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ORIGINAL PAPER

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Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout

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Abstract The purpose of this investigation has been the analysis of the long-term development of biological damage in natural populations of a model mammalian species, the bank vole (*Clethrionomys glareolus, Schreber*), which were chronically exposed to low doses of ionizing radiation over 22 animal generations within 10 years following the Chernobyl accident. The time course of the biological end-points (chromosome aberrations in bone marrow cells and embryonic lethality) was compared with the time course of the whole-body absorbed dose rate from external and internal exposure in the studied populations inhabiting monitoring sites in Belarus with different ground deposition of radionuclides. The yield of chromosome aberrations and, in lesser degree, embryonic lethality was associated with the radionuclide contamination of the monitoring areas in a dose-dependent manner. As a main feature of the long-term development of biological damage under low dose rate irradiation, permanently elevated levels of chromosome aberrations and an increasing frequency of embryonic lethality have developed over 22 animal generations. This contrasts with the assumption that the biological damage would gradually disappear since in the same period of time the whole-body absorbed dose rate decreased exponentially with a half-value time of about 2.5–3 years. Furthermore, gravid females were captured, and their offspring, born and grown up under contamination-free laboratory conditions, showed the same enhanced level of chromosome aberrations. Therefore the authors suggest that, along with the biological damage attributable to the individual exposure of each animal, the observed cellular and systemic effects reflect the transgenerational transmission and accumulation, via genetic and/or epigenetic pathways, of damage attributable to the chronic low-dose rate exposure of the preceding generations of animals. They also suggest that the level of the accumulated transmissible damage in the investigated populations will decrease in future due to the further recession of the chronic exposure and as a consequence of selection processes.

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Introduction

The Chernobyl accident in April 1986 caused the deposition of radionuclides across Europe, followed by a long-term artificial increase of the radiation background [1]. In addition to the classical subject of mutagenesis after acute radiation exposure [2], the study of the time course of biological damage associated with chronic low-dose radiation exposure of mammals and the endeavors to predict biological damage in consecutive generations have become a relevant issue. Since there is little information on this topic [3, 4], the present work addresses these important questions.

Starting with 1986, we were engaged in studying biological effects of chronic low dose radiation in natural populations of bank vole (*Clethrionomys glareolus, Schreber*) in a series of many animal generations. The bank vole is a widespread rodent species that is used as indicator of environmental quality. It is a convenient object for many genetic tests, which originally have been devised for the laboratory mouse [5]. Comparison of own and literature data on doubling doses of acute irradiation for chromosome injuries had shown that the sensitivity of somatic cells of the bank vole to ionizing radiation is very similar to the sensitivity of human lymphocytes and germ cells of laboratory mice [6]. This finding confirms that the bank vole is a suitable model species for assessment of genetic radiation risks in mammals.

According to the Atlas of Caesium Deposition in Europe [1], practically the whole Belarusan territory was

contaminated by ^{137}Cs above the level of global fallout. A ground deposition of ^{137}Cs equal to 37 kBq/m^2 (1 Ci/km^2) was chosen to discriminate between so-called "clean" regions and contaminated regions. We selected monitoring sites representing large areas of Belarus with a strong gradient of radionuclide ground deposition, from 8 to 8,500 kBq/m^2 of ^{137}Cs, i.e., from light contamination in "clean" regions to high contamination in the evacuated zone. In comparison with the pre-Chernobyl data, increased frequencies of chromosome aberrations and genomic mutations in somatic cells, abnormal sperm heads and embryonic losses were observed in bank vole populations at the monitoring sites [7, 8].

The aim of the present study was to analyze the long-term development of chromosome aberration frequency and embryonic lethality in bank vole populations over 22 animal generations living in 1986–1996 and to compare it with the time course of the whole-body absorbed dose rate.

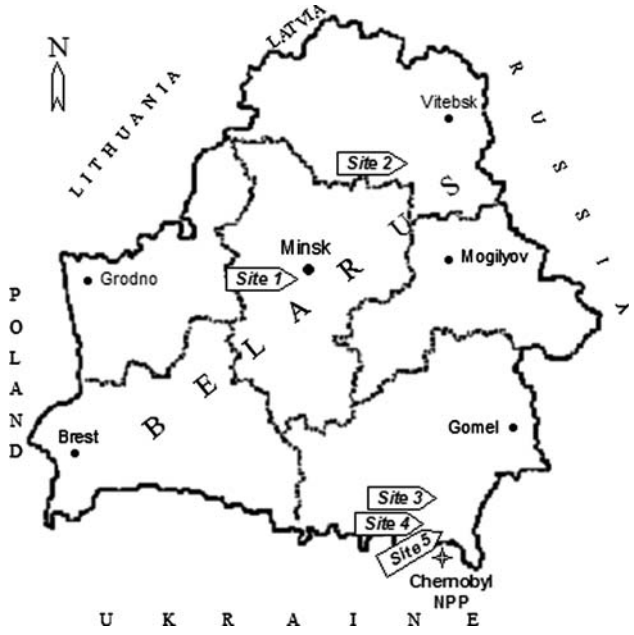

Fig. 1 Map of Belarus with localization of the monitoring sites. The position of the Chernobyl nuclear power plant (ChNPP) in the neighboring Ukraine is also indicated

Materials and methods

Monitoring sites

The animals were collected at five monitoring sites in large forestry areas with limited human activities, far from possible sources of industrial or domestic pollution. These sites are located at different distances and directions from the Chernobyl nuclear power plant (Fig. 1) and represent different levels of radionuclide contamination: site 1—the Priluksky Reserve, Minsk region, 330 km NW, site 2—the Berezinsky Biosphere Reserve, Vitebsk region, 400 km NNW, site 3—the vicinity of Majsk village, Bragin district, Gomel region, 60 km N, site 4—the vicinity of the evacuated Babchin village, Khoiniki district, Gomel region, 40 km NNW and site 5—the vicinity of the evacuated Radin village, Khoiniki district, Gomel region, 18 km N. The initial levels of radionuclide contamination of soil at these sites were determined earlier [9] and are shown in Table 1. The animals were captured using live traps with bait. The first animals were captured at sites 1, 3, and 4 in September 1986, i.e., about 5 months after the period of acute irradiation by short- and long-lived radionuclides. In the subsequent years, the animals were as well usually collected in the period of August–September in order to exclude the influence on the data of seasonal changes in the age structure of populations. Data obtained outside this collection period (e.g., the data from site 1 in 1991 and 1996 [7, 8]) were not included in this study. Investigations at sites 2 and 5 started in 1991 and 1996, respectively, i.e., 5 and 10 years after the accident.

