



Consequences of Polluted Environments on Population Structure: The Bank Vole (*Clethrionomys glareolus*) at Chornobyl

R. J. BAKER^{1,*}, A. M. BICKHAM¹, M. BONDARKOV², S. P. GASCHAK², C. W. MATSON³,
B. E. RODGERS¹, J. K. WICKLIFFE¹ AND R. K. CHESSER^{2,4}

¹Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, USA

²International Radioecology Laboratory, Slavutych, UA

³Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77840, USA

⁴Department of Genetics, University of Georgia, Athens, GA 30605, USA

Accepted 17 December 2000

Abstract. The accident at the Chornobyl Nuclear Power Plant in April 1986, released 100–200 million Curies of radioactive material into the surrounding environment. To investigate the possible genetic and population effects resulting from chronic exposure to this environmental radiation, we have examined mitochondrial DNA (control region) sequences from bank voles, *Clethrionomys glareolus*, inhabiting contaminated sites. Our analysis indicates genetic diversity is elevated in the contaminated sites when compared to relatively uncontaminated reference sites. This may be attributed to either an increased mutation rate in the mtDNA control region or immigration of individuals from surrounding areas into the contaminated environment. Although our observations do indicate that the contaminated areas represent sink populations, we cannot statistically discriminate between these two alternatives at this time. In addition, we have been unable to attribute any significant detrimental effects to bank vole populations inhabiting the contaminated Chornobyl environment based on these data. This is particularly paradoxical considering bank voles in the contaminated areas harbor the highest radiocesium (¹³⁷Cs) body burdens and external dose rates of any mammal ever measured. Our long-term research on the bank vole indicates that several factors, including contaminants, may affect haplotype dynamics both spatially and temporally. These multifarious influences subsequently affect population genetic estimates typically used to address the effects of environmental pollution on animal populations. Finally, we provide a general framework for designing experiments investigating the role contaminants play in altering the genetic characteristics of exposed populations.

Keywords: Chornobyl; bank vole; mtDNA control region; ionizing radiation; population genetics

Introduction

If populations in a contaminated environment are affected such that fitness, life expectancy, and health are adversely impacted, then the effect(s) should be detectable by population genetic and ecological studies.

Our research using the bank vole (*Clethrionomys glareolus*) from Chornobyl documents that many factors other than the existence of contaminants may affect population genetic structure making it difficult to provide statistically significant resolution when comparing populations living in contaminated to those inhabiting relatively uncontaminated sites. Therefore, the population genetic consequences of living in a contaminated environment are difficult to resolve. The significance

*To whom correspondence should be addressed: Tel.: 806-742-2702;
Fax: 806-742-2963; E-mail: rjbaker@ttu.edu

of this observation is not trivial. These approaches will ultimately provide a foundation for establishing estimates of human and wildlife risk to environmental contamination and many other critical economic and ecologic decisions (e.g., determining which environments should be remediated). Herein, we examine the population genetics of the bank vole using the mtDNA control region to elucidate the spatial and temporal dynamics of maternal lineages in radioactively contaminated and uncontaminated reference habitats near Chernobyl, Ukraine. The null hypothesis states that no significant genetic variation will distinguish populations living in contaminated environments from those living in reference environments. However, it is difficult to establish an adequate alternative hypothesis to test for the effects of radiation on the interface between ecology and population genetics. The difficulty in defining a falsifiable hypothesis is a function of the numerous possible outcomes which exist. One possibility is that the radiation released in April 1986 was so detrimental that local populations of bank voles were extirpated resulting in extreme bottlenecks that reduced haplotype diversity within these impacted populations. Second, individuals in these populations exposed to high levels of radiation may have experienced an elevated mutation rate. The populations at contaminated sites may then exhibit increased haplotype diversity. Third, assuming local populations were extirpated in 1986, founders of the present populations in the contaminated sites may represent a mixture of immigrants from surrounding metapopulations unaffected by radiation. This phenomenon could serve to increase or decrease haplotype diversity at contaminated sites depending upon the immigration rates and structure of these contributing metapopulations. Fourth, intense selection for radioresistance may have reduced genetic variation because only a subset of individuals possessed the "most fit" genotype. However, this alternative is not likely to confound population genetic studies using unlinked loci which are effectively in allelic equilibrium with the gene under selection. These conclusions are simplistic and certainly not exhaustive. Additionally, these alternatives are not mutually exclusive and may act in concert or in opposition to one another. The interface between ecology and population genetics is a fluctuating equilibrium of many forces. Therefore, the use of population genetics to study the effects of contaminants on a species is extremely complex and requires exceptional experimental design. Below, we briefly present an interpretation of data

regarding the population genetics of the bank vole inhabiting the Chernobyl region of northern Ukraine. We then present some essential criteria for the design of research investigating population genetics as a means of understanding the consequences of inhabiting polluted environments.

