DECLINE OF REPRODUCTION AS UNIVERSAL POPULATION ANSWER TO THE INCREASE OF IONIZING IRRADIATION LEVEL

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Abstract

Analysis of the population-genetic consequences of technogenic catastrophes, e.g., Chernobyl, represents special interest in connection with development of global ecological changes and rising technogenic contamination. Research of dynamics of cytogenetic anomalies in bone marrow cells of different Rodentia species (trapped in alienation zone near Chernobyl's NPP in places with different levels of contamination from 5 lip to 1000 Ci/km2) and in peripheral blood cells of cattle generations of experimental herd (Pripjat, 200 Ci/km2) were carried out. Information was accumulated about the differential cell sensitiveness to the same doses of ionizing irradiations, in depending of the level and direction of cytodifferentiation, rates of cellular division, belonging to different organisms of the same species, and to different species. The damaging effects dependent also of the initial level of ionizing irradiation. Substantial differences in the increase of frequency of mutational events at the irradiation were dependent on the specificity of examined molecular-genetic marker, and also on genotypic background of organism. The increase of ionizing irradiations induced in mice of different laboratory lines and in wild voles the frequency increase on those cytogenetic anomalies on which the line- (for mice) and species-specific enhanceable changeability in controls conditions was revealed. Complication of analysis of such mutational spectra was multiplied yet that investigational animals had the expressed preferable involving of individual chromosomes in the certain types of cytogenetic anomalies, both in the controls groups and after ionizing irradiations. The accumulation of resistance individuals in different species of voles after changing 26 - 30 generations in areas of high-density radionuclide contamination («Red forest», area of alienation of Chernobyl's NPP, 1000 Ci/km), but not in less polluted regions (100 - 200 Ci/km') was revealed. The changes of genetic structure in cattle generations were analyzed employing family analysis of allele's transfer in structural genes and ISSR-PCR markers. Increases of mutant animals were not detected, but reversal of genetic structure in cattle generations from an initial breed to ones, typical for more primitive breed was revealed. Our results indicate that ionizing radiation does not induce new genetic anomalies but allows realization of inherently unstable species- and individual-specific genetic characteristics. Contradictions between plenty of the experimental information accumulated in scientific subdivisions of different countries, presumably, related with a number of reasons - in particular, that not always it is used the differential approach to the estimation of consequences of increase of ionizing irradiations at different age-dependent groups, and also in depending on the initial ionizing background of residence. Usually the multifactorness of intracellular answer and changes of composition of heterogeneous cellular populations of mammalian species were not taken into account on the analysis of damaging effects of ionizing irradiation. The search of integral traits of damaging after actions of ionizing irradiations for mammalian species is the key problem of impartial evaluation of consequences of the Chernobyl's catastrophe for the population. It may be the changes in population reproduction, the most important trait for population future.

Key words: Population-genetic adaptation, ionizing irradiation, protein polymorphism, ISSR-PCR, cytogenctic anomalies, population-genetic adaptation to stress.

Introduction

The life on the Earth has arisen and developed in the presence of the natural radioactive background (RB), a constant external abiotic factor. RB for human, calculated for altitude of 1 meter above ground, fluctuates in world-wide average annual exposure near 3.5 mSv [9]. Exposure to natural sources is characterized by very large fluctuations, not excluding a range covering two orders of magnitude. The availability of the territories on Earth, in which RB differs dozens and hundred times from world average [6], testifies the relativity of concepts of injuring dozes of ionizing radiation.

Genetic consequences of residing in radioactive provinces have been investigated for many years. The broad research of the human populations in the areas with increased RB (for example, several states in India, Ramsar in Iran) where the exposure dose of the population for one year measures from 35 to 260 mSv, have not revealed an increased level of hereditary diseases in the populations [3]. Sudden significant elevation and persistence of sub-lethal radioactive exposure caused by human error as in Chernobyl can be considered a "changed" ecological condition. Not the new local elevated RB, but abruptness of the change, is a real long-term problem related to Chernobyl accident. The "novelty" of ecological conditions caused by human activity is the specific trait of modem time. Chernobyl serves as invaluable model for the study of the effects of fast changes in the environment on the well-being of humans and ecosystems.

Investigations of cytogenetic anomaly frequencies in somatic cells in connection with ionizing irradiation in the last 50 years were widely conducted on the people, plants, small-sized *Rodentia* species and others. The high individual variability in tested species in the same conditions, and also the absence of precise relations between quantity of cytogenetic damages in somatic cells and dozes of ionizing irradiation were the singularity of the accumulated data. Usually it is supposed that the increase of accuracy of methods will allow to reveal more precise correlations between low dozes of irradiation and induced mutation events. First data, confirming this hypothesis were obtained by Dubrova et al. [1] and concerned the occurrence of new minisatellite loci mutations in children of the liquidators. However, the increase of mutation frequencies was observed only in 3 from 8 investigated microsatellite loci [1]. In other research [13] the increase of new mutations was detected by RAPD-PCR, but not by ISSR-PCR DNA markers. So, revealing of mutation events on DNA level in these investigations determined by specificity of variability in investigated minisatellite loci [1] and in DNA fragments, flanking by particular decanucleotide or microsatellite invert repeats (RAPD-PCR, ISSR-PCR) [13]. Hence, as in cytogenetic investigation, DNA marker analysis fails to provide unambiguous data about genetic effects of low dozes of ionizing irradiation, which would not depend on specificity of initial-variability of separate character.