Assessment of animal age and change of animal generations

For age determination, root, cusp, and height of the first mandibular molar (M_2) were measured according to Bashenina [10]. The specimens were divided into seven age groups, which corresponded to ages of approximately 2 weeks, 1, 2, 3, and 4 months and 1 and 1.5 years.

The bank vole, like other rodent species, is known to have a short generation time with a complete change of about 2–3 generations per year [10]. According to data of Rozhdestvenskaya [11] and our own estimates of animal participation in reproduction, this holds also for the

Table 1 Densities of radionuclide contamination of soil (kBq/m^2) at five monitoring sites (data of April–May 1986 for ^{137}Cs, ^{134}Cs, ^{106}Ru, ^{144}Ce and data of August 1996 for ^{90}Sr and transuranic radionuclides) according to data [9]

Site	April–May 1986				August 1996				
	^{137}Cs	^{134}Cs	^{106}Ru	^{144}Ce	^{90}Sr	^{238}Pu	$^{239,240}Pu$	^{241}Pu	^{241}Am
1	8	4	5	0	4	0.04	0.10	2.98	0.14
2	18	9	12	0	5	0.07	0.14	5.10	0.19
3	220	140	150	440	39	0.62	1.28	48.80	1.81
4	1,530	1,020	1,090	3,050	117	1.17	2.35	86.70	3.21
5	8,500	5,650	5,790	1,7200	1,200	4.90	11.00	420.00	15.00

populations studied in the present work. Due to seasonal reproduction and short life-span, in the trapping periods of late summer and early autumn only few animals born in the preceding year were observed at the monitoring sites, showing that the populations were almost completely renewed every subsequent year by the time of observation.

The populations living at the time of the accident, in early spring of 1986, consisted of adult animals born in the previous year. Due to the turnover of generations, our investigations, beginning in September 1986, started with the first and second post-accidental generations of bank vole. In the period from 1986 to 1996, at least 22 generations were studied.

Determination of radionuclide concentration and estimation of absorbed dose rate

Concentrations of radionuclides in the soil and the whole-body animal samples were assessed as described in [9]. Briefly, the γ-spectrometry of samples was performed in the Hydrometeorological Centre of Belarus in Minsk using the γ-spectrometer ADCAM-300 equipped with a high-purity germanium detector GEM-30185 (EG&G Ortec, USA). Specific activities of ^{90}Sr and transuranic elements were determined by the staff of the Institute of Radiobiology, National Academy of Sciences of Belarus, using radiochemical methods and the α-spectrometer Ortec 576A with the silicon surface barrier detector (EG&G Ortec, USA), the liquid scintillation counter Tricarb 2700TR (Packard Company) and a gas-flow counter (Tesla Automat, Slovakia).

Dose rates due to internal and external exposure were assessed in the studied specimens as described previously [9]. The whole-body absorbed dose rate from incorporated γ-emitting radionuclides was calculated according to the absorbed fraction model. For the dose rate attributable to incorporated α- and β-emitters, we used the local absorption model. The whole-body absorbed dose was calculated as the product of the individual whole-body absorbed dose rate and the age of the animal at the time of capture.

Metaphase analysis of chromosome aberrations

Cytogenetic effects in bank vole somatic cells were studied by metaphase analysis of chromosome aberrations in red bone marrow cells according to a standard protocol [12] as described [7, 8]. Briefly, visually healthy animals of different age, sex, and state of maturation were randomly chosen for this test from numerous groups of animals captured shortly after trapping. Colchicine at concentration of 0.1 mg per 10 g animal weight was injected intraperitoneally for 1.5 h to accumulate metaphases. Animals were sacrificed by ethyl ether and cervical dislocation, and the marrow was aspirated from the femurs using inactivated fetal bovine serum. The marrow suspension was incubated at 37°C for 20 min, treated with 0.56% potassium chloride for the next 20 min and fixed in methanol:acetic acid (3:1 v/v). Fixed cells were spread on clean slides, flame-dried and stained with Giemsa. Coded slides were screened for chromatid- and chromosome-type aberrations in approximately 100 well-spread metaphases per specimen using standard criteria [12, 13]. Achromatic lesions (gaps) were not included in the statistical analysis.

Selected age groups

The chromosome aberrations and embryonic losses were also recorded from selected cohorts of mature animals with age from 2 to 4 months in order to exclude any age-dependent bias of the biological effects.

Furthermore, two gravid females were taken to a laboratory in Minsk, where they gave litters shortly after capture. Mother animals and their offspring were fed with uncontaminated food and were studied for chromosome aberrations in red bone marrow cells when the age of the offspring reached 1.5 months.

Analysis of embryonic losses

Assessment of embryonic mortality was performed in all captured and visually healthy females at 7–22 days of pregnancy using the conventional approaches [14]. The content of the uteri was examined to determine the number of implants including live and dead embryos. The early (pre-implantation) losses were calculated as the ratio (number of corpora lutea minus number of implants)/(number of corpora lutea). The late (post-implantation) losses were calculated as the ratio of the number of dead embryos to the number of implants. The total losses were determined as the ratio (number of corpora lutea minus number of live embryos)/(number of corpora lutea). All ratios are expressed in percent.

