Utility of Chromosomal Position of Heterochromatin as a Biomarker of Radiation-Induced Genetic Damage: A Study of Chornobyl Voles (*Microtus* sp.)

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**Abstract.** Biomarkers that effectively document effects of chronic multi-generational exposure to contaminated environments on chromosomes would be valuable in risk assessment, remediation, and environmental decisions. Native, free-ranging populations of voles inhabiting the highly radioactive regions surrounding Reactor 4 of the Chornobyl Nuclear Power Station provide a model system to evaluate biological and chromosomal effects of chronic multi-generational exposure to radioactivity and other reactor meltdown-related pollutants. Here, we explore the utility of heterochromatic elements as potentially informative biomarkers for genetic damage in voles from the radioactive environments surrounding Chornobyl. We analyzed chromosomal positions of heterochromatin from *Microtus arvalis* and *M. rossiaemeridionalis* using fluorescent *in situ* hybridization. Although intrapopulational variation existed in chromosomal position and abundance of heterochromatin, none of that variation could be assigned to environmental exposure.

**Keywords:** biomarkers; chernobyl; chornobyl; heterochromatin; *Microtus*

Numerous studies have documented chromosomal rearrangements and abnormalities induced by acute radiation dosage in laboratory settings, especially the **in vitro** response of cultured cells irradiated with acute doses of X-rays or γ-rays (Olivieri et al., 1984; Edwards et al., 1996; Natarajan et al., 1996; Tease and Fisher, 1996). Other studies have used an **in vivo** approach with laboratory mice receiving acute whole body radiation doses from a single external source (usually X- or γ-rays) providing a constant dose rate (primarily from 1 to 2 Gray [Gy]/min) with total doses of 0.5–4 Gy (Cooper and Hsu, 1971; Tateno et al., 1996). However, chromosomal effects of multi-generational chronic exposure to environmental radiation have proven difficult to quantitatively document (Baker et al., 1996; Rodgers and Baker, 2000; Rodgers et al., 2001). Sensitive biomarkers that could help assess the biological consequences of living in...
polluted environments are critical to the field of evolutionary toxicology and applicable to risk management (Shugart et al., 1992; Bickham and Smolen, 1994; Shugart and Theodorakis, 1998).

Evidence from several sources suggests that heterochromatic regions are more variable and more rapidly evolving than euchromatic regions. Heterochromatin has been shown to be a dynamic genomic element that can undergo significant changes between closely related taxa and among individuals in a single population (Wichman et al., 1991). Hamilton et al. (1990) demonstrated that within the subgenus Reithrodontomys (harvest mice) there was significant variation in amount and chromosomal placement of heterochromatin. Additionally, the repeat unit in heterochromatic sequences was defined by different restriction enzymes among these species of harvest mice. The Hamilton et al. (1990) study demonstrated considerable intragenomic movement and sequence evolution among closely related species of harvest mice. Variation at the intragenomic and inter-populational levels in amount and position of heterochromatic segments has been demonstrated in numerous taxonomic groups including rodents, insects and plants (Gamperl et al., 1982; Jamilena et al., 1990; Marchi and Mezzanotte, 1990; Bella et al., 1993; de Frietas, 1994; Basso et al., 1995).

Heterochromatic regions are also among the most common sites for chromosomal abnormalities to occur. Exposure to nickel has been shown to preferentially induce changes and chromosomal abnormalities in heterochromatic regions (Costa et al., 1992). Chromosomal breakage in cultured plant cells has been linked to heterochromatic blocks (Fluminhan et al., 1996). In humans, alterations of heterochromatic segments have been associated with various types of cancer and genetic instability syndromes (Kovaleva et al., 1993; Tsezou et al., 1993).

Cooper and Hsu (1971) documented that heterochromatin is highly fragmented by acute doses of radiation. Their study focused on the primarily heterochromatic sex chromosomes of the vole Microtus agrestis and found that frequencies of deletions and translocations were significantly elevated following acute exposure to X-ray dosages of 350 rad. However, aberrations involving heterochromatic regions did not seem to be detrimental to the cells. This observation implies that changes occurring in heterochromatic regions should be easier to maintain in populations of cells and therefore easier to detect. Because of the dynamic nature of heterochromatic regions and the proclivity for these regions to show chromosomal damage, we explored the possibility that heterochromatin might be an effective marker of possible chromosomal effects due to environmental contaminants, specifically the radioactive environment at Chernobyl.

The 26 April 1986 meltdown of Reactor 4 of the Chernobyl Nuclear Power Station, Ukraine, is the world’s worst nuclear power plant accident to date. An estimated 50–250 M Ci were released into the environment as a result of the accident (Mourad and Snell, 1987; Powers et al., 1987) and some areas adjacent to the remains of Reactor 4 continue to be among the most radioactively polluted sites in the world. Two exclusion zones with radii of 10 and 30 km have been established around the reactor. The most radioactive regions are within the 10 km zone. Although areas within the exclusion zones are highly contaminated with radioisotopes and other contaminants, the abundance and diversity of small mammals within the exclusion zones is equal to or greater than that in similar areas designated as control localities which are beyond the boundary of the 30 km zone (Baker et al., 1996). This abundance of rodents in the 10 km zone is not the result of radiation per se having a positive effect on the ecosystem. Rather the benefit is derived from the abundance of suitable habitat resulting from the elimination of human activities such as overgrazing and farming (Baker et al., 1996; Baker and Chesser, 2000).