We propose several methods to solve this problem. First, we need to search for the markers of the individual "resistance" to ecological changes, evaluate the changes in the fitness of genotypes facing changed conditions. This could be done by analyzing generation-to-generation changes in allelic composition of populations of several species reproducing in the conditions of ecological catastrophe (it is impossible in human populations because of large life duration). It is reasonable to expect that a set of molecular genetic markers distributed across the genome whose variability has nonrandom character in generations under conditions of increased ionizing radiation can be identified.

Thus, we propose our model of the control of population-genetic changes in Chernobyl's zone using several species, for example, species *Rodentia.* Among its advantages are fast changc of generations, this object is convenient for direct cytogenetic analysis of dividing cells in bone marrow and population genetic research. Among the

shortages of this object are short cycle of reproduction, complex migration processes, and impossibility of the family analysis of animals in field conditions.

Another good object for such investigations is the cattle of the experimental farm Novoshepelichi (Pripjat). Advantages of this object: each animal has strictly determined genealogy and is useful for family analysis; all generations bom after Chernobyl's catastrophe in conditions of a zone of alienation of Chernobyl's accident are available. The cattle of the same breed in rather pure regions is available as control. Large quantity of molecular genetic markers in cattle is available and their localization in chromosomes in cattle is well characterized. In addition, similarity between cattle and human in gene syntheny is noted. Shortages of this object could be low number of offspring and special service required.

Materials and Methods

Animals. The mice of laboratory lines BALB/c (35 animals), C57BL/6j (35 animals) and CC57W/Mv (27 animals) were investigated in two age groups (2-3 monthes and 12-18 monthes) in control conditions (vivarium of Institute in Kiev, Ukraine and their sibs in experimental vivarium near Chernobyl's NPP, in which they exposed to chronic low ionizing irradiation (in summary near 0,6 Sv,). The investigation was carried out in 1993 - 1999 y in different seasons.

Representatives of a *Microtus oeconomus* (10 animals), *Clethrionomvs glareolus* (29 animals) and *Microtus arvalis* (43 animals) were trapped in places of zone alienation of Chernobyl's NPP, distinguished by radio nuclide pollution from high (Red Forest, 1000 Ci/km^2), middle (Janov, near 200 Ci/km^2) and to low (Ivankov, Nedanchichi 1-3 Ci/km^2) levels.

Cytogenetic and population-genetic investigation were performed on Holstein cattle at Novoshepelichi farm located within 5 km zone surrounding the Chernobyl nuclear power plant (near 200 Ci/km^2). As result of the accident the zone showed dramatically increased ionizing radiation (-200 Ci/km^2). These cattle are further referred as "exposed" group. Parent's generation (FP), born in "pure" zones and founded the experimental herd in farm "Novoshepelichi", included two subgroups.

(1) Bull *Uran* and three cows -*Alfa, Beta* and *Gamma* trapped in 1987 near the Chernobyl reactor when the accident in 1986 y happened. These animals (FP) were founders of FI, F2 and F3 generations, bom in "Novoshepelichi".

(2) Another subgroup of cows (FP) brought to Novoshepelichi from "pure" zones of Ukraine in 1990-1993 years being founders of another FI and F2 generations, bom in experimental farm near the Chernobyl's reactor.

In both subgroups, FP as well as FI and F2 cows were mated exclusively to only one bull -*Uran* belonging to FP. In summary, the experimental exposed herd included 17 parents, 96 animals FI and 50 ones F2 (first and second generations bom in conditions of chronic influences of low doze of ionizing irradiation).

Cattle of the same breed kept in an uncontaminated region (Dnepropetrovsk, Ukraine) served as control, in whole 46 animals. The cattle group (36 animals) of Grey Ukrainian breed (from "pure" Kherson region, Ukraine) was included in analysis as example of more primitive breed in comparison of Holstein ones.

Cytogenetic investigations. The preparations of bone marrow cells of representatives of *Rodentia* species and peripheral blood cells of cattle (with the use of short cell cultivation throughout 72 hours with phytohcmagglutinin) were obtained by standard technique without colchicine. In *Rodentia* species the bone marrow from back legs was washed away by hypothonic solution of KC1 (0,54 %), fixed by a mix of

methanol spirit and ice acetic acid (3:1), three times changing a fixing solution, than cells spread out the cold glass slides, dried up and colored by dye Gimsa ("Merck", Germany), further analyzed with the help of binocular microscope Karl Ceiss at increase in 1000 times. Metaphase plates were photographed on a film “Micrat-300”. The frequency of metaphase plates (in %) with following cytogenetic characteristic were counted (in %): metaphase with aneuploidy, polyploidy (PP), chromosome aberrations (CHA), interchromosome associations on a type of Robertsonian translocation (RB), with asynchronous separation of centromere chromosome region (ASCR). Aneuploidy was evaluated in two variants: general aneuploidy (Al) and aneuploidy (A2) on one chromosome (2n±l).

Quantity of metaphase plates (MI) in 1000 cells, binuclear leukocytes (BL) and leukocytes with the micronuclei (LM) calculated on the same preparations in cells with saved cytoplasm (in ‰). Additionally the smears of cattle peripheral blood were done and frequency of MI, BL, LM and the erythrocytes with micronuclei (EM) in them was analyzed. Statistical reliability of between group differences was evaluated with use of Student criterion (t_s).

Population-genetic analysis. In cattle, we used electrophoretic method of protein separation in vertical PAGE gels on the according to the modified technique of Gahne (Gahne et al., 1977) for the analysis of the polymorphism of transferrin (TF), posttransferrin-2 (PTF-2) and receptor of vitamin D (GC) loci. The analysis of hemoglobin (HB), ceruloplasmin (CP), amylase-1 and purin nucleoside phosphorylase (PN) loci was carried out by a method of horizontal starch gel electrophoresis with subsequent histology-chemical dyeing using the standard techniques [2, 5].