Statistics

Chi-square and *U* tests, regression and correlation analysis were employed as a part of the Statistica software package (StatSoft Inc., USA). Figure 2 gives an example of the scatter of the individual aberration frequency and whole-body dose rate data at a given site and given season of the study (site 4, analyzed 5 years after the accident). In the following, these fluctuations are represented by the mean value and the standard deviation of the mean.

Results

Time course of the absorbed dose rate

The time course of the absorbed dose rates in populations of bank vole at the monitoring sites was determined previously [9] using numerous groups of captured animals. It was shown that external γ-irradiation and

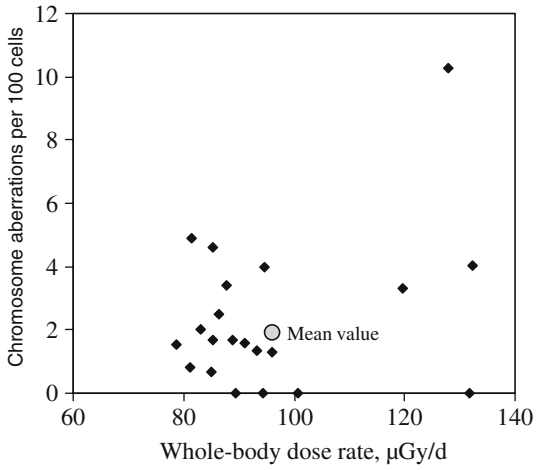

Fig. 2 Correlation of the chromosome aberration frequency in bone marrow cells of bank voles inhabiting site 4, with the individual whole-body absorbed dose rates 5 years after the Chernobyl accident. The dose rates are due to external γ- and internal γ + β-radiation of incorporated ^{137}Cs and ^{134}Cs

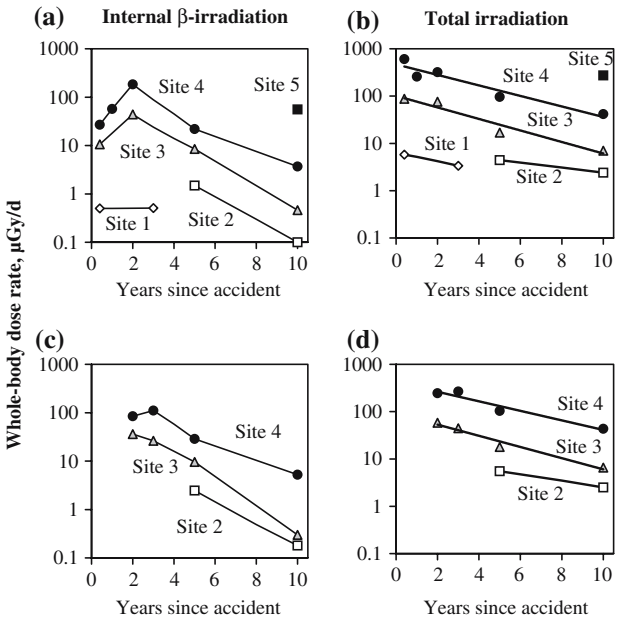

Fig. 3 Time course of the mean whole-body absorbed dose rates in bank vole specimens captured at sites 1–5 and studied for chromosome aberrations (**a**, **b**) and for embryonic mortality (**c**, **d**). The contributions by internal β-irradiation from incorporated ^{137}Cs and ^{134}Cs are presented in **a** and **c**, the total whole-body dose rates by external γ-irradiation and internal γ + β-irradiation from incorporated ^{137}Cs and ^{134}Cs are shown, together with exponential approximation curves, in **b** and **d**. Error bars are not shown for clarity

internal β-irradiation by incorporated ^{137}Cs and ^{134}Cs delivered the most prominent contributions to the whole-body dose rate over the monitoring period, which started 5 months after the accident with the first and second post-accident animal generations and ended 1996 after about 22 animal generations [9]. Here, we analyzed the time course of the mean whole-body absorbed dose rate due to internal β-irradiation and to total irradiation specifically for those animals which, out of the whole cohorts, were randomly chosen for assessment of chromosome aberrations (Table 2; Fig. 3a, b) and embryonic mortality (Table 3; Fig. 3c, d). The values of the whole-body dose rates and their temporal development in these sub-cohorts of animals were similar to those in the larger groups of bank vole used in the earlier dosimetrical investigations of monitored populations [9].

Table 2 Chromosome aberrations in bone marrow cells of bank vole at five monitoring sites

