pripyat and Dnieper river systems segregating sites) are probable contributors to existing levels of genetic discontinuity between these and other populations.

More pronounced levels of genetic structure are evident in the western sites Rakhiv and Ezyaslav. The populations in these areas are comprised primarily of unique haplotypes, and the haplotypes most common to other Ukrainian populations are absent (Tables 1 and 3). Pairwise comparisons and AMOVA results indicate that the populations are highly differentiated compared to those of other sites and to one another (Tables 4 and 6). Furthermore, genetic and geographic structures correlate well in Rakhiv, as evidenced by both the rooted and unrooted genealogies (Fig. 4). A similar level of correlation is less apparent for Ezyaslav, but ambiguity in terms of visible geographic structure may be related to differing times of divergence for the two populations. The combined results imply that a strong genetic discontinuity between these and other Ukrainian populations, presumably engendered by the accumulation of local mutations with little or no subsequent gene exchange (Table 6). Mismatch distributions reveal similar disparities in the two western regions, because observed distributions are indicative of long-term stability (data not shown). The demographic histories of the two western populations are clearly atypical compared to the histories characterizing the remaining study populations.

Deffontaine et al. [53] and Kotlík et al. [28] suggested that the Carpathian Mountains, bounding the westernmost portion of Ukraine, served as a refugium for woodland species during

the last glacial period. Several features of the westernmost Rakhiv population, including localized distribution of haplotypes, divergence from other populations before the last glacial period, and truncated gene exchange with other populations, are compatible with their hypothesis. Haplotype and nucleotide diversity estimates for the present study, however, are lower than those reported in the aforementioned studies [28,53]. The data suggest that current patterns of genetic diversity in Ukraine were strongly influenced by the northward movement of the boreal zone following the last glacial period, with the subsequent creation of a diversity center in northern populations. Southern populations likely were marginalized because of diminished gene exchange with areas of higher density. More sampling of Carpathian sites, as well as sites west of the Carpathians, will provide data to further test the glacial refugium concept.

Ecological or physical mechanisms dictating the apparent uniqueness of the Ezyaslav population are less straightforward, although F_{ST} and Nm values indicate that the most probable explanation is reduced historical gene flow. The site is proximal to a major tributary of the Pripyat River and, thus, is unlikely to maintain consistent levels of cover for woodland species because of occasional flooding in watershed regions. As such, bank vole populations in Ezyaslav, one of the highest points in the region, may be effectively insular so that gene exchange with other populations is rare. Alternatively, the unique genetic composition in Ezyaslav may be correlated with historical land use patterns, because the floodplain regions were among the populous areas of Ukraine from the 9th to 17th centuries. Limited information on local fauna prohibits deeper exploration of this topic, and more sampling in the region is necessary before our hypotheses regarding genetic discontinuity in Ezyaslav can be validated.

The data demonstrate that the mechanisms dictating genetic structure in Ukraine are influenced by natural processes producing subdivision across both broad and relatively limited geographic scales. The expanded data set thus provides a better understanding about the breadth of demographic histories that define Ukrainian populations. Genetic partitioning is clear in the populations from western Ukraine that are far removed from other collection sites, but it also is apparent in closely situated populations inhabiting northern Ukraine. If radiation exposure is the primary force shaping Chernobyl populations, then contaminated areas should contain the most atypical populations in terms of levels and patterns of diversity. In fact, however, populations from radioactive sites are genetically similar to the majority of other Ukrainian sites, whereas the populations exhibiting uncharacteristic structure are those residing in reference sites. These results underscore the importance of carefully evaluating populations that are selected for comparative studies.

Biogeographic perspective: Genetic diversity in Chernobyl

Researchers have drawn disparate conclusions based on the results of the population genetics studies conducted in Chernobyl. On one hand, a considerable body of research suggests that resident populations are experiencing an elevated mutational load as a result of exposure to ionizing radiation [11–13,29,31,32,54–57]. Such deleterious effects, however, are not indicated by the results of an equally substantial number of studies conducted in the most radioactive regions [4,15–17,58–63]. Below, the results of previous Chernobyl studies are revisited with a discussion about how the data may be useful

for evaluating the appropriateness of experiments designed to discern the biological effects of exposure to chronic radiation.

In four previous studies, the research team examined variation in the mitochondrial control region of exposed and unexposed bank vole populations in northern Ukraine and found that genetic diversity was elevated in radioactively contaminated areas as compared to uncontaminated reference regions. For the present study, haplotype and nucleotide diversity was examined in a number of additional sites, and diversity estimates varied considerably between sites and across regions (Table 1). Populations in northern Ukraine generally have higher levels of diversity compared to populations in other areas, but no relationship between exposure to ambient radiation and diversity is evident. Indeed, the areas of highest diversity are the northern collection site Korostyshev and the central site Nemirov, both of which are more than 100 km away from the nearest radioactive locality. When Ukrainian populations are viewed within a biogeographic context, genetic diversity in contaminated areas is not apparently elevated relative to that in uncontaminated sites.