Materials and methods

Experimental design

The following is an extension of and includes the data from Matson et al. (2000). The bank vole, *Clethrionomys glareolus*, was chosen as the model system in which to investigate population genetic characteristics (i.e., genetic diversity, haplotype frequencies) because this species exhibits the highest internal radioactivity of any rodent species inhabiting the Chernobyl environment (Chesser et al., 2000). See Matson et al. (2000) for details regarding molecular and analytical methodology. The mtDNA region (control region or D-loop) used in Matson et al. (2000) and in this extension of their study is considered to be highly variable and useful for population genetic analyses (Bickham et al., 1996; Bickham et al., 1998; Matson et al., 2000). Genetic diversity (h) was estimated for each locale using the Arlequin ver. 2.000 program (Schneider et al., 2000). Phenetic relationships among locales were analyzed using the TFPGA ver 1.3 program (Miller, 1997). Specifically, genetic distances (Nei, 1972; Nei unbiased 1978) were clustered using the UPGMA to visualize relationships among both spatial and temporal samples. Internal radiocesium activity and dose rate were estimated for many of the individuals from contaminated and reference sites (Chesser et al., 2000).

Collecting sites

We examined bank voles from two highly contaminated radioactive sites (the Red Forest 36295545 U5697040 and Glyboke Lake 36296254 U5703608). These two sites are separated by the Prypiat river and are approximately 7 km apart. These sites represent the two most contaminated sites studied thus far within the 10-km restriction zone. Three reference sites (Oranoe 36301009 U5658635, Chista 36351221 U5717505, and Nedanchichy 36455 U5707606), where little or no radioactive contamination exists, were chosen outside of the 30-km exclusion zone. At all of these sites, bank

voles were the predominant rodent species collected (Baker et al., 1996).

Results

Population genetic/mtDNA analyses

We examined 291 base pairs of the mitochondrial DNA (mtDNA) control region from 315 bank voles. A total of 11 different haplotypes were identified. Frequencies of each haplotype from each sampling effort are presented in Table 1. Genetic diversity estimates (*h*) are presented in Fig. 1. Genetic diversity was consistently higher in the contaminated sites relative

to the reference sites. Phenetic relationships among spatial and temporal samples are presented in Fig. 2. All temporal samples (i.e., multiple samples from the same locale) clustered together at a maximum genetic distance of 0.16. Spatial relationships suggested two predominant clusters. One included two reference sites, Chista and Nedanchichy which are separated from the other cluster and remaining sites by the Dnieper River (genetic distance of 0.61). A second “subcluster” was evident in the cluster containing the Red Forest, Oranoe, and Glyboke Lake samples. The Glyboke Lake locale is separated (genetic distance of 0.41) from the Red Forest and Oranoe locales by the Prypiat River.

Table 1. Mitochondrial DNA control region haplotypes in bank voles (*n* = 315), *Clethrionomys glareolus*, from northern Ukraine. Populations represent collecting locales (experimental sites-Red Forest and Glyboke Lake; reference sites-Chista, Nedanchichy, and Oranoe). No relationship among haplotypes is implied by the numbering scheme

Population	Haplotype										
	1	2	3	4	5	6	7	8	9	10	11
Chista (1999) <i>n</i> = 17	0.24	—	—	0.59	0.06	—	—	0.12	—	—	—
Nedanchichy (1999) <i>n</i> = 28	—	—	—	0.89	—	—	—	0.07	—	—	0.04
Oranoe (1998) <i>n</i> = 43	0.44	—	0.02	0.44	—	—	0.09	—	—	—	—
Oranoe (1999) <i>n</i> = 22	0.50	—	—	0.36	—	—	0.14	—	—	—	—
Glyboke (1995) <i>n</i> = 28	0.50	—	0.04	—	0.04	0.29	—	—	0.11	0.04	—
Red Forest (1997) <i>n</i> = 105	0.42	0.15	—	0.17	0.24	0.01	—	0.01	—	—	—
Red Forest (1998) <i>n</i> = 8	0.38	0.25	—	0.25	—	—	—	0.13	—	—	—
Red Forest (Fall 1998) <i>n</i> = 24	0.43	0.05	—	0.20	0.08	0.03	—	0.23	—	—	—
Red Forest (1999) <i>n</i> = 40	0.42	0.21	—	0.21	—	0.04	—	0.13	—	—	—

“—” indicates this haplotype was not observed in these samples.