Site	Year	Animal generation since the accident	Whole-body absorbed dose rate (μGy/day)	Number of analyzed		Aberrations per 100 cells ± SD			Aberrant cells, %
				Animals	Cells	Chromatid type	Chromosome type	Total	
1	1986	1–2	6.44 ± 0.14	10	992	0.30 ± 0.14	0.10 ± 0.09	0.40 ± 0.22	0.40 ± 0.22
	1988	5–6	3.36 ± 0.02	3	310	0.64 ± 0.32	0	0.64 ± 0.32	0.64 ± 0.32
2	1981–1983[a]	Pre-accident	–	24	2,437	0.41 ± 0.12	0	0.41 ± 0.12	0.41 ± 0.12
	1991	11–12	4.43 ± 0.38	19	1,945	1.19 ± 0.37	0.03 ± 0.03	1.22 ± 0.37[b]	1.22 ± 0.37
	1992	13–14	–	17	1,962	0.90 ± 0.27	0.27 ± 0.17	1.17 ± 0.13	1.09 ± 0.24
	1996	21–22	2.41 ± 0.03	8	585	1.06 ± 0.54	0.11 ± 0.11	1.17 ± 0.53	1.17 ± 0.53
3	1986	1–2	87.52 ± 4.50	18	1,987	1.36 ± 0.45	0.76 ± 0.28	2.12 ± 0.59	1.99 ± 0.53
	1988	5–6	76.06 ± 8.90	16	1,630	1.22 ± 0.49	0.52 ± 0.18	1.74 ± 0.57	1.51 ± 0.03
	1991	11–12	16.83 ± 2.27	16	1,655	2.67 ± 0.81	0.20 ± 0.13	2.87 ± 0.83	2.49 ± 0.76
	1996	21–22	7.02 ± 0.27	11	1,121	1.86 ± 0.41	0.18 ± 0.10	2.04 ± 0.39	1.95 ± 0.38
4	1986	1–2	605.46 ± 7.75	16	1,739	1.45 ± 0.43	0.06 ± 0.06	1.51 ± 0.45	1.21 ± 0.10
	1987	3–4	258.57 ± 11.33	36	3,675	0.79 ± 0.18	0.44 ± 0.15	1.24 ± 0.24	1.10 ± 0.22
	1988	5–6	321.64 ± 33.12	21	1,942	1.75 ± 0.39	0.17 ± 0.09	1.92 ± 0.40	1.86 ± 0.04
	1991	11–12	95.93 ± 3.69	31	3,780	1.63 ± 0.34	0.31 ± 0.10	1.94 ± 0.40	1.84 ± 0.36
	1996	21–22	41.80 ± 0.96	14	1,821	1.90 ± 0.49	0.24 ± 0.15	2.14 ± 0.42	2.04 ± 0.38
5	1996	21–22	274.52 ± 7.27	11	492	4.74 ± 0.74	1.47 ± 0.61	6.21 ± 0.85	5.10 ± 0.78

[a] Pre-accident data according to [15]
[b] All data on total frequencies of aberrations and aberrant cells in animals at sites 2–5 in 1986–1996 were significantly higher in comparison with pre-accident data by Chi-square test

Table 3 Embryonic lethality in bank vole populations at three monitoring sites

Site	Year	Animal generation since the accident	Whole-body absorbed dose rate (μGy/day)	Number of			Mean embryonic lethality, % (95% binomial confidence limits)		
				Animals analyzed	Yellow bodies	Embryos analyzed/ dead embryos	Before implantation	After implantation	Total
2	1981–1983[a]	Pre-accident	–	45	249	235/4	5.62 (3.11–9.25)	1.70 (0.47–4.30)	7.23 (4.34–11.18)
	1991	11–12	5.5 ± 0	12	56	56/3	0 (0.00–5.21)	5.36 (1.12–14.87)	5.36 (1.10–14.87)
	1992m	13–14	–	19	92	90/2	2.17 (0.26–7.68)	2.22 (0.27–7.80)	4.35 (1.20–10.76)
	1996	21–22	2.51 ± 0.13	8	44	39/0	11.36 (3.79–24.56)	0 (0.00–9.03)	11.36 (3.79–24.56)
3	1988	5–6	58.01 ± 5.40	7	36	36/0	0 (0.00–7.98)	0 (0.00–7.98)	0 (0.00–7.98)
	1989	7–8	44.38 ± 3.71	30	138	130/1	5.80 (2.50–11.10)	0.77 (0.02–4.21)	6.52 (3.03–12.08)
	1991	11–12	17.95 ± 2.53	14	71	65/1	8.45 (3.16–17.49)	1.54 (0.04–8.28)	9.86 (4.06–19.26)
	1996	21–22	6.58 ± 0.07	3	15	12/1	20.00* (4.33–48.09)	8.33 (0.21–38.48)	26.67* (7.74–55.10)
4	1988	5–6	245.6 ± 20.21	14	63	61/1	3.17 (0.39–11.00)	1.64 (0.04–8.80)	4.76 (0.99–13.29)
	1989	7–8	265.63 ± 34.53	40	201	192/3	4.48 (2.07–8.33)	1.56 (0.32–4.50)	5.97 (3.12–10.20)
	1991	11–12	103.78 ± 5.96	21	103	91/4	11.65* (6.27–19.76)	4.40 (1.21–10.87)	15.53* (9.15–24.00)
	1996	21–22	43.47 ± 0.87	11	51	44/4	13.73* (5.70–26.26)	9.09* (2.53–21.67)	21.57** (11.29–35.32)

[a] Pre-accident level according to [16]
* $P < 0.05$ and ** $P < 0.01$ in comparison with pre-accident data (Chi-square test)

The maxima of internal β-irradiation due to incorporated ^{137}Cs and ^{134}Cs were observed in the second year after the accident (Fig. 3a, c) due to the increasing biological availability of cesium isotopes from the biota [7-9], followed by a decrease by one order of magnitude in the subsequent period. In [9] it was shown that external γ-irradiation was the essential determinant for the time course of the total whole-body dose rate; this was also true for the animals analyzed for chromosome aberrations (Fig. 3b) or embryonic losses (Fig. 3d). The highest values of total dose rate were observed in the year of the accident, followed by an approximately exponential decrease over the next 10 years ($r^2 = 0.95$, $P < 0.05$ and $r^2 = 0.91$, $P < 0.05$ for sites 3 and 4, respectively, in Fig. 3b). The reduction factor since the accident was about 15, corresponding to a half-life of 2.5–3 years. These data show that after the primary insult in the year of the accident, every successive generation of animals was exposed to lower whole-body dose rates of ionizing radiation than the preceding one.