Increased mutation rates resulting from exposure to radiation could manifest as a greater number of alleles at a given locus. Because the mitochondrial genome is inherited in its entirety, the number of alleles for mtDNA motifs is measured as the number of haplotypes identified in the population. An increased mutational load resulting from exposure to radiation therefore could be detectable as a greater number of locally confined haplotypes. On the contrary, uncontaminated sites were found to contain more unique haplotypes, and the ratio of unique to total haplotypes also tends to be greater (Table 1). Only two haplotypes exclusive to populations in radioactive sites were detected, despite extensive sampling over multiple years. Data from additional uncontaminated sites thus alters the conclusions drawn from previous Chernobyl research [4,6,16,17], in which radioactively contaminated sites were shown to harbor a greater number of endemic lineages.

Certain studies conducted in Chernobyl have indicated that populations in contaminated areas are experiencing genetic change consistent with adaptation to the radioactive environment [64,65]. Whereas biological responses are not unexpected in populations residing in polluted areas for several generations, the present study suggests that multigenerational changes causally related to environmental radiation exposure will produce distinctive genomic signatures. Populations in contaminated sites should contain novel combinations of alleles that are different from those in ecologically matched reference sites with similar demographic histories (i.e., with recent common ancestry), and levels of genetic diversity in contaminated sites should be notably dissimilar, with exposed populations exhibiting a greater range of variation. Diversity in radioactive areas may decline as a result of selective sweeps favoring alleles with optimal fitness for the contaminated environment or because of population bottlenecks related to decreased survival and/or reproduction in radioactive areas. Conversely, diversity may be enhanced because of positive selection for increased genetic diversity in populations chronically exposed to environmental stressors or recurrent colonization from multiple source populations outside contaminated zones. The current scientific literature suggests that any of these scenarios are plausible [66–68]. The populations of the present study, however, fail to satisfy the conditions as outlined above. Haplotype composition in radioactive sites is

equivalent to that documented in other populations throughout much of Ukraine, including the most proximal reference sites in northern Ukraine, suggesting that the dynamics shaping genetic composition in the sites are similar. Additionally, haplotype frequencies have remained essentially static throughout multiple sampling periods in contaminated regions. Finally, as noted above, levels of diversity in exposed populations are unremarkable compared to other populations in Ukraine, indicating that the ostensibly increased mutational pressure resulting from exposure is not manifested in the most labile region of the mitochondrial genome. The genetic data from bank vole populations in Ukraine fail to support the hypothesis that mutational changes in contaminated regions are the product of multigenerational exposure to Chernobyl radiation.

The present results differ from those of previous studies implicating chronic radiation exposure in a number of genotoxic effects. Based on information gathered across an expansive geographic as well as temporal range, the present study shows that patterns of diversity in Ukraine are more reasonably explained by typical demographic and ecological processes, such as range expansion and dispersal, than by the levels of exposure currently experienced in Chernobyl.

Data from the present study underscore the importance of adequate geographic sampling in ecotoxicological studies. Ecologically equivalent sites proximal to contaminated sites are expected to provide the most valid comparisons; however, a careful and considered approach to the selection of reference sites should include an understanding of generalized patterns of genetic diversity for the species under study. Without the benefit of an expanded perspective, it can be difficult or impossible to distinguish between variation resulting from natural processes and that resulting from exposure to an environmental toxicant. Population changes may be putatively attributed to site contamination, but any resultant data from spatially or temporally limited studies must be reevaluated in the context of repeated temporal sampling in multiple collection sites to take into account the genetic variability present in natural populations.

Acknowledgement—We gratefully acknowledge all the individuals who participated in Ukrainian fieldwork expeditions from 1995 to 2004, which have resulted in the database generated for the present study. We thank Mikhail Bondarkov, Julia Makluk, and the International Radioecology Laboratory at Slavutych, Ukraine. We also acknowledge Jeffrey K. Wickliffe and Hugo Mantilla-Meluk. Finally, we thank Heath Garner, Kathy MacDonald, and the Natural Science and Research Laboratory at Texas Tech University.

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APPENDIX

List of *Myodes glareolus* specimens collected in Ukraine and their corresponding localities. Voucher specimens and tissues are catalogued in the TK database of the Natural Sciences Research Laboratory of the Museum of Texas Tech University (TTU) in Lubbock, Texas, USA. Vouchers are housed in either TTU or the International Radioecology Laboratory in Slavutych, Ukraine. Specimens are grouped according to general locality (Fig. 1) and are subsequently arranged according to haplotype number (Table 2). Haplotypes 1, 2, 4, 5, 6, 7, 8, 12, 13, 14, and 15 are archived in GenBank under accession numbers AY449642 to AY449649 and EF421408 to EF421411. Representative specimens for those haplotypes correspond to TK67632, TK67631, TK67657, TK68040, TK67881, TK68032, TK67730, TK95824, TK95792, TK95818, and TK95814.

