The molecular genetics of radiation-induced changes at the gene, genome and transcriptome level in Drosophila melanogaster
PROJECT RADIOGENE

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Contents

Annotation

As a continuation of the previous project “Radiogene”, the main goals of the planned project are: 1) study of the molecular nature of structural changes of the gene without and in association with the aberration rupture during inversions and translocations in the genome of sperm cells of male Drosophila melanogaster induced by γ-radiation and neutrons using a set of methods, including In situ hybridization and filtering Southern, polymerase chain reaction (PCR) and sequencing and carrying out general genomic analysis of radiation-induced DNA changes in the offspring of the first generation from irradiated males after the action of γ-radiation in different doses, using the shotgun sequencing method with a comparison of the results obtained with similar literature data in mice; 2) To study the transcript (a set of all RNA) in the control Drosophila lines contrasting in radiosensitivity using the device Afimetrix equipment

For the first time, the results of the Project will solve open questions of structural mutagenesis of the gene, evaluate the nature and degree of infestation of the inherited genome at the molecular level at different doses of γ-radiation, which will allow to evaluate the effectiveness of this type of radiation in inducing similar DNA micro changes underlying human inherited genetic diseases (data from HGMD, www.hgmd.org), and also allows for the first time to identify a cluster of genes that control the radiosensitivity of the genome in Drosophila melanogaster. These new fundamental data for animal organisms are planned to be published in the domestic and foreign press.

During the previous phase of the project (2017-2019), 3 articles were published, several reports were made at conferences and meetings.

The project budget is 144 thousand US dollars for 2020-2022.

Introdaction

The ability to predict the genetic consequences of radiation on the progeny is one of the most important problem that humanity has been facing for over 50 years. The impossibility of its solution directly on a man predetermined the importance and necessity of radiation-genetic studies in this direction on model objects, among which the main ones are Drosophila melanogaster and Mus musculis. Understanding that the gene is the basis of life, the efforts of geneticists have been directed at studying the nature and frequency of inherited gene mutations in irradiated germline cells of these test organisms. In the first period of the 90-year history of radiation genetics, genetics and cytogenetics of inherited gene mutations were obtained after action of sparsely-ionizing radiation. Modern progress in DNA technology opens up a new page in the radiation genetics of these test objects and broad prospects for studying mutational changes at both gene and genome levels. At the gene level our investigations were aimed to study the molecular nature of intragenic DNA alterations at inherited gene/point mutations of five genes. However, as shown by the results of genetic and cytogenetic studies that we have carried out, “point” mutations are only part of the spectrum of radiation induced gene mutations. Another part of this spectrum is represented by structural intragenic and chromosomal alterations the molecular nature of which is unknown so far. Taking this into account, the first task of the planned researches, as a continuation of the Radiogene project, is to study the molecular nature of such structural mutations using *In situ* FISH hybridization, PCR and sequencing techniques. Simultaneously, as the second task, genome-wide consequences for offspring of γ-irradiated males of D. melanogaster is planned to study using the shotgun sequencing method with subsequent comparison of these results with mouse date. Although the size of the targets (gene and genome) are different, subject of study (DNA changes), the methodologies and methods are common to them. It lets to formulate a common goal of the project for these targets, namely, molecular analysis of radiation-induced changes in the structure of the gene and genome. The results of this part of the Project allow to solve opened questions of the nature and frequency of radiation-induced inherited DNA mutations at the gene and genome level. This new date will make it possible to evaluate the effectiveness of radiation under study in the induction of similar micro changes in DNA underlying human inherited genetic diseases (data from HGMD, www.hgmd.org). The second goal of the Project is to study the genetic bases of different radiosensitivity in two high-inbred Drosophila melanogaster laboratory lines using RNA (transcriptome) analysis by the Afimetrix. The results of this part of the Project will allow for the first time to identify a gene family that controls the radiosensitivity of the Drosophila genome.

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The state of research on the problems studied in the project