The mean whole-body absorbed doses in the studied cohorts of animals were also maximal in the year of radionuclide deposition. The highest individual value, determined in the first and second post-accidental generations of animals at site 4, was 73 mGy. By the end of the monitoring period, the mean whole-body doses were about 0.3, 0.7, 12, and 25 mGy for sites 2, 3, 4, and 5, respectively [9].

At any given time of the observation period, absorbed dose rates (Fig. 3) and absorbed doses at the different sites differed by about two orders of magnitude, thereby representing a strong gradient in the level of animal exposure.

Temporal development of the chromosome aberration frequency

The time course of chromosome aberration frequencies in the studied animals is presented in Table 2. The frequency of chromosome aberrations in bone marrow cells of the bank vole inhabiting the Berezinsky Biosphere Reserve, i.e., site 2 in our study, was first recorded 3–5 years before the accident [15] and can be taken as a pre-accident (historical) control for our data. The data of this pre-accident control are similar to those observed at the less contaminated site 1 (Minsk region) in the years 1986 and 1988. Before the accident, only chromatid-type aberrations were observed, and the cells never contained multiple aberrations. In contrast, in the post-accident period, the chromosome anomalies consisted of both chromatid- and chromosome-type aberrations, containing single and paired fragments in the majority of cases, but also rare Robertsonian translocations (fusion of acrocentric chromosomes at the centromeres). In animals inhabiting contaminated sites, the mean frequencies of both chromosome aberrations and aberrant cells were significantly higher ($P < 0.01$, Chi-square test) than the pre-accident value, and were increased in a dose-dependent manner, by a factor of 3–7 at sites 2, 3, and 4, and of about 15 at site 5. Also Pearson correlation analysis of the total aberration frequencies and those of the Robertsonian translocations at sites 2, 3 4, and 5 in 1996 (Fig. 4a, b) hints at a relationship between mean aberration frequencies and the whole-body dose rates at the time of capture. The increased frequency of chromosome aberrations observed in animals inhabiting contaminated areas appears to remain relatively constant over the years of the investigation (Table 2).

The data in Table 2 comprise the level of chromosome aberrations in animals varying by age, sex and maturity. These animals represent the studied populations, from where they were chosen at random. Generally, the mean age of the animals in each group did not differ from the usual mean age of about 3–4 months at the season of capture. There was a single exception at site

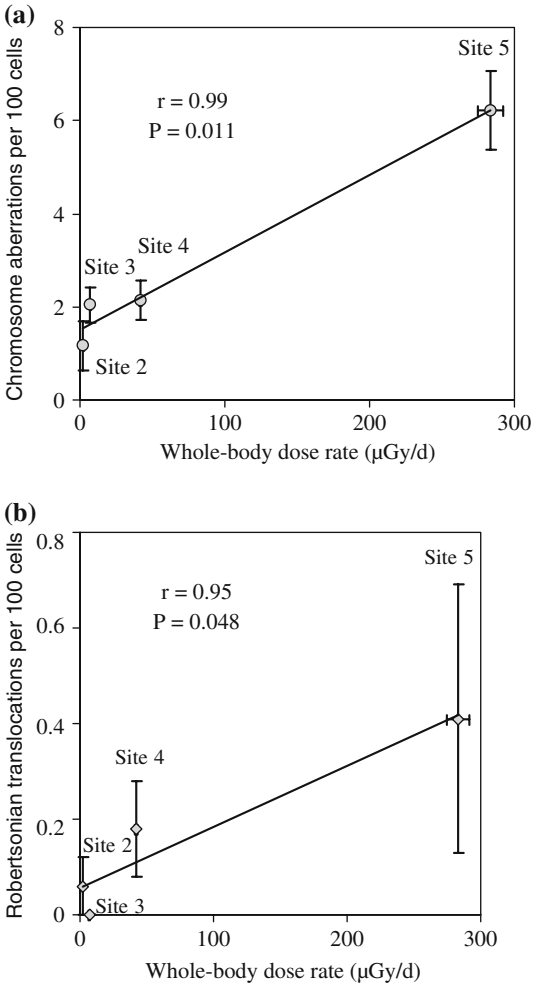

Fig. 4 Correlation between mean frequency values of all types of chromosome aberrations (**a**) and of Robertsonian translocation (**b**) with the mean values of the whole-body absorbed dose rate in bank vole populations inhabiting sites 2–5, 10 years after the accident. Standard deviations of the mean values are indicated as error bars

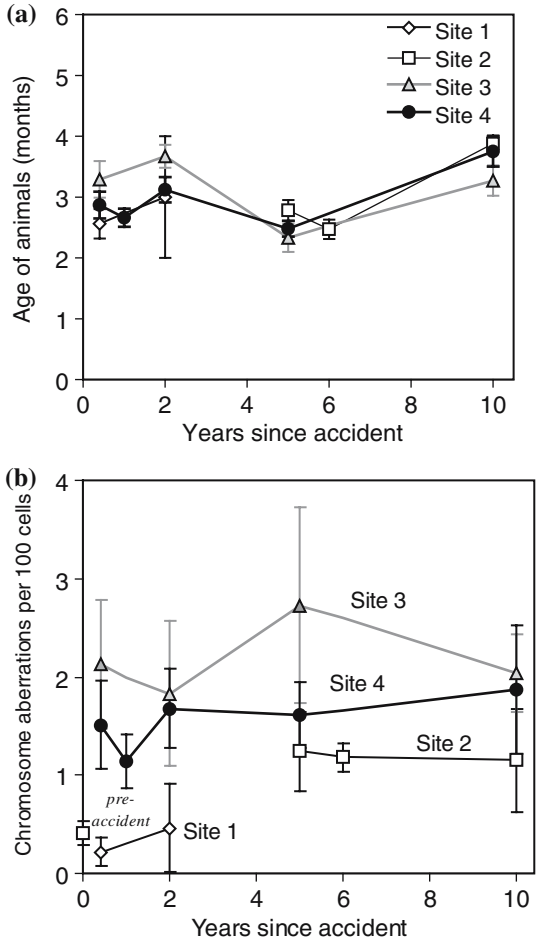

Fig. 5 Time course of animal age (**a**) and chromosome aberration frequency (**b**) in a sub-cohort of adult, 2–4 months old bank voles at four sites. Mean data and standard deviation are shown. Pre-accident data according to [15]