Polymorphism kappa-casein genes were investigated with the use of PCR-RFLP analysis. For PCR-amplification of a fragment of a kappa-casein gene used the following primers: Bocas A: 5 ' - ATGTGCTGAGCAGGTATCCTAGTTATGG - 3 ' and Bocas B: 5 ' - CCAAAAGTAGAGTGCAACAACACTGG - 3 ', picked up so that the DNA fragment between them included Pst I site specific for A and B allelic variants [14]. PCR-amplification carried out in the following mode: denaturation - 60 sec at + 92 C, subsequent 35 cycles - 60 sec at + 62 C, 90 sec at + 72 C. 5 mcl of amplified product was used for restriction analysis which was carried out within 4 hours at + 37 C with restrictase Pst I in the buffer of firm Sibenzime in volume 15 mcl. Allele CSN3 A contains the site to restrictase Pst I, and B - don’t contain. The restriction products divided by a method of electrophoresis in 1,5%-s' agarose gels with addition of ethidium bromide and testing under ultra-violet light. The mix for PCR contained in all cases 50 ng of DNA, 15 pmol of each primer, 2,5 mcl 10 x buffer (700 mmol/1 TRIS-HCL, pH 8,8 at +25oC, 170 mmol sulfate ammonium, 1,7 mg/ml BCA, 0,3 mmol/1 Mg2Cl), on 200 mcmol/1 desoxinucleoside triphosphates, and also on 1,5 U of Taq polymerase ("Bion", Moscow). PCR was carried out in volume 25 mcl in thermocycler PTC-100 MJ Research, Inc. (USA).

We used also the method proposed by Zietkiewicz E. et al., 1994 [15] for PCR-amplification of DNA fragments, flanked by microsatellite repeats (ISSR-PCR), with the use as primers dinucleotide repeats - $(CA)_{10}G$, (CG)9G and three nucleotide repeats - $GT(CAC)_7$, (CAC)vT, $(AGC)_6C$, $(AGC)_6G$.

Statistical analysis (accounts of allelic and genotype frequencies, gcnetic distances on M.Nei’s method, estimation of gene balance according to the Hardy-Weinberg’s law, cluster analysis) was carried out with use of the standard computer program "BIOSYS-I", the statistical reliability between frequencies of allelic variants and phenotypes on various loci paid off with use of Fisher’s criterion.

Results and discussion

1) Constitutive (inherited) mutations

Our results indicated the absence of constitutive mutations in the zone of alienation of Chernobyl's NPP in *Rodentia* species and increased resistance to radiation from 1994 to 2001 years on frequency of cells with cytogenetic anomalies in bone marrow cells. The constitutive mutations were not detected under exposure of mice lines (C57BL/6, BALB/c, CC57W/Mv) to increased (approximately 100 times) level of ionizing radiation in special vivarium [4], in species of red and common voles, and in oeconomus voles, surprisingly, including those, trapped in the Red Forest.

In cattle, in one animal (from 160 investigated), the mutation in transferrin gene was revealed and only in the second animal generation, which was born in conditions of increased contamination by radio nuclides (200 Ci/km2).

Carriers of Robertsonian translocation were not detected in mice and cattle in Chernobyl's zone, in spite of the presence of such mutation quite often was observed even in "pure" zones in the genomes of species with acrocentric autosomes.

2) Cytogenetic anomalies in somatic cells

Laboratory lines of mice. In laboratory experiments on mice lines (in special vivarium near Chernobyl's reactor), we observed an increase of cytogenetic anomalies in the bone marrow cells subjected to ionizing radiation (near 0,6 Sv). However, only those types of cytogenetic anomalies were increased which were spontaneously highly variable in an age- or season-dependent manner in the same mice lines not subjected to radiation. For example, from 8 investigated cytogenetic characters only the frequency of binucleated leukocytes (BL) and leukocytes with micronuclei (LM) varied in relation with season of analyzing (winter, summer and autumn) and age of BALB/c mice in control conditions, and only BL and LM were increased in BALB/c exposed experimental population. In control conditions only aneuploidy (A1 and A2 types) was varied in relation with investigation season and age in C57BL/6j mice and only aneuploidy increased in exposed population. The increase of LM and metaphase plates with chromosome aberration (CHA) in old mice and in winter in comparison with summer was revealed in CC57W/Mv mice, and CHA and LM were increased in CC57W/Mv mice in special Chernobyl's vivarium. Moreover, in the group of "old" linear mice CC57W/Mv (aged 16-18 months), some cytogenetic anomalies (LM) were less frequent **(5,0±0,8‰)** than in the mice of the same age in the control group **(9,0±l,2‰).** This corroborates the findings of an increased rates of cell division (MI, updating of cell populations in bone morrow and elimination of defect cells) in Chernobyl's animal populations **(7,0±l,8‰)** in comparison with control group **(4,0±0,7‰).** Therefore, our results indicate that ionizing radiation does not induce new anomalies in laboratory mice lines, but strengthens realization of inherently unstable line-specific cytogenetic characteristics in the investigated lines of mice.

Species of voles. In other experiments, three species of voles (*Microtus arvalis, Microtus oeconomus, Clethrionomvs glareolus)* were investigated. Among them, the evolutionary youngest species of common vole (*Microtus arvalis*) characterized by comparative high karyotype instability in area was the most sensitive to ionizing radiation [7].

This interspecies comparison thus confirmed that an increase of ionizing radiation does not induce new genetic damages, but destabilizes the preexisting genomic "hot spots" that are either species-specific (and more characteristic to evolutionary young species) or genotype-specific (for example, different laboratory lines of mice).