The free-ranging populations of native rodents living in highly contaminated areas surrounding Reactor 4 provide an opportunity to assess the biological effects of the Chernobyl meltdown. Understanding the effects of the Chernobyl disaster will provide insight into the potential human health risks associated with exposure to highly contaminated environments. Generating knowledge about the biological effects of the Chernobyl meltdown may be valuable in shaping policies and strategies regarding nuclear power, waste management, and remediation of contaminated environments.

We examined two species of voles, Microtus arvalis and M. rossiaemeridionalis, to further examine the chromosomal effects of chronic exposure to Chernobyl pollution. These two species are appropriate model systems because they are abundant in both the experimental and control regions and because their mean dose rates are near the median for the
eight species for which data have been collected (Chesser et al., 2000). Combined internal and external dose estimates suggest that Microtus living in the more radioactive area within the 30 km zone would receive daily doses ranging from 19.4 to 53.3 milli Gray per day (mGy d⁻¹) (Chesser et al., 2000). Therefore, the radiation doses expected over six months for these mice are 3.5–9.6 Gy (Chesser et al., 2000).

The chronic radiation doses (lifetime total six months or greater of internal and external) received by voles at Chornobyl are comparable in magnitude to the acute radiation doses that have been shown to increase chromosomal breaks and rearrangements in Microtus (Cooper and Hsu, 1971) and approach the LD50/30 for voles (10 Gy) (Chesser et al., 2000). However, no significant difference in the frequency of chromosomal breaks between experimental and control populations was observed in any of the three species of Microtus examined from the Chornobyl region (Baker et al., 1996). A reciprocal translocation in a single individual of Microtus oeconomus collected from the Chornobyl area was reported (Nadzhafova et al., 1994), but as only single individuals from both the contaminated and control regions were examined, the evidence to attribute the presence of the rearrangement to the radioactive environment at Chornobyl is anecdotal. Nadzhafova et al. (1994) utilized differential staining of chromosomes to reveal a small structural change in a single individual, whereas Baker et al. (1996) focused on gross karyotypic changes in multiple individuals.

The apparent discrepancy between predictions based on laboratory studies of radiation exposure and the preliminary results of gross karyotypic characteristics of Microtus from the Chornobyl region may be due to differences in the effects of acute versus chronic exposure (Sorensen et al., 2000). Because of the potential for heterochromatic elements to be informative biomarkers of chronic multi-generational radiation-induced damage, we utilized fluorescent in situ hybridization (FISH) with heterochromatic probes isolated from M. arvalis and M. rossiaemeridionalis. Examination of heterochromatin by FISH provides two advantages. First, FISH enhances detection of potential differences because of the clear presence/absence signal generated by this technique. Second, it allows direct examination of heterochromatic regions to provide greater resolution in determining the chromosomal effects of chronic exposure to the Chornobyl environment.

Materials and methods

Sample collection

Collecting localities for both the experimental and control populations are described in Baker et al. (1996). Experimental populations were collected from the following locations with the following coordinates: Gluboyke Lake 51°26'49.1"N, 30°04'04.2"E; Chistogolovka 51°22'48.4"N, 30°01'29.3"E; Red Forest 51°22'23.6"N, 30°05'49.0"E; Orchard 51°22'29.3"N, 30°08'25.4"E; Rechitsa 51°18'34.1"N, 29°55'44.6"E. Control populations came from the following localities with the following coordinates: Control Site 51°04'29.4"N, 30°20'19.6"E; Burning Field 51°04'05.7"N, 30°20'19.6"E; The Shop 51°05'20.0"N, 30°06'27.6"E; White Tree 51°50'55.6"N, 30°20'21.6"E; Cow Pasture 51°50'29.2"N, 30°21'06.9"E. The control sites are separated from the experimental sites by an average of 32 km. Specimens were trapped from natural populations using Sherman live traps (Baker et al., 1996). Karyotypes were prepared from bone marrow and tissues were obtained as described in Baker et al. (1996). Internal doses of radioactivity for control animals were beyond the resolution of the Geiger counter. The internal dose of individuals from the experimental regions ranged from 150 to 22,000 disintegrations per minute. Individuals from the experimental site would also be experiencing a dose from the environment (Chesser et al., 2000). The amount of environmental radiation present at Chornobyl between 1986 and 1995 had decreased through radioactive decay by over 95%. The significance of this observation is that earlier generations of voles living in this environment would have encountered much higher doses of radiation. Our field data suggest that 2–3 generations occur in natural populations during an annual breeding season. Extending these data the number of generations between the nuclear meltdown in 1986 and the collection of these samples in 1994–1995 would range from 16 to 27 vole generations.