4, 10 years after the accident, when by chance 10 of 14 animals randomly sampled for cytogenetic analysis were at the age of one year or older, whereas in the more numerous group of specimens captured at this site for dosimetrical investigations [9] there was no detectable change in age distribution. In order to exclude any possible influence of variations in the age distribution on the observed frequencies of chromosome aberration, analysis of the time course of the chromosome aberration frequency was limited to mature 2–4 months old animals, whose mean values and temporal development of age over the period of this study is shown in Fig. 5a. The temporal pattern of the frequency of chromosome aberrations in the 2–4 months old animals (Fig. 5b) was almost identical with the pattern observed in all animals (Table 2).

As is evident from Fig. 5, the frequencies of chromosome aberrations observed in 2–4 months old bank voles remain fairly constant over time, in spite of the approximately exponential decrease of the whole-body dose rate. This suggests that the induction of aberrations depends not only on the actual exposure level. To test this, aberration frequencies were investigated in the offspring of mice captured in 1988 at sites 3 and 4, which were born and brought up in the laboratory and fed with uncontaminated food. The chromosome aberration frequency observed in the offspring animals showed no significant difference to that observed in animals from the same sites that had grown up in the contaminated environment, and to the aberration frequency of their mother animals (Fig. 6).

Embryonic mortality

Studies on embryonic losses in the monitored populations were started 2 years after the accident. Since at sites 1 and 5 no gravid females after the stage of embryonic implantation were captured, the analysis on embryonic mortality is limited to sites 2, 3, and 4. Figure 7a shows that there was no increase over the monitoring period in the age of gravid females, which could have affected the values of embryonic lethality. Rather, the mean age of

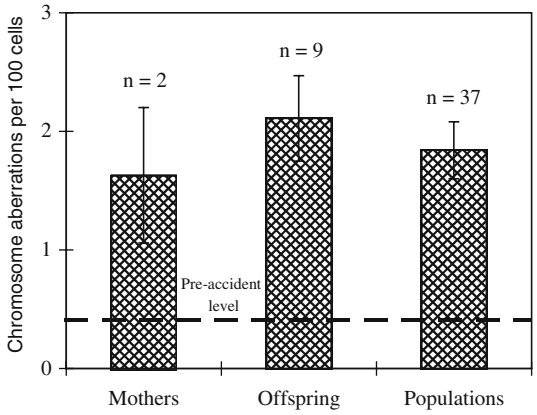

Fig. 6 Chromosome aberration frequencies in bank vole females captured at sites 3 and 4 in 1988, and of their offspring, born, and raised under laboratory conditions for about 1.5 months before analysis. Data are compared in bank vole populations living at sites 3 and 4 at the same period. Means, standard deviations of the means and the number of analyzed animals are shown

the gravid females tended to decrease over the period of monitoring. The frequencies of embryonic losses in the population inhabiting the Berezinsky Biosphere Reserve 3–5 years prior to the accident [16] were used as pre-accident control (Table 3; Fig. 7b). A comparison of pre- and post-accident data shows that both pre- and post-implantation embryonic mortality in populations in the moderately contaminated site 2 and in the more highly contaminated sites 3 and 4 remained on the level of pre-accident mortality frequencies during the first years of observation. After 5–10 years, however, a tendency towards increasing embryonic lethality was found in all populations studied, reaching a statistically significant increase in samples from sites 3 and 4 after 10 years (by a factor 2–5 in comparison with pre-accident data).

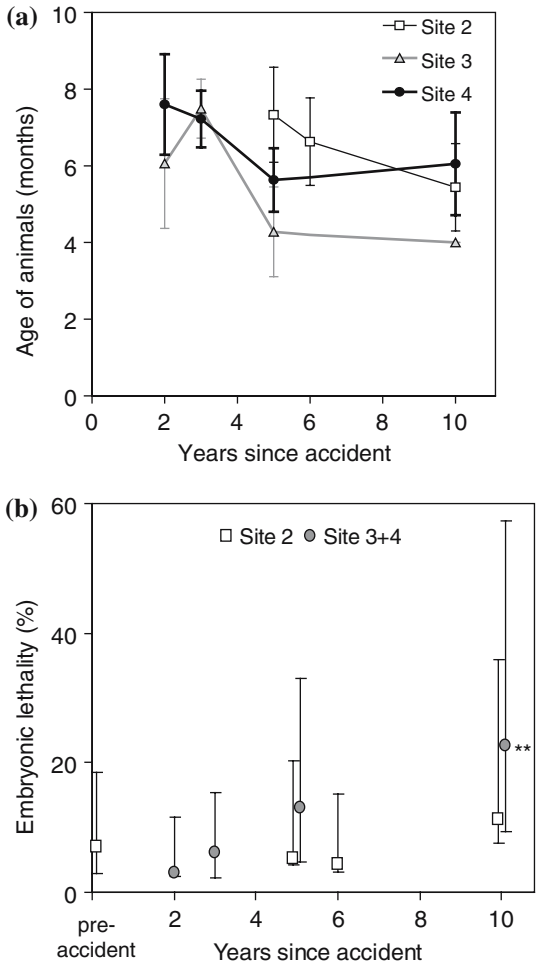

Fig. 7 **a**: Time course of the age of gravid bank vole females analyzed for embryonic losses. Means and standard deviation of the means are show. **b**: Time course of embryonic lethality in animals at site 2 and at sites 3 and 4 (pooled). Means and binomial 95% confidence limits are indicated. Pre-accident data according to [16]

