



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

LARA E. WIGGINS,^{1,†} RONALD A. VAN DEN BUSSCHE,² MEREDITH J. HAMILTON,²
RONALD K. CHESSER^{3,‡} AND ROBERT J. BAKER^{1*}

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

²*Department of Zoology, 430 Life Sciences West, Oklahoma State University, Stillwater, OK 74078, USA*

³*Savannah River Ecology Laboratory, Aiken SC 29802, USA*

Accepted 31 August 2001

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 Chernobyl 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 Chernobyl. We analyzed chromosomal positions of heterochromatin from *Microtus arvalis* and *M. rossiaemeridionalis* using fluorescent *in situ* hybridization. Although intrapopulation 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

*To whom correspondence should be addressed:

†Present address: Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

‡Present address: Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, USA.

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 intra- and inter-population 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 MCi 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 Chernobyl 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 Chernobyl region (Baker et al., 1996). A reciprocal translocation in a single individual of *Microtus oeconomus* collected from the Chernobyl 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 Chernobyl 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 Chernobyl 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 Chernobyl 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; Chistogalovka 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 Chernobyl 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-population variation in the chromosomal position of sex-chromosome-specific heterochromatin in *M. rossiaemeridionalis* (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 Chernobyl and is a naturally occurring polymorphism. The observation that chromosomal polymorphisms in heterochromatin revealed by FISH are maintained in natural populations of *M. rossiaemeridionalis* 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 Chernobyl 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 Chernobyl 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 Chernobyl 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 Chernobyl 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, Chernobyl voles receive both β and γ radiation (Chesser et al., 2000) whereas the study of Cooper and Hsu (1971) utilized only γ radiation. These differences in acquisition of dose, nature of dose (β and γ radiation) and method of exposure may be important in explaining the lack of chromosomal aberrations and changes in the Chernobyl 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 Chernobyl 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 Chernobyl 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 Chernobyl.

Another possibility is that differences in frequencies of chromosomal rearrangements exist in the Chernobyl 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.

Acknowledgements

Elena Bundova, Andrew DeWoody, Sergei Gaschak, Galena Rudenskaya, and Derrick Sugg provided assistance in the collection and preparation of the specimens utilized in this study. Thanks to Brenda Rodgers and Deidre Parish for their assistance with figure preparation and for critically reviewing this manuscript. Support for this research was provided (in part) by a grant from the Howard Hughes Medical Institute through the Biological Sciences Undergraduate Education Program at Texas Tech University, the Clark Scholars Program, and by contract DE-FC09-96SR18546 between the United States Department of Energy and the University of Georgia Savannah River Ecology Laboratory.

References

- Baker, R.J. and Chesser, R.K. (2000). The Chernobyl nuclear disaster and subsequent creation of a wildlife preserve. *Environ. Toxicol. Chem.* **19**(5), 1231–2.
- Baker, R.J. and Wichman, H.A. (1990). Retrotransposon *mys* is concentrated on the sex chromosomes: implications for copy number containment. *Evolution* **44**(8), 2083–8.
- 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. Mammal.* **77**, 155–70.
- Basso, A., Lifschitz, E. and Manso, F. (1995). Determination of intraspecific variation in sex heterochromatin of *Ceratitis capitata* (Wied.) by C-banding. *Cytobios* **83**, 237–44.
- Bella, J.L., Serrano, L., Hewitt, G.M. and Gosalvez, J. (1993). Heterochromatin heterogeneity and rapid divergence of the sex chromosomes in *Chorthippus parallelus parallelus* and *C. p. erythropus* (Orthoptera). *Genome* **36**, 542–7.
- 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**(Suppl. 12), 25–8.
- 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 134,137 Cesium and 90 Strontium in small mammals at Chernobyl, Ukraine. *Environ. Toxicol. Chem.* **19**(2), 305–12.
- Cooper, J.E.K. and Hsu, T.C. (1971). Radiation-induced deletions and translocations of *Microtus agrestis* sex chromosomes *in vivo*. *Exp. Cell Res.* **67**, 343–51.
- Costa, M., Conway, K., Imbra, R. and Wei Wang, X. (1992). Involvement of heterochromatin damage in nickel-induced transformation and resistance. In E. Nieboer and J. Nriagu (eds). *Nickel and Human Health: Current Perspectives*, pp. 295–303. New York: Wiley.
- de Frietas, T.R.O. (1994). Geographical variation of heterochromatin in *Ctenomys flamarioni* (Rodentia-Octodontidae) and its cytogenetic relationships with other species of the genus. *Cytogenet. Cell Genet.* **67**, 193–8.
- Edwards, A.A., Virsik-Peuckert, P. and Bryant, P. (1996). Mechanisms of radiation-induced chromosome aberrations. *Mutat. Res.* **366**, 117–28.
- Fluminhan, A., de Aguilar-Perecin, M.L.R. and dos Santos, J.A. (1996). Evidence for heterochromatin involvement in chromosome breakage in maize callus culture. *Ann. Bot.* **78**, 73–81.
- Gamperl, R., Ehmann, C. and Bachmann, K. (1982). Genome size and heterochromatin in rodents. *Genetica* **58**, 199–212.
- Hamilton, M.J., Honeycutt, R.L. and Baker, R.J. (1990). Intragenomic movement, sequence amplification, and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys*: evidence from *in situ* hybridization. *Chromosoma* **99**, 321–9.
- Il'enko, A.I. and Krapivko, T.P. (1994). Radioresistance of populations of bank voles *Clethrionomys glareolus* in radionuclide-contaminated areas. *Doklady. Biol. Sci.* **336**, 262–6.
- Jamilena, M., Ruiz Rejón, C. and Ruiz Rejón, M. (1990). Variation in heterochromatin and nucleolar organizing regions of *Allium subvillosum* L. (Liliaceae). *Genome* **33**, 779–84.
- Kovaleva, N.V., Butomo, I.V., Pavlova, M.N. and Khitrikova, L.E. (1993). Centromeric heterochromatin polymorphism in the etiology of human aneuploidy. *Genetika* **29**(9), 1536–43.
- Kovalchuk, O., Arkhipov, A., Barylyak, I., Karachov, I., Titov, V., Hohn, B. and Kovalchuk, I. (2000). Plants experiencing chronic internal exposure to ionizing radiation exhibit higher frequency of recombination than acutely irradiated plants. *Mutat. Res.* **449**, 47–56.