We have revealed selection of radio resistant animals in environments with a high level of radio nuclide contamination. Among red and common voles trapped in Chernobyl

contaminated zone in places with high (Red Forest, **1000** Ci/km2) and middle (Janov, near **200** Ci/km2) levels of radiation, in **1994-1996,** or **16-20** generations after explosion, increased frequency of cytogenetic anomalies in bone marrow cells was revealed. In bone marrow cells of *Microtus arvalis,* trapped in contaminated zones in **1996** y, the frequencies of aneuploid metaphases **(A2,** 2n±l) and **LM** were **17,9±4,4%** and **6,8±0,5%o** in comparison with **A2=8,6±2,8%** and **LM=3,0±0,4%o** in control group (trapped in "pure" zones). In voles of *Clethrionomys glareolus* in these zones in **1996** y the frequency of metaphases with chromosome aberration was **7,3±3,4%** in comparison with **1,2±0,7%** in animals from "pure" zones. In **1999-2001,** after **26-30** generations, no distinguishes from control groups on the frequency of cytogenetic anomalies in bone marrow cells were revealed in animals, trapped in Red Forest. So, the frequencies of **A2** and **LM** in *Microtus arvalis* were **3,1±0,8%** and **3,1+0,5%o; CHA** in *Clethrionomys glareolus* - **0,9±0,3%.**

The intensity of selection for radio resistance was mostly expressed in a Red Forest (1000 Сл/км2). The slower speed of such selection was observed under middle level of radio nuclide contamination. In 1999, in locations with radioactive contamination level of 200 Сл/км2 (Janov), higher individual variability and increased frequency of cytogenetic anomalies in comparison with the control group from the "pure" zone (lower than 5 Ci/km2) and population from a Red Forest in 1999-2001 year were revealed. For example, the frequency of metaphases with CHA in bone marrow cells of *Clethrionomys glareolus,* trapped in 1999 y in Janov, was CHA=8,1±4,0%.

Cattle. In the parent cattle generation in the experimental economy "Novoshepelichi", frequency of leukocytes with the micronuclei (LM) in blood smears was significantly higher ($P < 0,001$), than in the first, second and third generations of animals that were born in the zone of increased radio nuclide contamination. This characteristic in the cattle of the third generation was significantly lower ($P < 0,001$) than in the second generation. 6 animals from parent generation (LM=4,5±0,3%o), 15 cattle from FI generation, bom in experimental farm (LM=l,l±0,8%o), 12 animals form F2 generation (3,0±0,3%o) and 3 animals from F3 generation (l,5±0,4%o) were included in analysis. Frequency of binuclear leukocytes (BL) in smears of peripheral blood also was significantly higher in the parent generation than in the first and in second generations of animals. That is, on the frequencies of cytogenetic anomalies in smears of peripheral blood in generations of cattle, which were bom in conditions of increased ionizing radiation, the clear increase of radio resistance of animals was observed also. We investigated also the fertility of cows (in average number of calves, bom by one cow in one year) in parent's and FI generations. Fertility of cows in the first generations after Chernobyl explosion on the experimental farm located in contaminated zone was reduced approximately in 5 time in comparison with the parent generation (on average, from 0,93 up to 0,12 calves per cow per year). So, 16 cows of parent's population produced 96 calves (0,93±0,03 calf per cow per year); 20 of them (21%) died before 3 month age. However, the sterile cows were absent in parent's population. In FI, between 36 cows 21 ones (58%) were sterile; only 15 cows of FI bom animals of F2 generation: 50 calves (27§ and 23,з) in 8 years. 13 of them died before 3 month age (26%). If calculated for all 36 cows of FI, the cow's fertility decreased from 0,93 in parent's cow generation to 0,12 calves per cow per year in FI cows. If calculated the number of bom calves only on 15 fertile cows of the F1 generation, the decrease would be less, to 0,73±0,06 calves per cow per year, but some fertility decrease (t_s=2,86; $P<0,01$) was revealed. Four cows of F2 in summary bom 10 calves (F3) for 4 - 2 years, in average, 0,94±0,06 calf per year per cow. It allowed to suppose, that the fertility of F2 cows could be increased in comparison with FI cows (t_s=2,67; $P<0,02$). The data obtained can result from selection pressure in FI generation, related with new conditions of cattle reproduction (the increase level of ionizing irradiation), which lead to elimination of some genotypes.

3) Population-genetic changes in cattle's generations

Analysis of the allele inheritance in different genes and DNA fragments, flanked by microsatellite loci (ISSR-PCR) in cattle's generations that were bom under increased radio nuclide contamination was carried out. Data on allele frequencies observed in control and exposed groups of cattle are presented in Table 1. The homozygosity of HB locus and low level of polymorphism in PN locus are the specific traits of Holstein breed in different countries. It was true for exposed experimental herd also and hence the data of HB and PN loci was not included in following comparative analysis of the genetic structures of cattle groups. It is interesting that the mean heterozygosity in the exposed group (by one sire, bull *Uran)* was not lower than that in the control group sired by a number of bulls (Tab.l). The investigations covered allelic variants of the following polymorphous loci traced in the exposed group: *TF, CP, GC, AM-I, PTF-2,* and *CSN3.* At the *TF locus* three allelic variants -A, D1 and D2 were found. The rare allele Tf E and specific for ancestor breed Grey Ukraininan allele Tf F were not revealed in experimental herd. Two allelic

