REPORT on the

Project RADIOGENE: "Experimental justification of radiation genetic risk estimation according to the frequency of heritable DNA changes in human and animal structural genes".

The goal of the Project is to obtain a new experimental data on the molecular regularities and mechanisms of radiation mutagenesis in animal germ cells. This data could be a base for a new gene-molecular approach to an estimation of genetic risk of the ionizing radiation. To achieve this goal it was necessary to resolve the following tasks:

1. To study the spectrum and frequency of the inherited DNA changes induced by γ- rays and neutrons at five structural genes (Fig.1) using PCR technique and to assess the dose-dependence effects for different DNA changes detected;

2. To study the quality and frequency patterns of DNA micro-changes using the sequence approach;

3. To determine the frequency of the inherited DNA base substitution induction on the basis per 1Gy per 1 nucleotide;

4. Taking into account the frequency of DNA base substitution per 1Gy per 1 nucleotide and the literature data on the spontaneous frequency of such changes to determine the doubling dose as the basic criteria of the genetic risk of ionizing radiation for animal and human populations.

The genetic and cytogenetic analysis of γ-ray- and neutron-induced mutations at five genes under study (Fig. 1) reviled a complex spectrum of gene mutations among which two main classes of genetic changes were regularly observed, namely, the gene mutations with a gross genome changes and gene/point mutations with intragenic alterations which are inherited by the Mendelian type (Fig 2).

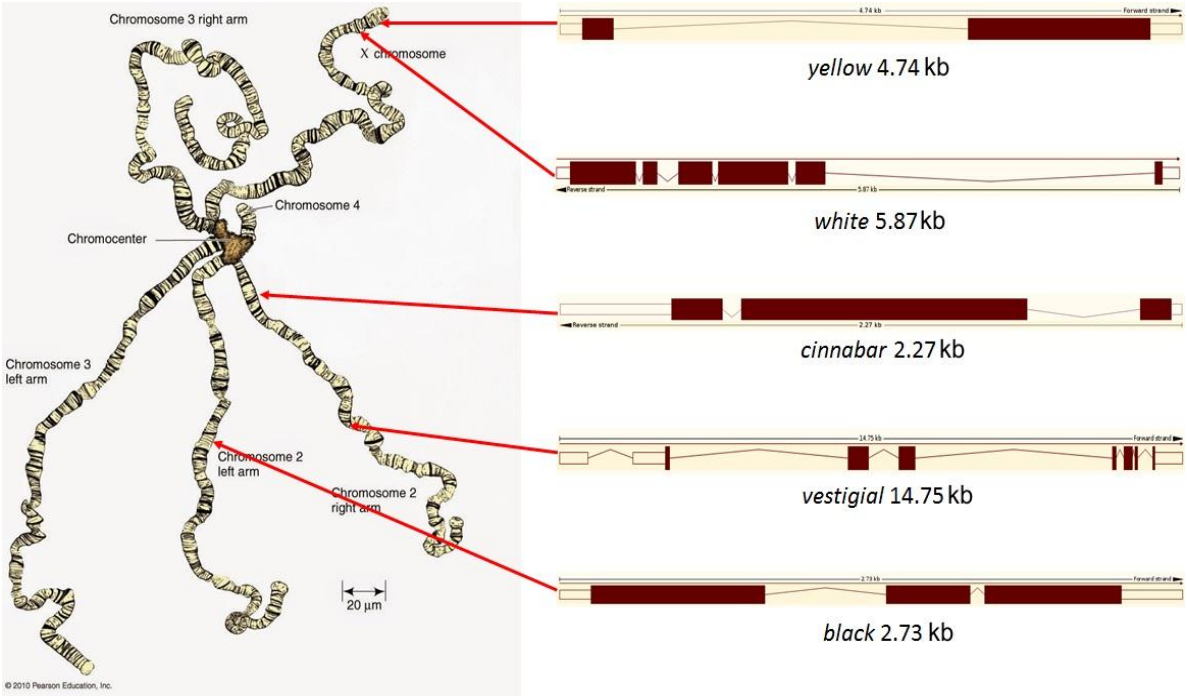

Fig. 1. The size, molecular organization and location on the polytene chromosomes of the five *Drosophila melanogaster* genes radiation-induced mutations of which were studied.