- Krapivko, T.P. and Il'enko, A.I. (1988). First features of radioadaptation in a population of red-backed voles (*Clethrionomys glareolus*) in a radiation biogeocenosis. *Doklady Akademii Nauk SSSR* **302**(5), 1272–4.
- Longmire, J.L., Maltbie, M. and Baker, R.J. (1997). Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers, The Museum of Texas Tech Univ.* **163**, 1–3.
- Marchi, A. and Mezzanotte, R. (1990). Inter- and intraspecific heterochromatin variation detected by restriction endonuclease digestion in two sibling species of the *Anopheles Maculipennis* complex. *Heredity* **65**, 135–42.
- Mourad, R. and Snell, V. (1987). Source term and radiological consequences of the Chernobyl accident. *Trans. Amer. Nucl. Soc.* **54**, 226–8.
- Nadzhafova, R.S., Bulatove, N.Sh., Kozlovskii, A.I. and Ryahov, I.N. (1994). Identification of a structural chromosomal rearrangements in the karyotype of a root vole from Chernobyl. *Russian J. Genet.* **30**(3), 318–22.
- Natarajan, A.T., Balajee, A.S., Boei, J.J.W.A., Darroudi, F., Dominguez, I., Hande, M.P., Meijers, M., Slijepcevic, P., Vermeulen, S. and Xiao, Y. (1996). Mechanisms of induction of chromosomal aberrations and their detection by fluorescent *in situ* hybridization. *Mutat. Res.* **372**, 247–58.
- OCED (1998). Report on developments in radiation health science and technology and their impact on radiation protection Nuclear Energy Agency Committee on Radiation Protection and Public Health. OCED, Paris.
- Olivieri, G., Bodycote, J. and Wolff, S. (1984). Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* **223**, 594–7.
- Powers, D.A., Kress, T.S. and Jankowski, M.W. (1987). The Chernobyl source term. *Nuclear Safety* **28**, 10.
- Rodgers, B.E. and Baker, R.J. (2000). Frequencies of micronuclei in bank voles from zones of high radiation at Chernobyl, Ukraine. *Environ. Toxicol. Chem.* **19**(6), 1644–8.
- Rodgers, B.E., Wickliffe, J.K., Phillips, C.J., Chesser, R.K. and Baker, R.J. (2001). Experimental exposure of naïve bank voles, (*Clethrionomys glareolus*) to the Chernobyl environment: a test of radioresistance. *Environ. Toxicol. Chem.* **20**(9), 1936–41.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Plainview NY: Cold Spring Harbor Laboratory Press.
- Shugart, L.R., McCarthy, J.F. and Halbrook, R.S. (1992). Biological markers of environmental and ecological contamination: an overview. *Risk Anal.* **12**(3), 353–60.
- Shugart, L.R. and Theodorakis, C.W. (1998). New trends in biological monitoring: application of biomarkers to genetic ecotoxicology. *Biotherapy* **11**(2–3), 119–27.
- Sorensen, K.J., Zetterberg, L.A., Nelson, D.O., Grawe, J. and Tucker, J.D. (2000). The *in vivo* dose rate effect of chronic gamma radiation in mice: translocation and micronucleus analyses. *Mutat. Res.* **457**, 125–36.
- Tateno, H., Kamiguchi, Y., Shimada, M. and Mikamo, K. (1996). Difference in types of radiation-induced structural chromosome aberrations and their incidences between Chinese and Syrian hamster spermatazoa. *Mutat. Res.* **350**, 339–48.
- Tease, C. and Fisher, G. (1996). Cytogenetic and genetic studies of radiation-induced chromosome damage in mouse oocytes. Numerical and structural chromosome anomalies in metaphase II oocytes, pre- and post-implantation embryos. *Mutat. Res.* **349**, 145–53.
- Tsezou, A., Kitsiou-Tzeli, S., Kosmidis, K., Paidousi, K., Katsouyanni, K. and Sinaniotis, C. (1993). Constitutive heterochromatin polymorphisms in children with acute lymphoblastoid leukemia. *Pediat. Hematol. Oncol.* **10**, 7–11.
- Van Den Bussche, R.A., Longmire, J.L. and Baker, R.J. (1995). How bats achieve a small C-value: frequency of repetitive DNA in *Macrotus*. *Mamm. Genome.* **6**, 521–5.
- Wichman, H.A., Payne, C.T., Ryder, O.A., Hamilton, M.J., Maltbie, M. and Baker, R.J. (1991). Genomic distribution of heterochromatic sequences in equids: implications to rapid chromosomal evolution. *J. Hered.* **82**, 369–77.