Discussion

In this study free-living small mammals, the bank voles, were used as indicator organisms for the monitoring of environmental effects. The studied populations lived at different distances from the Chernobyl nuclear power plant. The investigations were started 5 months after the accident, from the 1 and 2 post-accident generations of animals that were irradiated at doses one order of magnitude less than animals in the acute period after the accident. The particular subject of this study was to compare the time course of biological damage in animals, representing up to 22 generations living during 10 years following the Chernobyl accident, with the time course of the radiation exposure experienced by these animals. The main results of this study are (a) a dose-dependent increase in the frequencies of chromosome aberrations and embryonic losses in animals living in contaminated areas as compared to historic controls and to animals living in an area with the lowest level of contamination, (b) the fact that the chromosome aberration frequencies remained on elevated levels (Fig. 5) and the frequencies of embryonic losses even increased (Fig. 7) over 10 years after the accident, although the whole-body absorbed dose rates experienced by the animals declined almost exponentially (Fig. 3).

Historic controls raise the concern that they might not directly be comparable to the samples under study. In this study the frequencies of chromosome aberrations observed in 1986 and 1988 in animals living at the almost uncontaminated site 1 can be used as controls, and they were similar to those assessed in the historic control (Table 2; Fig. 3). Thereby we can exclude that the increases in chromosome aberration frequency seen in the contaminated areas have reasons other than the higher radiation exposure. Unfortunately, we have no data from site 1 for the years 1991 and 1996 collected in the period August–September, but the fact that all investigations at the contaminated sites were done by the same team guarantees that the methods of investigation

have not changed in these years. Therefore, and in line with the unequivocal dose-rate dependency displayed in Figs. 4, 5 and 7, we propose a radiogenic etiology of the observed biological effects.

Steady decrease of the whole-body dose rate over the monitoring period of 1986-1996

At all sites investigated, whole-body absorbed dose rates were found to decrease over about one order of magnitude in 10 years, which is attributable to the gradual disappearance of the radioactive fall-out material from the biota [9]. More specifically, we show that the whole-body dose rate decreased approximately according to an exponential function with a half-life of 2.5–3 years (Fig. 3b, d). As the observed biological damage did not decrease in the same animals over the studied period, it is important to exclude that the exposure levels in the later years of the investigation, due to long-lived α-emitting transuranic radionuclides and/or increasing ^{90}Sr concentrations in the studied populations, were underestimated. Our analyses of radionuclide concentrations in animals showed that 10 years after the accident the contribution of transuranic elements and ^{90}Sr to the whole-body dose rate in the studied specimens of bank vole did not exceed 3 and 8%, respectively [9]. This contribution was estimated to be two orders of magnitude lower than the whole-body dose rates during the year of the accident [9]. The contribution of ^{90}Sr to local doses in bone tissue in the later years of the investigation was about 2–10× lower than that of external γ-irradiation. The absorbed dose rate in the red bone marrow due to ^{90}Sr β-radiation, which is approximately equal to the absorbed dose rates averaged over the bone tissues, was of the same small magnitude, comparable to that of $^{137+134}$Cs β-radiation in the later years and an order of magnitude lower than β-radiation of $^{137+134}$Cs in early period after the accident. We conclude that doses at late time points were not underestimated by our approach.

Dose dependence of the observed effects

Data presented in Tables 2 and 3, as well as in Figs. 5 and 7, hint at a strong dependence of the observed biological effects on radiation exposure. This is manifested by relatively low levels of aberrations and, where present, embryonic losses at the less contaminated sites 1 and 2, the medium levels at sites 3 and 4, and the relatively high level at the most contaminated site 5. Furthermore, there is a statistically significant linear correlation between mean values of the chromosome aberration frequency observed 10 years post-accident and the mean whole-body dose rates prevailing in the studied animals at the same time (Fig. 4). A similar association of mean micronuclei frequencies with exposure at low dose rates (4.22–39.4 μGy/day) has been shown in bank vole populations studied 2 years after the accident at four Swedish regions contaminated by Chernobyl fallout at deposition levels of 1.8, 22, 90, and 145 kBq/m^2 [17], i.e., levels similar to those at our monitoring sites 1–3 (Table 1). Mean levels of chromosome aberrations were also increased in a dose-dependent manner in laboratory mice, exposed in our monitoring areas during 4 months to whole-body dose rates from external and internal irradiation of 3–145 μGy/day [8], i.e., at exposure levels close to those in free-living animals.

Dose dependence of the biological damage is a basic requirement for conclusions concerning the causal role of radiation exposure for the observed effects. This includes the possibility of direct as well as indirect modes of action, in other words, the induction of the effects seen in a given generation by radiation exposure of the same or of the preceding generations of animals. In such a way, a linear correlation observed between mean values of chromosome aberration frequencies and the dose rates in late generations of animals 10 years after the accident can as well demonstrate a linear correlation of the aberration frequencies with the dose rates in all previous years, because the dose rates at the various sites remained in constant proportions to each other, while they decreased in an exponential fashion over the monitoring period (Fig. 4). On the other hand, the scatter plots of the individual frequency of chromosome aberrations versus the individual level of the whole-body absorbed dose rate or whole-body dose may hint at a dose dependence of the biological effect even on the exposure of the individual animal (Fig. 2 and data not shown). An analogous correlation has been observed between the individual frequencies of micronuclei and the individual radiation exposures in the same population of animals [6]. These dose-effect relationships should be studied in future.