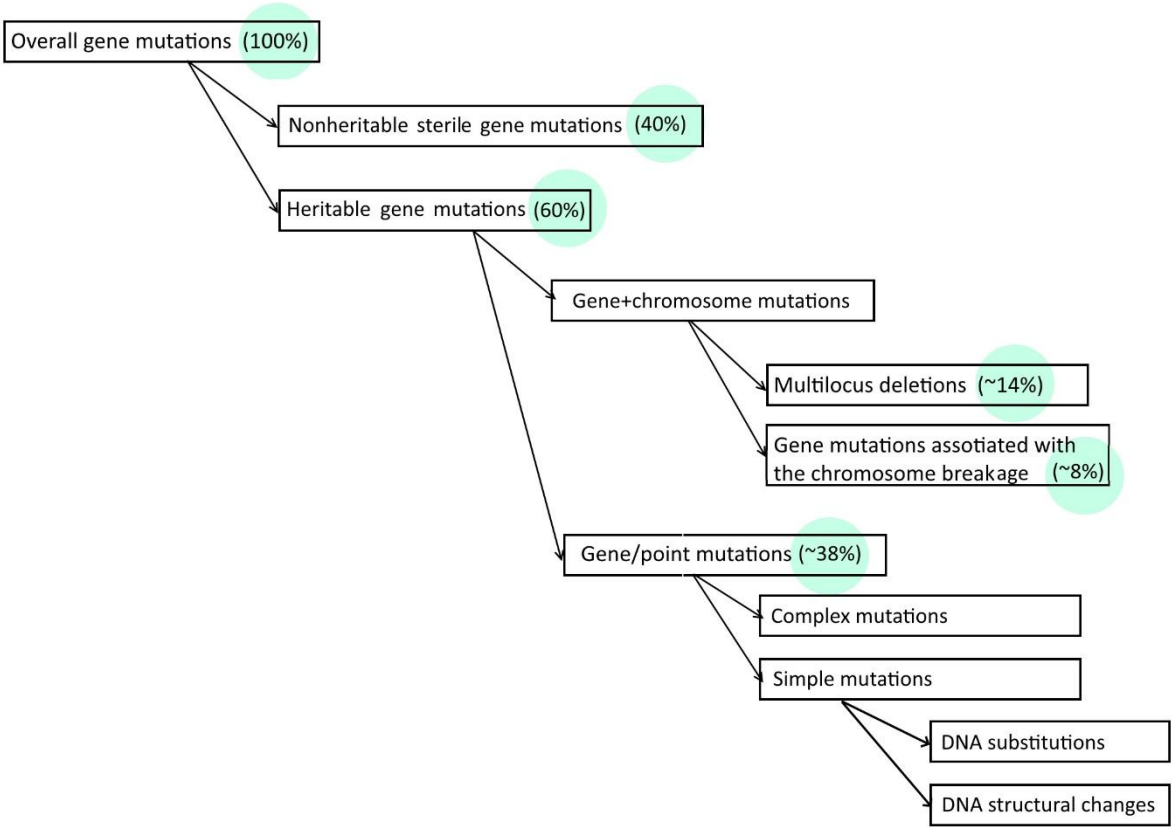

Fig. 2. Spectrum of γ-ray- or neutron-induced locus-specific mutations in the sperm cells of *Drosophila melanogaster* recovered by the genetic, cytogenetic and PCR-analysis.

It is important to note that these gene/point mutations underlay almost one-half of human genetic diseases. In this regard, the study of the molecular nature of spontaneous and radiation-induced "point" mutations has become the main focus of experimental researches in this project.

As the first step, the inherited DNA changes of five genes were studied using PCR technique. According to the results obtained, based on 2500 PCR reactions for all five genes, the overall picture of the recorded changes closely matches and includes two main categories of mutations. The first of them is based on DNA micro-changes that are not detected by this method (PCR^+-mutants), and the second is based on changes as deletions of various sizes and location on the gene map (PCR^--mutants) (Fig 3).