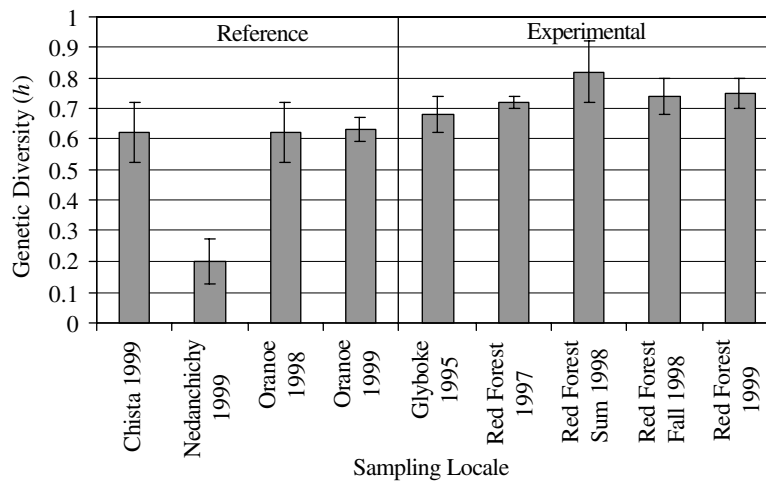


Figure 1. Genetic diversity (*h*) estimates for bank voles sampled in northern Ukraine. Glyboke and Red Forest represent radioactively contaminated experimental sites. Chista, Nedanchichy, and Oranoe represent reference sites. Genetic diversity estimates were generated in the Arlequin ver 2.000 program (Schneider et al., 2000). Bars are standard errors.

Radiocesium levels

Internal radioactivity ($^{134,137}\text{Cs}$) burdens were estimated for 230 individuals from both contaminated sites ($n = 203$ -Red Forest, $n = 27$ -Glyboke Lake) and 15 individuals from two of the reference sites ($n = 3$ -Chista, $n = 12$ -Nechanchichy; Chesser et al., 2000). Individuals from the contaminated sites exhibited an average internal radiocesium activity and dose rate of 2.8×10^4 Becquerels(Bq)/g and 20 milliGray(mGy)/day, respectively. The range of internal radiocesium activity in the experimental samples was from 0.0 to 1.3×10^5 Bq/g. Individuals from the relatively non-radioactive reference sites exhibited an average internal radiocesium activity and dose rate of 4.4×10^{-1} Bq/g and 3.1×10^{-4} mGy/day. The range of internal radiocesium activity in the reference samples was from 0.0 to 4.6 Bq/g.

Discussion

In the past two decades, there has been a revolution in molecular biology and molecular genetics (e.g., Polymerase Chain Reaction (PCR), automated DNA sequencing and genotyping). This revolution has permitted scientists to investigate genetic changes within populations. In addition, theoretical models of gene and haplotype dynamics in a population genetics framework have furthered the empirical application and interpretation of these types of data (Cockerham, 1973; Chesser and Baker, 1996; Nei, 1987; Chesser, 1998). Ecotoxicologists use standard parameter estimates generated from molecular data to compare exposed and unexposed populations in an effort to understand the impacts of environmental pollution primarily on populations but also on the DNA itself and individuals (Bickham and Smolen, 1994; Shugart and Theodorakis, 1996; Theodorakis et al., 1999; Bickham et al., 2000). These parameters include haplotype or allele frequencies, genetic diversity, heterozygosity, phylogenetic affiliations among haplotypes or alleles, and phenetic relationships among populations. We have examined spatial genetic variation among five locations (the two most radioactive sites known at Chernobyl, and three reference sites) as well as temporal genetic variation within the Red Forest. These examinations are based on 315 individuals with a resulting database of control region sequence data totalling 91,665 nucleotides. Our fundamental

goal was, and continues to be, assessing the possible impacts radioactive contamination has on populations of bank voles inhabiting the Chernobyl environment. Our conclusions thus far include the following:

- (1) Ecological components (i.e., habitat quality, community structure, etc.) vary to such an extent that distinguishing the effects of contaminants on population structure from non-contaminant effects will require massive datasets and long-term studies;
- (2) Spatial and temporal dynamics are substantially affected by stochastic influences over space and time. These may result in significant differences between populations. Additionally, geographic influences and proximity substantially affect population relationships (Fig. 2);
- (3) The single conclusion that potentially has significant implications to our primary research objective (the effects of contaminants on population genetics) is the increase in genetic diversity at the two experimental sites. With the data available at this time, we cannot discriminate between two alternative explanations (elevated mutation rate or increased variation through immigration). Assuming that the addition of new haplotypes is continuing, it will be possible to discriminate between the two by long-term mark-recapture studies coupled with detailed molecular genetic analyses.

Application of the following criteria should enable robust experimental design when analyzing and interpreting genetic data from populations inhabiting contaminated areas.

- (1) Sample multiple experimental sites, if possible, where the distribution of the contaminant(s) is documented.
- (2) Sample multiple reference sites where the contaminant(s) is relatively absent.
- (3) Sample from different geographic locations over the same time period.
- (4) Sample sites over time to establish patterns resulting from non-contaminant factors such as climate and population cycles.
- (5) Dose and/or body burden for exposed specimens should be calculated to estimate both exposure and variance in exposure.
- (6) Accurate and precise location data (e.g., GIS coordinates) should be recorded for each specimen collected.

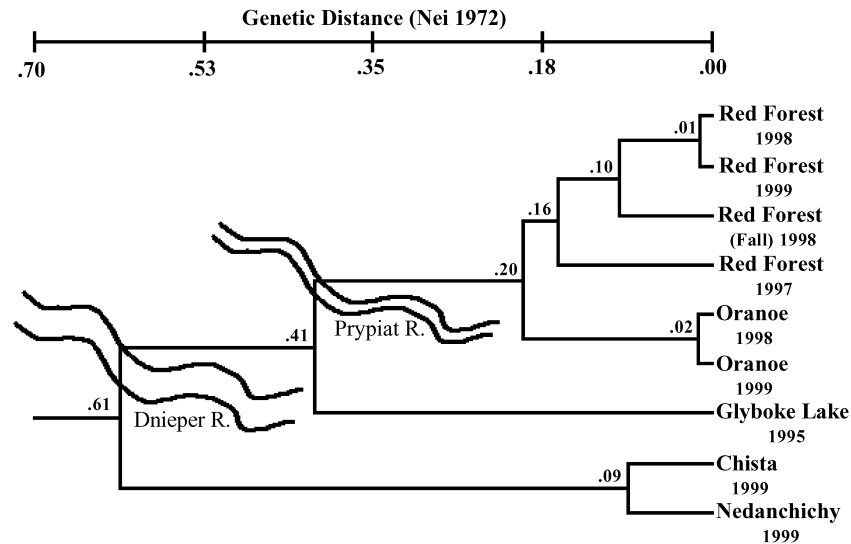


Figure 2. UPGMA phenogram of genetic distances (Nei, 1972) between temporal and/or spatial samples of bank voles from northern Ukraine. Genetic distances are based on mtDNA control region haplotype frequencies (TGPGA Version 1.3). Units at each node represent genetic distances. Glyboke Lake and Red Forest locales are experimental sites; Chista, Nedanchichy, and Oranoe locales are reference sites. All samples were collected in the summer (June, July, or August) of their respective year unless otherwise indicated. Nei unbiased (1978) did not differ from Nei (1972). A distance value of 1.00 represents fixation for different haplotypes.

- (7) Samples (e.g., tissues, DNA, voucher specimens) should be archived for future studies as the number and sensitivity of biomarkers increases and the ability to differentiate contaminant-related effects increases.

These criteria are not meant to be exhaustive, rather they provide a framework for addressing the effects of environmental pollution on populations of organisms. We emphasize that population genetics is an effective tool and is well suited for understanding these effects. Unfortunately, without accounting for the array of biotic and abiotic factors (i.e., non-contaminant) influencing gene dynamics, conclusions regarding these effects can be compromised and misguided. We feel the only way that ecotoxicologists can properly address contaminant issues at the population level is through multiyear, multidisciplinary, robust experimental design.