T a b l e 1

The allele distribution in parent's generation (FP), first (F1) and second (F2) generations of cattle born in experimental farm «Newshepelichi» in zone of alienation of Chernobyl's NPP (cattle group exposed to ionizing irradiation), in ancestor cattle breed Grey Ukrainian (from "pure" zone, Kherson, Ukraine) and in Holstein "control" group (from farm in "pure" zone, Dnepropetrovsk region, Ukraine) on TF, AMI, CP, GC, PTF-2 and CSN3 loci

	CATTLE'S GROUPS				
	EXPOSED CATTLE's HERD IN EXPERIMENTAL FARM "NOVOSHEPELICHI"			GREY UKRAINIAN	HOLSTEIN ("control" group)
	Parent's generation (sum of 13 genotypes of mothers and 13 the same genotypes from one bull Uran)	F1 offspring	F2 offspring		
Locus					
TF (N)	26	34	21	30	45
A	.423	.412	.333	.167	.411
D1	.346	.279	.476	.000	.267
D2	.231	.309	.190	.667	.300
E	.000	.000	.000	.117	.022
F	.000	.000	.000	.050	.000
AM-1 (N)	26	34	21	30	46
A	.000	.000	.000	.000	.000
B	.038	.103	.143	.933	.283
C	.962	.897	.857	.067	.717
CP (N)	26	34	21	30	46
A	.577	.412	.524	.733	.435
B	.423	.588	.476	.267	.565
GC (N)	26	34	21	30	35
A	.327	.324	.405	.183	.057
B	.673	.676	.595	.817	.943
PTF2 (N)	26	34	21	30	35
F	.596	.529	.357	.850	.529
S	.404	.471	.643	.150	.471
CSN3 (N)	23	15	8	36	43
A	.848	.733	.625	.653	.895
B	.152	.267	.375	.347	.105
The averege heterozygosity	.575 (S.E. .144)	.474 (S.E. .086)	.440 (S.E. .081)	.379 (S.E. .080)	.398 (S.E. .087)

(N) - quantity of investigated animals.

variants were revealed at the *CP* locus - A and B. Polymorphism at the GC locus was due to two alleles: A and B. *AM-1* was represented by variants B and C. *PTF-2* locus showed fast and slow allelic variants - F and S, respectively. *CSN3* had two variants -A and B. For the first time an animal was revealed with a constitutive mutation, the carrier of a unique variant at the *TF* locus, having electrophoretical mobility different from the other five *TF* variants, including the parental and rare ones, revealed in Holstein from "pure" zone (Tf E) and in Grey Ukrainian breed (Tf F). Mutated allele (mut) had faster electrophoretic mobility than allele E, but slower than allele D2. Its genetic nature was confirmed by data on its inheritance (Tab.2). Neither in literature nor in the control animals (bred in a relatively clean environment) was a similar allelic variant found, thus confirming the uniqueness of the mutation. Basing on the available data, it is not possible to establish precisely whether the mutation came from the dam or the sire. One may only assume, that the mutation had appeared in cow No.49 (FI. the daughter of cow Beta) and next was inherited by No. 113 (F2) and her daughter No. 155 (F3), but did not appear in any other progeny of Beta and Uran (Tab. 2).

The distribution of allelic variants at the loci studied in parents and their progeny of different generations is presented in Tables 2 and 3. An analysis of the transfer of allelic variants from heterozygous parents to their progeny was carried out. Theoretically, both alleles have an equal chance of being transferred from the parents to the offspring.

In parent generation (FP) genetic structure was described as the sum of 13 different mother's genotypes and 13 identical genotypes of bull Uran, which was the father in all cases (Tab. 1). Excess of heterozygosity in some loci was observed. However, among parents excess of heterozygosity in TF locus was revealed on Uran's Tf ADI genotype (Chi-square= 15.384; p=0,002), but in FI - on Tf AD2 genotype (Chi-square=8.975; p=0,030). In four out of the investigated six loci deviations were found from the expected parent —> offspring transfer values in two generations, being significant, however only for AMI and *CP* loci (t_s=2,0, P<0.05 and t_s=2,8, P<0.01, respectively) and only in FI derived from cows *Alfa* and *Gamma* the preference of Tf D2 allele transferring from mothers to offspring was obvious (Tab. 2,. 4). In general, in the case of the *TF* locus in FI, allele A was more often transferred to the offspring from the sire (ADI genotype) while allele D2 - from the dams. In F2 the allelic transfer from *Uran* was closer to that expected (Tab. 2, 3, 4). In case of CSN3 locus the increase of B allele frequency in FI in comparison with FP was observed also, but the differences were more small (t_s=T,77, P<0.10). It is very important to note that the comparative high frequencies of allele D2 in TF, B in AMI, B in CSN3 loci are the specific traits of gene pools of ancestor Grey Ukrainian breed (Tab.l). So, the data obtained demonstrated some sift of gene pools in FI offspring, born in conditions of ionizing irradiation, from parent generation to ones, typical for more primitive cattle breed, Grey Ukrainian.

An analysis of the changes in allele frequencies occurring over the two generations demonstrated a clear disturbance in their distribution in FI, as compared to that characteristic for the parental (FP) generation. In generation F2, the observed frequencies were close to those expected. The reason for the inheritance disequilibrium observed in FI may be related to a change in selection, as, also, the fertility decrease of FI cows. Effects of an abiotic stress may lead to selection in different stages of offspring forming from parental generation which was subjected of increased level of ionizing irradiation in the zone of alienation around Chernobyl. Thus, the change of environment could directly affect the preferable reproduction of some genotype combination of the parental animal gametes, and also change the pattern of genetic structure in FI. The effect of the environment on preferable genotype reproduction is most clearly demonstrated in the disruption of the expected allelic transfer.