№	Mutations	Origin	The *vestigial* gene fragment studied															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
44	vg88b59	n, 2.5 Gy	+*	+		+	+	+	+	+	+	+	+	+	+		+	+
45	vg88c3	»	**	+	+	+	+	+		+	+	+	+	+	+	+	+	+
46	vg88c64	»	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
47	vg88c87b	»	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
48	vg88c96	»	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
49	vg88b10	n, 5 Gy	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
50	vg88b32	»		+		+	+	+		+	+	+	+	+	+	+	+	+
51	vg88c30	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
52	vg79d6	n, 10 Gy	+	+	+	+	+	+			+	+	+	+	+	+	+	+
53	vg88c45	»	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
54	vg88c62	»	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
55	vg88c102	»	+	+	+	+	+	+						+	+			
56	vg80l2	»	+	+	+	+	+	+				+	+	+	+	+	+	+
57	vg83l1	»	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
58	vg83d	cf, 7 Gy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
59	vg82c13	cf 14 Gy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60	vg88f21	n + γ, 15 Gy	+	+	+	+	+	+		+	+							
61	vg88g40	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
62	vg79d5	n + γ, 20 Gy	+	+	+	+	+	+			+	+	+	+	+	+	+	+
63	vg88f66	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64	vg88g108	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
65	vg88h11	»	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
66	vg88f33	n + γ, 30 Gy	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
67	vg88g38	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
68	vg88e94	»	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 3. As example, the size and location of intragenic deletions induced by different doses of neutrons at the *vestigial* gene of *Drosophila melanogaster* recovered by the PCR technique. *-a normal fragment, **- lack of fragment

Analysis of the ratio of the two mutant categories for individual genes showed that in all cases PCR^+-mutants occur more often than PCR^-. This picture is preserved at all studied doses of γ-radiation ^{60}Co (Fig. 4).

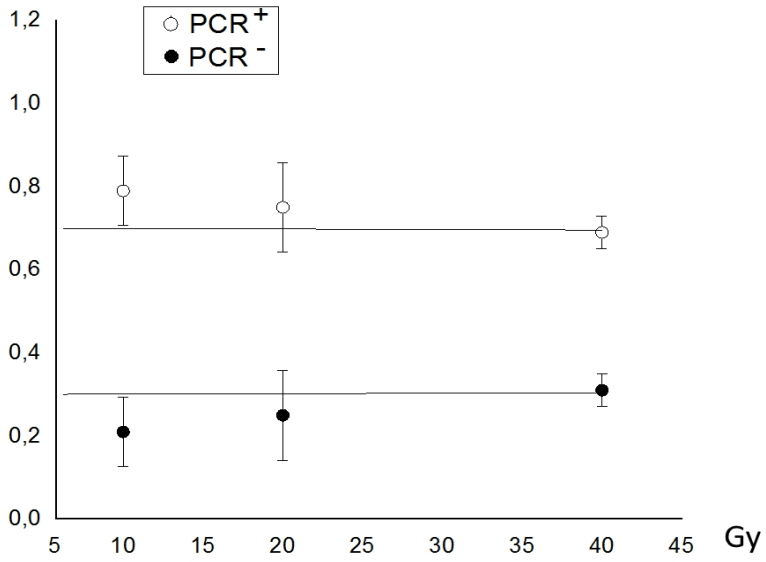

Fig. 4. The dose-effect dependence of PCR^+ and PCR^- mutants for five *Drosophila melanogaster* gene studied after action of ^{60}Co γ- irradiation.

However, the picture of the relative frequency of neutron-induced PCR^+ -and PCR^- - mutants is quite different from that for γ- irradiated. (Fig. 5). In particular, the share of PCR^+-mutants increases, whereas the share of PCR^-- mutants decreases with dose increasing. Moreover, neutrons are twice as effective in the induction of PCR^+ - mutants as compared with PCR^- - mutants in all doses in sum.