Acknowledgements

We thank V. Kholosha for granting permission to conduct research within the Chernobyl exclusion zone. We acknowledge C.J. Phillips, I. Shohalevitch, and I. Zhichevsky for their help in collecting and preparing specimens and for research support. We also thank

the Scientific and Technical Center at Chernobyl, the Chernobyl Center for Nuclear Safety, Radioactive Waste and Radioecology, the Administration of the Chernobyl Exclusion Zone, and the Ministry of Emergency Situations and Protection of the Population from the Consequences of the Chernobyl Nuclear Accident for their cooperation, use of their facilities, and access to the exclusion zone. We thank the International Radioecology Laboratory, Ukraine, for assistance and laboratory facilities. Funding for this study was provided in part by a contract (DE-FC09-96SR18546) between the United States Department of Energy and the University of Georgia. This research was supported in part by a Howard Hughes Medical Institute grant through the Undergraduate Biological Sciences Education Program to Texas Tech University.

References

- Baker, R.J., Hamilton, M.J., Van Den Bussche, R.A., Wiggins, L.E., Sugg, D.W., Smith, M.H., Lomakin, M.D., Gaschak, S.P., Bundova, E.G., Rudenskaya, G.A. and Chesser R.K. (1996). Small mammals from the most radioactive sites near the Chernobyl Nuclear Power Plant. *J. Mamm.* **77**, 155–70.
- Bickham, J.W. and Smolen, M.J. (1994). Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. *Environ. Health Perspect.* **102**, 25–8.

- Bickham, J.W., Patton, J.C. and Loughlin, T.R. (1996). High variability for control-region sequences in marine mammal: implications for conservation and biogeography of Steller sea lions (*Eumetopias jubatus*). *J. Mamm.* **77**, 95–108.
- Bickham, J.W., Loughlin, T.R., Calkins, D.G., Wickliffe, J.K. and Patton, J.C. (1998). Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. *J. Mamm.* **79**, 1390–5.
- Bickham, J.W., Sandhu, S., Hebert, P.D.N., Chikhi, L. and Athwal, R. (2000). Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutat. Res.* **463**, 33–51.
- Chesser, R.K. and Baker, R.J. (1996). Effective sizes and dynamics of uniparentally and diparentally inherited genes. *Genetics* **144**, 1225–36.
- Chesser, R.K. (1998). Heteroplasmy and organelle gene dynamics. *Genetics* **150**, 1309–28.
- Chesser, R.K., Sugg, D.W., Lomakin, M.D., Van Den Bussche, R.A., DeWoody, J.A., Jagoe, C.H., Dallas, C.E., Whicker, F.W., Smith, M.H., Gaschak, S.P., Chizhevsky, I.V., Lyabik, V.V., Buntova, E.G., Holloman, K. and Baker, R.J. (2000). Concentrations and dose rate estimates of ¹³⁴Cesium and ⁹⁰Strontium in small mammals at Chernobyl, Ukraine. *Environ. Toxicol. Chem.* **19**, 305–12.
- Cockerham, C.C. (1973). Analysis of gene frequencies. *Genetics* **74**, 679–700.
- Matson, C.W., Rodgers, B.E., Chesser, R.K. and Baker, R.J. (2000). Genetic diversity of *Clethrionomys glareolus* populations from highly contaminated sites in the Chernobyl region, Ukraine. *Environ. Toxicol. Chem.* **19**, 2130–5.
- Miller, M.P. (1997). Tools for population genetic analyses (TFGPA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by the author.
- Nei, M. (1972). Genetic distance between populations. *Am. Nat.* **106**, 283–92.
- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–90.
- Nei, M. (1987). *Molecular evolutionary genetics*, 512p. New York: Columbia University Press.
- Schneider, S., Roessli, D. and Excoffier, L. (2000). Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Shugart, L.R. and Theodorakis, C. (1996). Genetic ecotoxicology: the genotypic diversity approach. *Compar. Pharmacol.* **113**, 273–6.
- Theodorakis, C.W., Elbl, T. and Shugart, L.R. (1999). Genetic ecotoxicology IV: survival and DNA strand breakage is dependent on genotype in radionuclide-exposed mosquitofish. *Aquat. Toxicol.* **45**, 279–92.