A. Chernigov District

1. Nezamozhnyia (51°35.335'N, 30°51.138'E), $n = 22$

Haplotype 1: TK81441, TK81567, TK81572, TK81583, TK81601, TK81604

Haplotype 4: TK81342 to TK81345, TK81448, TK81449, TK81570, TK81573, TK81575, TK81576, TK81578, TK81582, TK81603, TK81617, TK81620

Haplotype 8: TK81571

2. Nedanchichy (51°30.002'N, 30°51.592'E), $n = 30$

Haplotype 1: TK81565

Haplotype 4: TK81529, TK81530, TK81532 to TK81539, TK81542 to TK81544, TK81546 to TK81554, TK81557, TK81559, TK81560, TK81563, TK81566

Haplotype 8: TK81540

Haplotype 11: TK81531

3. Oranoe (51°02.681'N, 30°09.670'E), $n = 65$

Haplotype 1: TK81015, TK81018, TK81019, TK81023 to TK81028, TK81088, TK81103, TK81104, TK81106, TK81107, TK81109, TK81116, TK81117, TK81127, TK81129, TK81642, TK81650, TK81651, TK81654, TK81657, TK81658, TK81670, TK81692 to TK81694, TK81709, TK81712, TK81770

Haplotype 3: TK81058, TK81565, TK81637, TK81660

Haplotype 4: TK81004, TK81016, TK81021, TK81022, TK81029, TK81030, TK81087, TK81089, TK81090, TK81091, TK81092, TK81108, TK81110, TK81119, TK81120, TK81122, TK81123, TK81125, TK81128, TK81629, TK81659, TK81662, TK81718

Haplotype 7: TK81017, TK81101, TK81115, TK81631, TK81635, TK81638

4. Red Forest (51°23.040'N, 30°03.780'E), $n = 231$

Haplotype 1: TK67027, TK67632, TK67634, TK67638, TK67681, TK67726, TK67729,

TK67791, TK67807, TK67808, TK67813, TK67877, TK67878, TK67914, TK67927, TK67974, TK67983, TK67987, TK67990, TK67992, TK67994, TK67995, TK68028 to TK68030, TK68034, TK68041, TK74007, TK74011, TK74062, TK74068, TK74082, TK74089, TK74093, TK74119, TK74120, TK74131, TK74133, TK74138, TK74151, TK74152, TK74160, TK74171, TK74172, TK74186, TK74188, TK74202, TK74204, TK74205, TK74214, TK74224, TK74233, TK74235, TK74238, TK74240, TK74242, TK74243, TK74254, TK74257, TK74264, TK74271, TK74272, TK74274, TK74276, TK74279, TK74289, TK74290, TK74291, TK74303, TK74304, TK74308, TK81148, TK81163, TK81178, TK81244, TK81245, TK81247, TK81257, TK81258, TK81260, TK81263, TK81265, TK81267, TK81270, TK81277, TK81279, TK81293 to TK81296, TK81310, TK81422, TK81423 to TK81425, TK81442, TK81444, TK81445, TK81452, TK81470, TK81471

Haplotype 2: TK67631, TK67633, TK67635, TK67636, TK67652, TK67670, TK67671, TK67673, TK67674, TK67676, TK67789, TK67883, TK67899, TK67901, TK67902, TK67915, TK67928, TK67975 to TK67977, TK67980, TK74059, TK74081, TK74099, TK74132, TK74157, TK74162, TK74200, TK74208, TK74210, TK74215, TK74223, TK74229, TK74253, TK74270, TK74281, TK74306, TK81151, TK81152, TK81259, TK81264, TK81461, TK81462, TK81472, TK81476, TK81481

Haplotype 4: TK67655, TK67657, TK67680, TK67732, TK74008, TK74012, TK74014, TK74043, TK74122, TK74123, TK74150, TK74165, TK74170, TK74203, TK74227, TK74232, TK74239, TK74244, TK74259, TK74261, TK74292, TK74302, TK81150, TK81162, TK81228, TK81229, TK81261, TK81272, TK81273, TK81278, TK81299, TK81304, TK81435, TK81443, TK81446, TK81450, TK81487

Haplotype 5: TK67895, TK68040, TK74009, TK74010, TK74129, TK74149, TK74169, TK74189, TK74190, TK74201, TK74212, TK74231, TK74234, TK74237, TK74251, TK74252, TK74256, TK74258, TK74260, TK74262, TK74265, TK74273, TK74287, TK81230, TK81269, TK81281