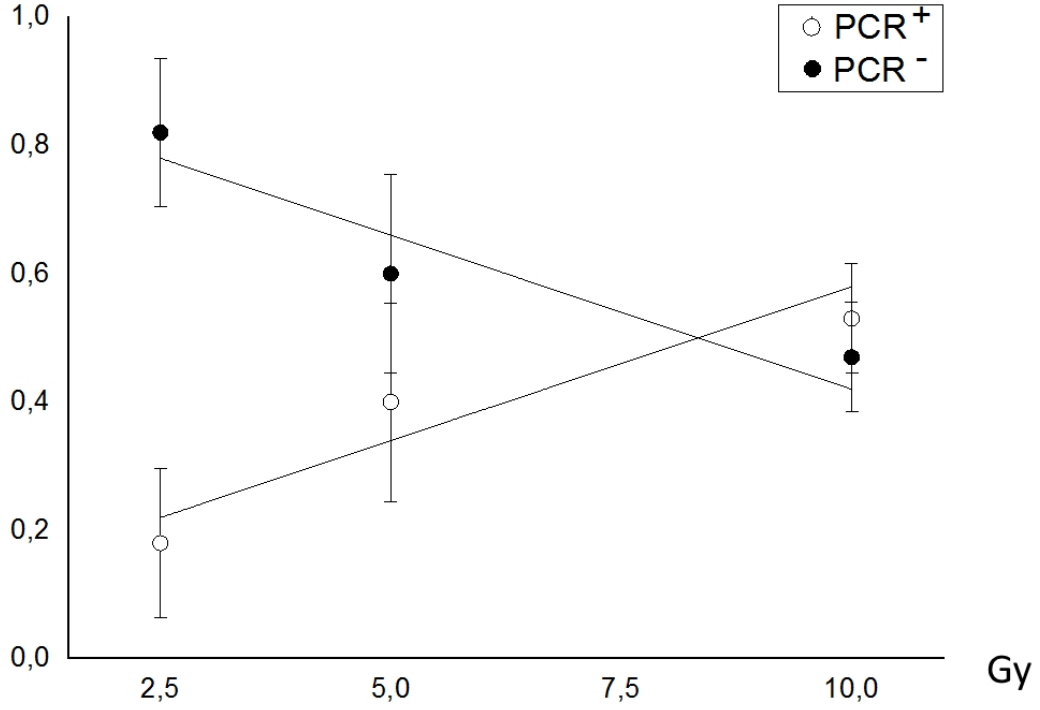

Fig. 5. The dose-effect dependence of PCR^+ and PCR^- mutants for five *Drosophila melanogaster* genes studied after action of fission neutrons.

This shows that PCR^- mutants can be based on structural changes in the gene, in the induction of which neutrons are more effective than γ-irradiation. In order to ascertain their nature independent research is required.

In the context of this project, the molecular nature of PCR^+-mutants was studied in detail by the sequencing technique.

Analysis of the results of the completed work on sequencing of spontaneous and induced PCR^+-mutants at the *yellow X*-linked gene (30 mutants were studied) and the autosomal *black* gene (34 mutants were studied) showed that these mutations can be based on various DNA changes, among which for two genes are common DNA base substitutions, microdeletions (1-53 bp), microinsertions (1-15 bp), deletions + insertions and large insertions (> 5 kbp) (Fig. 6,7). Substitutions of 2-3 adjacent DNA bases (6 cases among 45 detected changes or 13.3%), as well as gene conversion (6 cases out of 45 or 13.3%) were specific for the *black* gene (Fig. 7).