Table 2

The transferring of allelic variants of TF, CP, AM-I, GC, PTF2, CSN3 loci from cows-mothers Alpha, Gamma, Beta and bull Uran to offspring of 1, 2. 3 generation (F1, F2, F3), which were birthing in experimental economy of alienation zone of Chernobyl's accident Novoshepelichi"

Mother's name	Mother's genotype TF	Allele Tf from mother	№ offsp-ring	TF geno-type of offsp-ring	Allele Tf from bull Uran	CP geno-type of off-spring	Cp allele from father	AM-I genoty-pe of offspring	GC genotype mother-offspring		PTF2 genotype mother-offspring		CSN3 genotype mother-offspring	
Bull Uran					AD1		AB	CC	AB		FS		AB	
F1 from cow Alpha														
Alpha	AD2	D2	Rosa	AD2	A	AB	B	CC	BB	AB	FS	FF	AB	BB
Alpha	AD2	D2	Galka	AD2	A	AB	B	CC	BB	BB	FS	SS	AB	AA
Alpha	AD2	D2	105	AD2	A	AB	B	CC	BB	BB	FS	FF	AB	AA
Alpha	AD2	D2	120	AD2	A	AB	B	CC	BB	AB	FS	SS	AB	-
Alpha	AD2	D2	167	AD2	A	AA	A	CC	BB	BB	FS	FS	AB	-
F2 from cow Alpha														
Rosa	AD2	A	83	AD1	D1	AA	A	CC	AB	AA	FF	FF	BB	-
Rosa	AD2	A	116	AD1	D1	BB	B	CC	AB	AB	FF	FS	BB	-
Galka	AD2	D2	80	AD2	A	AA	A	CC	BB	AB	SS	FS	AA	AB
Galka	AD2	A	95	AD1	D1	BB	B	CC	BB	AB	SS	SS	AA	AB
Galka	AD2	A	11	AD1	D1	AB	?	CC	BB	AB	SS	FS	AA	AB
120	AD2	D2	152	D1D2	D1	BB	B	CC	AB	AA	SS	SS	-	-
F1from cow Gamma														
Gamma	AD2	D2	Maika	AD2	A	AB	B	CC	BB	BB	FS	FS	AA	-
Gamma	AD2	D2	32	AD2	A	AA	A	CC	BB	BB	FS	FF	AA	AA
Gamma	AD2	D2	15	AD2	A	AB	B	CC	BB	BB	FS	FF	AA	AB
F2 from cow Gamma														
Maika	AD2	D2	85	D1D2	D1	BB	B	CC	BB	BB	FS	FS	-	-
Maika	AD2	A	99	AD1	D1	AB	?	CC	BB	AB	FS	FS	-	AB
32	AD2	D2	93	AD2	A	AA	A	CC	BB	AB	FF	FF	AA	AA
15	AD2	A	81	AD1	D1	BB	B	CC	BB	BB	FF	FF	AB	AA
15	AD2	A	100	AA	A	AB	?	CC	BB	BB	FF	FF	AB	BB
F1 from cow Beta														
Beta	AD1	A	103	AA	A	BB	B	BC	BB	BB	FF	FF	AB	-
Beta	AD1	?	49	AD1	?	AB	A	BC	BB	BB	FF	FS	AB	-
F2 from cow Beta														
49	AD1	?	92	AD1	?	AB	?	CC	BB	BB	FS	SS	-	AA
49	AD1	?	113	**Amut**	?	AA	A	CC	BB	BB	FS	SS	-	-
49	AD1	D1	144	D1D1	D1	AA	A	BC	BB	BB	FS	SS	-	-
49	AD1	D1	158	D1D1	D1	AA	A	BC	BB	AB	FS	FS	-	-
F3 from cow Beta														
113	**Amut**	**mut**	155	**Amut**	A	AB	B	CC	BB	AB	SS	SS	-	-
113	**Amut**	A	168	AD1	D1	AB	B	CC	BB	BB	SS	FS	-	-

The notes:

"?" - cases, in which it is impossible to establish, what allele is received from mother, and with what - from the f a t h e r ; - the data are absent, **«mut» -** mutation in transferrin's locus

Despite close inbreeding in the exposed herd (one bull serving several generations), the heterozygosity at the loci analyzed in generation F2 was close to the mean heterozygosity in FI. In the case of *PTF-2* and *CSN3* loci the mean heterozygosity in F2 was even higher than that in FP. Thus, over the two progeny generations examined the principal effect of inbreeding (increase in the number of homozygotes) was not observed. This phenomenon may possibly be explained by the involvement of mechanisms preserving a stable heterozygosity level, expressed as a disrupted allelic transfer from the parents to the offspring.

Table 3

The transferring of allelic variants of TF, CP, AM-I, GC, PTF2, CSN3 loci from cows-mothers, introduced in experimental economy «New-Shepelichi» of alienation zone of Chernobyl's accident in 1990-1993 years to offspring of 1 and 2. generation (F1, F2), which were birthing in condition of increased ionizing pollution