Long-term persistence of biological effects and possible mechanism of their transgenerational transmission

Our data (Tables 2, 3; Figs. 5, 7) suggest that biological effects in bank voles living in regions contaminated by the Chernobyl accident persisted on a stable level (chromosome aberrations) or even increased (embryonic loses) over 10 years, corresponding to at least 22 animal generations, although the dose rates have substantially decreased during this period. This observation is in fundamental contrast to the assumption that the biological injury would gradually disappear in direct connection with the exponential reduction of the whole-body dose rate. Thus, the biological effects cannot be explained alone by the exposure experienced by the individual animals. Rather, our observations call for a different interpretation, namely that the biological effects reflect a transgenerational transmission of radiation damage that occurred in early generations, especially in the generations living at the time of the accident and in the early post-accidental generations, and was further accumulated in the course of the chronic exposure of the following generations. The fact that offspring from captured females, irradiated in utero at contaminated areas, but born in the laboratory and fed with uncontaminated

food, did not show a significant reduction in aberration frequencies in comparison with animals born and grown up in the contaminated environment (Fig. 6) corroborates the hypothesis that some mechanism of transgenerational transmission of radiation damage has been manifested.

The occurrence of radiation damage in cells and tissues that have not been directly exposed to radiation is not without precedence. For example, genomic instability is a long-term radiation effect that has been intensively investigated. The term "genomic instability" refers to the de novo production of delayed responses of the genome in the progeny of the exposed cells in a cell culture or in an irradiated animal [18–22]. Genomic instability, including chromosomal instability in hemopoietic stem cells in vitro and in vivo, has been shown to be activated by high- and low-LET ionizing radiations at relatively low doses [21–25] and to exhibit a dose-dependent increase at higher doses [24]. Dose dependence was also observed for chromosomal instability in mice fetuses irradiated as zygotes [26]. Other authors did, however, not find a dose relationship for genomic instability over a wide range of radiation doses [27–29]. The expression of genomic instability was described to persist for up to or even more than 70–80 population doublings at higher doses [29, 30]. In the progeny of long-term repopulating haemopoietic stem cells of mice, chromosomal instability was observed over 24 months after cell irradiation and transplantation [22]. There is evidence that the genetic background or genotype can influence radiation-induced instability [25]. The mechanisms of genomic instability are largely unknown. At least part of them may rely on epigenetic transmission of information. For instance, genomic instability was suggested to reflect altered signal transduction pathways and changes in the DNA microenvironment [31, 32]. Since DNA methylation is linked to chromosome condensation [33], chromosome and chromatid fragments as well as Robertsonian translocations may be due explained by the radiation-induced increased fragility of the chromatin during chromosome condensation [34]. These types of chromosome aberrations have been suggested as hallmarks of induced genomic instability, although other types of chromosome aberration can also occur [24, 26, 27, 30].

However, in contrast to studies where long-term effects are investigated in the same animals, which had been exposed to radiation at an earlier stage of individual development, the interpretation of the effects seen in our study must deal with damage persisting over many generations of animals. In the literature, the term "transgenerational transmission of radiation damage" has mainly been used in a descriptive sense, including genetic and epigenetic as well as hitherto uncharacterized mechanisms of transmission damage to the progeny [21, 35–42]. Delayed radiation-induced responses attributable to genomic instability were revealed in the first few animal generations after irradiation of male germ cells [43] or zygotes [37]. Heritable tumors and anomalies were observed in descendants of exposed mice [35], and physiological or developmental disorders were seen in the progenies of irradiated parents, which resulted in embryonic and early postnatal death, fertility disturbances, congenital abnormalities or malformations [44]. Genomic instability in the first-generation offspring (F_1) of irradiated mice or rats covers diverse endpoints such as chromosome aberrations, micronuclei, point mutations and reversions in somatic cells (reviewed in [39]) as well as early (pre-implantation) and late (post-implantation) embryonic resorption [37], reduced fertilization rate [45], increased number of sterile females [37] and reduction in the proliferative ability of both F_1 and F_2 embryonic cells [36] and F_1 liver cells [38]. Transgenerational germline instability was also demonstrated in the offspring of irradiated mice (reviewed in [39]), as well as in children born from irradiated parents [46] and in barn swallows breeding not far (25–50 km) from the Chernobyl nuclear power plant [47]. In addition, transgenerational transmission of genomic and developmental effects was reported after parental exposure to certain chemicals [e.g., 35, 41].

In conclusion, we suggest that in the studied bank vole populations the radiation exposure of the parental generations has lead to an accumulated pool of germline mutations and/or of epigenetic changes, which resulted in the observed, persistently elevated levels of chromosome aberrations in somatic cells and in increased embryonic losses in later generations. With regard to the continuous build-up of transgenerationally-transmitted damage in the course of chronic radiation exposure of the parental generations, we will shortly speak of the *transgenerational accumulation* of transmitted biological damage.

The present report is the first in which the time course of biological damage in mammals chronically exposed to ionizing radiation over a series of generations has been studied. So far, the long-term development of transgenerationally transmitted radiation damage has been studied in a restricted number of consecutive generations of animals, generally in the first 1–2 generations after irradiation of parents [21, 31, 35–39, 43, 46]. Increased frequencies of chromosome aberrations were also observed in bone marrow cells after 25–30 generations of red vole [3] and after 75–80 generations of common vole [48] chronically irradiated in regions of heavy radioactive contamination in the Urals as well as in bordering areas. The latter observation was explained by hereditary chromosome instability [48], but there is no data on the quantitative time course of these effects. The question arises whether the levels of chromosomal aberrations and embryonic lethality that in our study were reached in 1996 after 22 animal generations, will be maintained permanently, or whether a phase of reduction will follow in subsequent animal generations. Our study indicates that a further reduction of chronic radiation exposure has to be anticipated, and that there exists an effective selection mechanism, the dose-dependent increased embryonic lethality in animal populations in radiocontaminated areas. Further research will be required to clarify this genetically and ecologically interesting point.

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