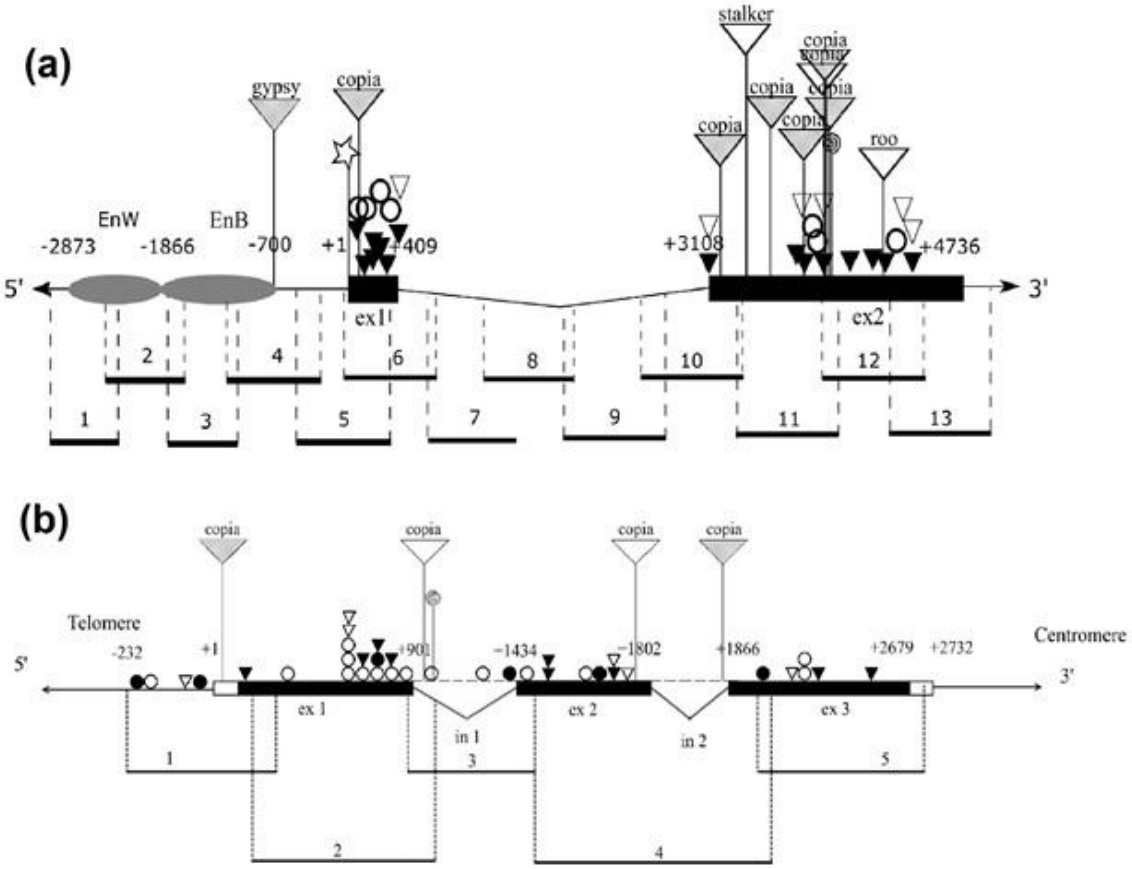

Fig. 6.Distribution of γ-ray-induced DNA changes on the linear map of the *yellow* (a) and *black* (b) genes of *Drosophila melanogaster*. ○ - base substitution, ● - substitution of > 2 bases, ▼ -micro-deletion, ▽ - micro-insertion, ▽, ◎ - spontaneous insertion of a large retrotransposons or other genomic DNA, copia - the same insertions in radiation-induced mutants.

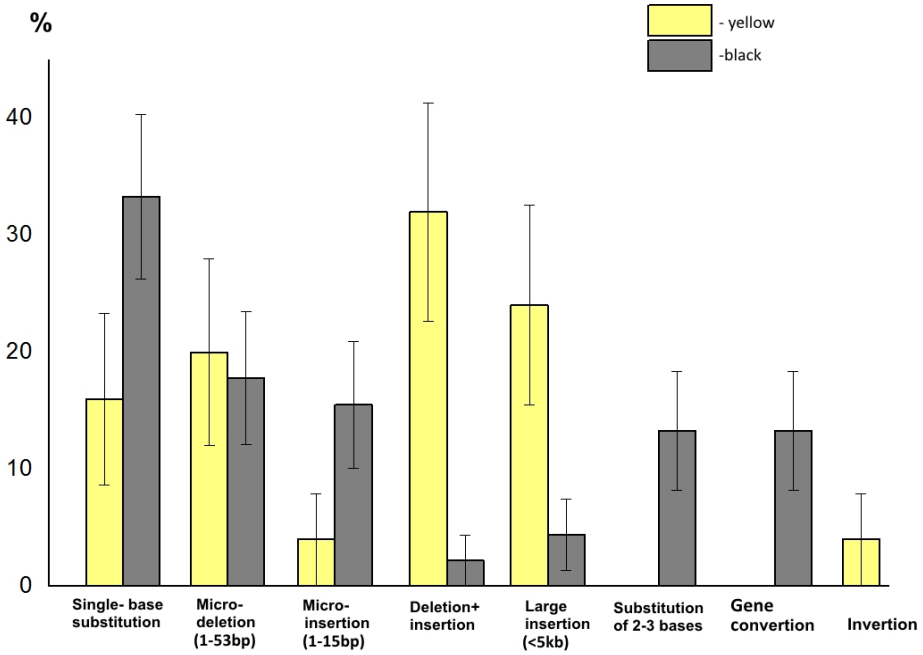

Fig.7. Spectra of γ-ray-induced DNA changes at the *yellow* and *black* genes of *Drosophila melanogaster*