Mother's name	Mother's genotype TF	Allele Tf from mother	№ offspring	TF genotype of offspring	Allele Tf from bull Uran	CP genotype of offspring	Cp allele from father	AM-I genotype of offspring	GC genotype mother-offspring	PTF2 genotype mother-offspring	CSN3 genotype mother-offspring
Bull Uran					AD1		AB	CC	AB	FS	AB
without name	-	D2	4768	AD2F1	A	AB	?	CC	?? AB	?? FS	- -
without name	-	D2	4776	D1D2F1	D1	AB	?	CC	?B BB	?S SS	- -
without name	-	D2	4789	AD2F2	A	AB	?	CC	?? AB	?F FF	- -
6843	AD2	A	91	AA F1	A	AA	A	CC	AB AB	FF FF	BB BB
6843	AD2	D2	160	D1D2 F1	D1	AB	?	CC	AB AB	FF FS	BB -
6841	D1D2	D2	40	AD2 F1	A	BB	B	CC	BB AB	FF FS	- AB
6827	D1D2	D2	42	AD2 F1	A	AB	B	CC	AB AB	SS FS	BB AB
6827	D1D2	D1	88	AD1 F1	A	BB	B	CC	AB AA	SS FF	BB -
6827	D1D2	D1	104	AD1 F1	A	AB	B	CC	AB AB	SS FS	BB AB
6827	D1D2	D1	166	D1D1 F1	D1	BB	B	BC	AB BB	SS SS	BB -
6824	D1D2	D1	162	D1D1 F1	D1	BB	B	BC	BB AB	FF FS	AA -
6824	D1D2	D2	149	D1D2 F1	D1	AB	?	BC	BB BB	FF FS	AA -
6824	D1D2	D1	79	AD1 F1	A	AB	B	BC	BB BB	FF FS	AA AA
6824	D1D2	D1	7Golk	AD1 F1	A	BB	B	BC	BB BB	FF FS	AA AA
6803	AD2	D2	102	D1D2 F1	D1	BB	B	CC	AB BB	FS FS	- AA
6803	AD2	D2	150	D1D2 F1	D1	AB	?	CC	AB AB	FS SS	- -
4789	D1D2	D2	86	AD2 F1	A	AA	A	CC	AA AB	FF FS	- AA
4789	D1D2	D1	94	AD1 F1	A	BB	B	CC	AB AA	FF FS	- AB
4789	D1D2	D1	169	AD1 F1	D1	BB	B	CC	AB AA	FF FS	- -
4776	D1D2	D2	87	D1D2 F1	D1	BB	B	CC	BB AA	FS FF	- -
4776	D1D2	D2	101	AD2 F1	A	AB	?	CC	BB BB	FS SS	- AA
4776	D1D2	D1	147	D1D1 F1	D1	AA	A	CC	BB BB	FS SS	- -
4768	AD2	D2	84	AD2 F1	A	BB	B	CC	AB BB	FS FS	AB -
4768	AD2	A	97	AA F1	A	AB	B	CC	AB AA	FS FS	AB AB
118	?D2	D2	151	AD2 F2	A	BB	B	BC	?? AB	?S SS	- -
118	?D2	D2	157	D1D2 F2	D1	BB	B	BC	?B BB	?S SS	- -
42	AD2	D2	136	D1D2 F2	D1	AA	A	CC	AB AA	FS SS	AB -
42	AD2	D2	154	D1D2 F2	D1	AA	A	CC	AB AA	FS SS	AB -
7 Golka	AD1	?	122	AD1 F2	?	AB	A	BC	BB AB	FS FS	AA -
7 Golka	AD1	D1	161	D1D1 F2	D1	AB	A	BC	BB BB	FS SS	AA -

The notes: "?" - cases, in which it is impossible to establish, what allele is received from mother, and with what - from the f a t h e r ; - the data are absent.

Table 4

The frequency of allele transfer in transferrin (TF) and ceruloplasmin (CP) loci from cows *Alpha, Gamma* and bull *Uran* to offspring in first (Fi) and second (F_2) generation

Allele		F1		F2	
sex	TF	offspring /parent	frequency of transfer	offspring /parent	frequency of transfer
Uran	A	8/8	1.00	3/11	0.27
cows	D2	8/8	1.00	4/11	0.36
sex	CP	offspring/parent	frequency of transfer	offspring /parent	frequency of transfer
Uran	B	6/8	0.75	5/8	0.62
cows	B	0/0	0.00	5/8	0.62

Thus, under changed environmental conditions the disrupted transfer of certain alleles from the parents to the offspring leads to a significant differences in allele frequencies from those expected in FI and to a stabilization of these differences in F2.The comparison of genetic structure of the same molecular-genetic markers between Holstein and Grey Ukrainian cattle breeds from "pure" zones, parent's generation of experimental herd and their children (bom under influences of ionizing irradiation) demonstrated the shift of the offspring's genetic structure in some loci from typical ones for parents (belonging to Holstein breed) to more primitive, ancestor breed, Grey Ukrainian cattle.

Early we revealed the increase of frequencies of cytogenetic anomalies which did not lead to cell death (such as inversions, inserts, reciprocal translocations) in blood cells of children (14 - 16 years age) who received the doze of ionizing radiation (0,3-0,4 Sv) in utero [10]. Obviously, it related with the clonal cell expansion after sharp ionizing irradiation. Our data about the cattle fertility decrease in FI, allowed to suppose, that children exposed to low dozes of ionizing radiation in utero, could face reproductive problems in the future. The analysis of polymorphism and heritability of some molecular genetic markers of anonymous sequences in DNA (ISSR-PCR) was carried out using the method described by Zi^tkicwicz *et al.* (1994) [15]. The following sequences were used as primers: dinucleotide repeats -(CA)IOG, (CG)9G and trinucleotide repeats - GT(CAC)7, (CAC)7T, (AGC)6C and (AGC)6G.