Library construction

In order to isolate evolutionarily active DNA fragments, plasmid libraries were constructed from genomic DNA isolated from a male specimen of each species collected from native vole populations in the most radioactive regions within the 10 km
All three patterns were present in both control and experimental populations and the frequency of each type did not differ significantly between control and experimental populations ($\chi^2 = 0.018$).

We have documented intra-populational variation in the chromosomal position of sex-chromosome-specific heterochromatin in *M. rossiemeridionalis* (Fig. 3). Because the distribution of the three patterns of heterochromatin on the Y chromosome does not differ significantly between the experimental and control populations, this variation cannot be attributed to the contaminated environment at Chornobyl and is a naturally occurring polymorphism. The observation that chromosomal polymorphisms in heterochromatin revealed by FISH are maintained in natural populations of *M. rossiemeridionalis* without obvious detrimental effects suggests that if there were numerous chromosomal rearrangements being produced as a result of exposure to radiation, some of these could be expected to survive and be maintained with chronic multi-generational exposure. Therefore, because of the absence of an elevated frequency of heterochromatic rearrangements in populations living and reproducing in the experimental site, we cannot reject the null hypothesis that chronic exposure to the Chornobyl environment has had no detectable effect on karyotypic position of heterochromatin in voles.

These data failed to reveal chromosomal breaks and rearrangements as had been reported previously in *Microtus* exposed to acute radiation doses (Cooper and Hsu, 1971) and to the Chornobyl environment (Nadzhafova et al., 1994). We consider three possible explanations for these results: chronic versus acute exposure, radioresistance, and inadequacy of the methods. The chronic radiation doses (lifetime total of internal and external) received by voles at Chornobyl are comparable in magnitude to the acute radiation doses that have been shown to increase chromosomal breaks and rearrangements in *Microtus* (Cooper and Hsu, 1971). If the linear non-threshold model is accurate (OCED, 1998), then we would anticipate seeing a number of rearrangements in older individuals comparable to that observed by Cooper and Hsu (1971). However, although the doses are of comparable magnitude, the method and time of exposure are significantly different in the laboratory studies as compared to the natural populations of Chornobyl voles. The voles living in the contaminated environment are chronically exposed to both internal and external sources of radiation throughout their ontogenies, whereas individuals in laboratory studies typically receive a single, acute, external dose. Additionally, Chornobyl voles receive both $\beta$ and $\gamma$ radiation (Chesser et al., 2000) whereas the study of Cooper and Hsu (1971) utilized only $\gamma$ radiation. These differences in acquisition of dose, nature of dose ($\beta$ and $\gamma$ radiation) and method of exposure may be important in explaining the lack of chromosomal aberrations and changes in the Chornobyl vole populations. Recently, Kovalchuk et al. (2000) have demonstrated an increased frequency of homologous recombination in plants exposed to chronic ionizing radiation as compared to those with acute exposure. This observation indicates that both chronic and acute exposure to ionizing radiation may have significant and unique genetic effects.

Another potential explanation for the apparent lack of gross chromosomal abnormalities and absence of differences in arrangement of heterochromatic segments is that the voles currently living near the Chornobyl reactor have some resistance to radioactive exposure. Radioresistance has been hypothesized for a number of rodent species (Il’enko and Krapivko, 1994; Krapivko and Il’enko, 1988). If the vole species used in this study have radioresistance, they may have an inherent spontaneous resistance to the effects of exposure to radiation. Alternatively, these vole populations living near the reactor at Chornobyl may have undergone selection for more efficient repair mechanisms than would be present in naive mice with no exposure to a contaminated environment. However, Rodgers et al. (2001) failed to document radioresistance in a population of *Clethrionomys glareolus* at Chornobyl.

Another possibility is that differences in frequencies of chromosomal rearrangements exist in the Chornobyl vole populations, but they have not been revealed by the heterochromatic sequences used in this analysis. The three heterochromatic probes used in this study cannot detect some types of chromosomal rearrangements. For example, translocations between chromosomal regions devoid of heterochromatic sequences would not be detectable with this technique. However, rearrangements would have to be small in size and selectively located in areas not closely associated with heterochromatic sequences in order not to be revealed by our methods.

The lack of variation in heterochromatic regions along with the absence of gross karyotypic changes in
native rodent populations (Baker et al., 1996) suggest that the long-term chromosomal effects of the Chernobyl disaster are not as great as has generally been predicted from previous laboratory studies. This data set does not resolve the question of whether exposure to the Chernobyl environment is having a significant effect on the chromosomes of native rodents; however, it does further document that the magnitude of chromosomal rearrangements in voles exposed to the Chernobyl environment is less than expected based on traditional paradigms. If long-term chromosomal effects from radiation are present in these rodent populations, more sensitive methods will be required to reveal them. Furthermore, these data suggest that studies of acute exposure to radiation in laboratory settings are not adequate predictors of the response of free-ranging populations of native rodents to chronic, multi-generational exposure.

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References