The frequency of gene conversion among the neutron-induced PCR$^+$-mutations of the *black* gene was unexpectedly high (18 cases among the 24 DNA changes or 75.0%). The remaining neutron-induced PCR$^+$-mutants, as in the case of γ-radiation, were based on large insertion of retrotransposon (1 case), microdeletions (1 case), deletion + insertion (1 case), base substitution (2 cases) and a large deletion (1 case) (Fig. 8). Gene conversion detection, (i.e. replacement of irradiated paternal *black*$^{+32}$ gene by the intact maternal *black*^{1} gene) became possible after sequencing of the *black*^{1} gene, which is differed from the *black*$^{+32}$ gene by 26 polymorphic sites throughout the gene. A complete, and in some cases partial, replacement of one gene by another shows that homologous recombination, is the repair mechanisms in early Drosophila zygote after junction of the maternal and paternal haploid genomes. The fact that after the action of neutrons the frequency of gene conversion is almost 6 times higher than after γ-radiation (Fig. 8) shows that the neutron induced primary DNA damage (local clusters of single and double-stranded breaks) in the genome of irradiated sperm are repaired, mainly by the mechanism of gene conversion.

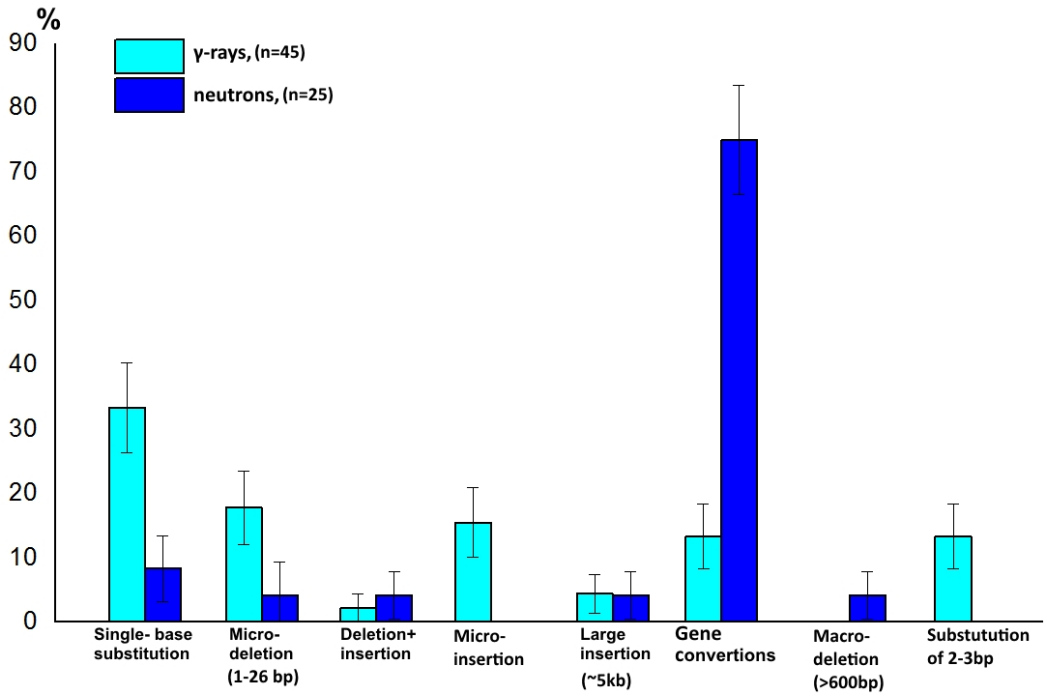

Fig.8. The relative frequency of different DNA changes induced by γ-rays and neutrons at the black gene of in *Drosophila melanogaster*

Analysis of the distribution of γ-induced DNA micro changes of different types identified by sequencing (Fig. 6) on the *yellow* and *black* gene maps suggested their non-random position with a tendency to clustering in certain regions of the gene. Testing the 0-hypothesis of the random distribution of such changes using the χ^2 criterion showed that it is valid for the *yellow* gene, but not for the *black* gene, for which clustering of DNA micro changes in the 3'-end of exon 1 gene is reliably observed ($p < 0.001$) (Fig. 9).