The amplification spectra were analyzed for two families of the exposed group -from cows *Alfa* and *Beta* mated, as always, to *Uran.* In the case of dinucleotide repeat (CA)IOG, in the amplification spectra of all investigated animals, eight distinct DNA fragments were observed -from 750 to 1900 bp. These fragments were identified in all animals -both in the parents and in the offspring, and neither individual variation nor the occurrence of new variants was observed in the progeny. The same pattern was observed when (CG)9G primer was used -the amplicon spectra had six precisely identified fragments 650-1500 bp long. Neither individual variation nor differences of amplicon spectra from parental variants were found when using the trinucleotide repeat (CAC)7T as a primer, and ten fragments were recorded in its amplicon spectrum -400-1600 bp long. Thus, considering each fragment of amplified DNA as a separate locus, it was possible to conclude that in all 24 DNA *loci* and in all animals analyzed no individual variation appeared and no new mutation variants occurred in the progeny bom under increased ionizing radiation.

The use of three other primers, consisting of trinucleotide microsatellite repeats - GT(CAC)7, (AGC)6C and (AGC)6G -resulted in the formation of polymorphous spectra of amplification products. Using the GT(CAC)7 primer produced ten fragments 650-2000 bp long, in the spectra of amplification products. The 2000 bp fragment was absent in cow *Alfa* and in her FI offspring (Nos. 120 and 105). The progeny of cow *Beta* (No.l 13 and 155, respectively) were also lacking this fragment. Fragments 1800 and 1700 bp long were absent in F2 and F3 (No.l 13 and 155, respectively) of cows *Alfa* and *Beta.* The 1500 bp fragment was absent in cows Nos. 49, 113, 144 and 155 *(Beta's* progeny), while fragment 1400 bp -in No.105 *(Alfa's* progeny). Thus, among ten fragments (loci) of GT(CAC)7 primer, five appeared polymorphous as they were present or absent from the amplicon spectra. Basing on the data obtained one may conclude that *Uran* was heterozygous with respect to the 2000 bp fragment, *Alfa* was homozygous as regards the absence of the fragment, while *Beta* homozygous as regards its presence. The distribution of this fragment in the progeny was close to that expected.

The use of the (AGC)6G primer resulted in a wide spectmm of amplification products, consisting in total of 27 fragments (600-2600 bp) from different animals. Again no amplification products which would point to the occurrence of mutations were found. Altogether, with the existing polymorphous spectra in different animals the use of these two trinucleotide primers resulted in 49 amplification products.

The most complex spectrum of amplification products was observed with (AGC)6C primer. Two amplification products were revealed in *Alfa's* daughter No. 105 that were absent in both parents. A poor reproducibility of the amplification spectra in this case was also marked. One may assume that such complexity of the amplification spectra and poor reproducibility were caused by the primer itself, in particular by the presence on its 3' end of the nucleotide combination GCC, which promotes formation of a "mini-pin". It could result in a poor annealing accuracy. For this reason it proved necessary to make a special family investigation establishing whether the new bands in animal No. 105 were a result of mutation events or artefacts of an inaccurate reannealing of the primers.

In total, using two dinucleotide and three trinucleotide primers, 73 amplification products were found in the progeny of cows *Alfa* and *Beta* mated to the bull *Uran.* No changes were observed which could be interpreted as a new mutation. Two unique bands found in one offspring of *Alfa* with the (AGC)6C primer could be the effect of the reduced accuracy of annealing, and thus requires further research. Interesting is the high heterozygosity of *Uran,* shown by the analysis of polymorphous spectra of anonymous DNA fragments and by the polymorphism of structural genes. It is possible that the prolonged and successful fertility of the bull under both increased ionizing radiation and inbreeding was caused by his high heterozygosity. It is important to note that observed shift of a genetic structure in cattle generations in the direction of the less specialized forms was in agreement with the literature data about a decrease of a number of behavioral specialized functions in voles (more primitive relatives of burrows) in conditions of increased radio nuclide contamination [8], and also with the data of the Danish investigators about disturbance of functions of associative thinking in Danish children after the first air explosions of nuclear bombs and after Chernobyl accident [11].

All these appearances corresponded to a rule of I.I. Shmalgauzen (1983) [12] that any change of the environment lead to preferable reproduction of the more primitive forms within a species. Thus, the main problem after the Chernobyl's catastrophe, as well as other ecological changes, lies not in the occurrence of the new mutant organisms, but in the long-term changes of the genetic structure of populations and, accordingly, in the appearance of the new interspecies interactions between the less specialized (marginal) representatives of each species in species communities.

Conclusions

1. Problem of Chernobyl's catastrophe is that the populations of different organisms were subjected to dozes of ionizing radiation which were new to them.

2. Increased level of ionizing radiation did not induce qualitatively new damages of the genetic material, but increased chromosomal instability in those species or at those cytogenetic anomalies that have been determined to be *apriori* more prone to appearance of cytogenetic defects than others. This provides a basis for the hypothesis that evolutionary younger species are more sensitive to the change of ecological conditions at the chromosome rearrangement level in comparison with older ones.

3. Effects of detrimental environmental changes have deferred realization in generations. Decrease of the reproduction function in cattle was observed in cows, which were bom in first generation in a zone of alienation of Chernobyl's NPP, possibly, in connection with the particularities of mammalian oogenesis (maturation of ooblasts to meiotic stage before birth). Strong selection for radio nuclide resistance in voles emerged through approximately 26 generations after the beginning of the ionizing radiation exposure and it was dose-dependent.

4. No increase in the quantity of constitutive mutations in investigated genes, ISSR-PCR markers or chromosomes in analyzed species (cattle and *Rodentia* species) was observed.

5. In generations of cattle, disturbance of equiprobable transmission of alleles of a number of molecular genetic markers, increase of heterozygosity and radio resistance were observed.

6. In family analysis the changes of genetic structure in cattle generations of experimental farm "Novoshepelichi", the shift of gene pool from typical for specialized parent dairy breed Holstein to that characteristic for the less specialized breeds was revealed (decrease in level of specialization)

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