Haplotype 6: TK67881, TK81459

Haplotype 7: TK68032

Haplotype 8: TK67730, TK67810, TK67979, TK74004, TK81166, TK81231, TK81232, TK81271, TK81276, TK81280, TK81309,

- TK81311 to TK81313, TK81406, TK81451, TK81460
5. Glyboke Lake (51°26.695'N, 30°03.826'E), $n = 28$
- Haplotype 1: TK50056, TK50065, TK50071, TK50072, TK50081, TK50083, TK50084, TK50088, TK50097, TK50098, TK50100, TK50109, TK50110
- Haplotype 3: TK50076
- Haplotype 5: TK50078, TK74114, TK74134, TK74161
- Haplotype 6: TK50059, TK50067, TK50080, TK50086, TK50089, TK50104, TK50105, TK50107, TK74207, TK81266
- Haplotype 9: TK50074, TK50079, TK50108
- Haplotype 10: TK50103
6. Krasnoye (51°27.409'N, 30°07.831'E), $n = 13$
- Haplotype 1: TK95755 to TK95758, TK95761, TK95762, TK95768
- Haplotype 3: TK95753, TK95754
- Haplotype 6: TK95759, TK95764
- Haplotype 15: TK95760, TK95763
7. Paryshev (51°18.805'N, 30°17.799'E), $n = 48$
- Haplotype 1: TK95775, TK95777, TK95786, TK95801, TK95803, TK95810, TK95811, TK95816, TK95819, TK95821, TK95822
- Haplotype 3: TK95802
- Haplotype 4: TK95776, TK95797
- Haplotype 6: TK95778 to TK95785, TK95787 to TK95791, TK95793 to TK95796, TK95798 to TK95800, TK95805 to TK95809, TK95812, TK95815, TK95825
- Haplotype 12: TK95824
- Haplotype 13: TK95792, TK95804
- Haplotype 14: TK95814
- Haplotype 15: TK95818, TK95820
8. Stupnikovo (51°22.245'N, 30°04.171'E), $n = 46$
- Haplotype 1: TK95827, TK95833, TK95836, TK95837, TK95838, TK95840, TK95842, TK95844, TK95846, TK95847, TK95849, TK133971, TK133973, TK133974, TK133976, TK133977, TK133978, TK133985, TK133988, TK133990, TK133991
- Haplotype 2: TK133975, TK133979
- Haplotype 4: TK95835
- Haplotype 5: TK95829, TK95830, TK95831, TK95832, TK95834, TK133967, TK133968, TK133970, TK133972, TK133986, TK133987, TK133989, TK133994, TK133995
- Haplotype 6: TK95828, TK95839, TK95841, TK95843, TK95845, TK133992, TK133993, TK133966
- B. Korostyshev, Zhytomir District (50°22.512'N, 29°12.104'E), $n = 10$
- Haplotype 4: TK96761
- Haplotype 12: TK96757, TK96762
- Haplotype 16: TK96760, TK96776
- Haplotype 17: TK96782
- Haplotype 18: TK96755, TK96756, TK96758, TK96759
- C. Ezyaslav, Khmelnytsky District (50°04.337'N, 26°38.396'E), $n = 14$
- Haplotype 19: TK96800, TK96806, TK96823, TK96833
- Haplotype 20: TK96801, TK96807, TK96810, TK96813, TK96814, TK96818, TK96826, TK96829, TK96831, TK96832
- D. Terebovlya (between villages Slobodka and Budanov), Ternopol District (49°11.083'N, 25°41.987'E), $n = 4$
- Haplotype 1: TK96842, TK96843, TK96853
- Haplotype 25: TK96863
- E. Smela, Cherkassy District (49°13.966'N, 32°02.610'E), $n = 9$
- Haplotype 1: TK96965 to TK96968, TK96970, TK96975
- Haplotype 4: TK96978, TK96979
- Haplotype 27: TK96969
- F. Nemirov (between villages Pechera and Sokoletc), Vinnitsa District (48°51.901'N, 28°44.657'E), $n = 4$
- Haplotype 1: TK96938
- Haplotype 3: TK96950
- Haplotype 21: TK96949
- Haplotype 26: TK96939
- G. Klementsyy, Chernivtsy District (48°34.170'N, 26°55.557'E), $n = 1$
- Haplotype 4: TK96916
- H. Rhakiv, Uzhgorod District (48°01.519'N, 24°10.63'E), $n = 16$
- Haplotype 21: TK96875, TK96877, TK96882, TK96883, TK96898, TK96900
- Haplotype 22: TK96872, TK96874, TK96879, TK96880, TK96881, TK96884, TK96897, TK96899
- Haplotype 23: TK96876
- Haplotype 24: TK96878