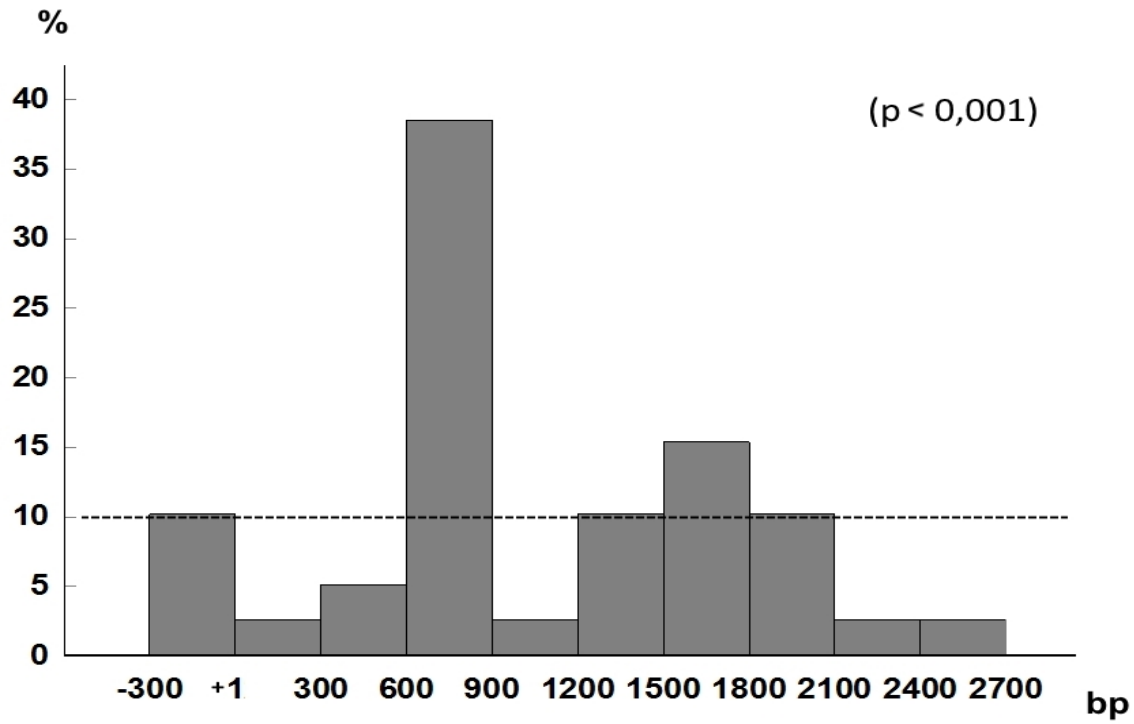

Fig. 9. Distribution of γ-ray-induced DNA micro-changes on the *b* gene map underlying the point mutations in *Drosophila melanogaster*

The results of sequencing of γ-ray-induced changes obtained for the *black* gene at 40Gy, as the most complete, allow determin the frequency of base substitutions on the bases per 1 Gy per 1 nucleotide, which turned out to be $2.1x10^{-9}$/Gy/nucleotide. Taking into account the spontaneous frequency of such substitutions in the *Drosophila melanogaster* genome ($3.5x10^{-9}$/nucleotide/generation, P.D. Keightley et all, 2017) and radiation-induced frequency of such changes named above, it is possible to obtain the first estimate of the doubling dose of sparsely ionizing radiation at the molecular level, which turns out to be $3.5x10^{-9}$ / $2.1x10^{-9}$/Gy= 1.7 Gy. This purely preliminary estimate of the doubling dose as a criterion for the genetic risk of ionizing radiation will be refined after completing the work on the sequencing of three other studied genes in the current year. These works are currently being successfully continued.

According to the results of the research conducted in the framework of the current project, 4 articles were published in the profile peer-reviewed journal "Radiation Biology. Radioecology", as well as oral presentations at several conferences and workshops.

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