Among the inherited genetic effects induced by ionizing radiation in the germline cells of higher organisms, the induced recessive gene mutations of the Mendelian type, along with the spontaneous, form the genetic burden that modern human and animal populations carry.Among the inherited radiation-induced genetic effects in higher eukaryotes, a Mendelian-type point mutations along with spontaneous ones form the most important and dangerous genetic load in current animal population.Evidence from the current molecular genetics of human genetic diseases (OMIM 2017, www.omim.org; HGMD 2018, www.hgmd.org), the classical radiation genetics of Drosophila melanogaster [1,2], the silkworm Bombyx mori [3], Neurospora crassa [4] and Mus musculis [5], as well as our long-term studies on the genetics and cytogenetics of radiation-induced inherited recessive mutations of Drosophila melanogaster five genes (Fig. 1) [6-9] allow us to make a conceptual conclusion that the diversity of recovered gene changes (Fig. 2) can be combined into two main classes: 1) “Point” mutations with introgenic changes of different types; 2) structural mutations such as multilocus deletions, inversion, translocation or transposition with one of the aberration breaks in the region of the gene under study. According to our data, the share of second-class radiation mutations in the total spectrum of observed changes varies from 20 to 80%, depending on the size and position of the gene and radiation (Fig. 3). Moreover, as our results of PCR analysis of “point” mutations of the studied genes have shown, structural changes in the form of deletions and other molecular rearrangements of different size are regularly observed in the heterogeneous picture of their DNA damage [6–9]. At the same time, the localization of the ends of such rearrangements often coincides for mutants induced by different doses of radiation (Fig. 4), forming “hot” points of radiation mutagenesis with their non-random position on the gene map [10]. Given the important role of “hot” DNA points with a specific context in the determination of human genetic diseases [11,12] and evolution [13], the molecular analysis of the nature of “hot” points at the DNA level in radiation-induced mutations of the studied Drosophila genes acquires not only a large basic significant, but also important predictive value. The basis of the radiation-induced structural changes of a gene in the form of deletions of different size that we observe using standard PCR can be based on different genetic changes. As our results of PCR analysis in the previous project have showed, the lack of one or more gene fragments may be due to such genetic changes as insertions or excision of a large mobile elements (Fig. 5), losses 5-7 or more b.p. in the region of primer annealing or molecular inversion/translocation. Clarification of this issue by PCR and sequencing is the first task of this part of the project. Radiation-induced mutations of a gene associated with an aberration break may also be based on several different genetic changes. In the first case, the aberration break is localized outside the gene, with the result that the gene is in a new genetic environment that suppresses its expression, which leads to a mutant phenotype. At the same time, the gene itself has not mutation lesion. In this case, we will deal with the classical “position effect”. In another case, the aberration break may be located within the gene and its localization on the gene map requires independent research using *in situ* FISH hybridization. Finally, in the third case, there are two independent hits, namely the 1st is an aberration break outside the gene and the 2nd is mutation damage within the gene leading to mutant phenotype. In this case, the aberration break and gene mutation are two mutational events of different physical nature as a result of the passage of a single ionizing particle, which is not so unlikely in the light of modern ideas about the structure of a track in a biological condensed medium [15]. The hypothesis of two or more independent mutational events at the genome level after passing of a single particle through the genome was earlier predicted and partially experimentally substantiated in our investigations [16]. Further experimental study of the three types of genetic

changes listed above is the second task of this part of the project, using *In situ* FISH hybridization, PCR and sequencing.

The progress of DNA technologies opens up a broad perspectives in studying the DNA changes a the wide-genome level in animal germline cells after action harmful environmental factors, including ionizing radiation. Such studies are just beginning, and the first results obtained in mice open a completely new look at the genetic consequences for the offspring of irradiated parents [17]. The spectrum of DNA genomic changes revealed in the offspring includes along with the known a new type of mutations as a clusters of DNA micro-changes. These first results suggest a much greater genetic risk of ionizing radiation for the offspring of irradiated parents than was previously thought. Since the results of genetic studies on fruit flies and mice have always been the basis for extrapolation to humans, in our Radiogene project, it is planned to carry out a genome-wide analysis of inherited DNA changes in offspring of males irradiated by different doses of γ-rays.

Research on the problem of genetic control of radiosensitivity in higher eukaryotes has so far been limited to identifying and characterizing the action of individual genes controlling repair and other important genetic processes [18]. Modern approaches to the gene expression study at the RNA level and technical progress open up the perspective of identifying of a gene set that control radiosensitivity. In this respect, the planned priority research studies of the transcriptome in Drosophila melanogaster lines with different radiosensitivity (Fig 6) using the Afimerrix are of great scientific and prognostic value.

Thus, in the planned priority studies it is proposed to study both fundamental aspects (structural and functional) of the problem of radiosensitivity of animal germline cells at the gene and genomic levels.

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Description of the planned research and their methods

Analysis of structural changes in the gene

To achieve the first goal of the project in terms of studying the molecular nature of intragenic rearrangements for “point” mutants and the characteristics of the target DNA for them, the following tasks are planned: 1) Based on the character data previously obtained for each of the five studied genes (Fig. 1) gene damage detected by PCR, to select mutants with changes in the form of loss of one, a number of adjacent or several different fragments on the gene map; 2) To carry out crossbreeding to obtain homo-or hemizygotes for each of the studied genes to obtain genomic DNA and subsequent molecular analysis; 3) Work on the isolation of high molecular weight DNA of each mutant under study and the creation of a genomic DNA bank of the *D. melanogaster* mutants under study; 4) To carry out the first stage of molecular analysis (PCR) to determine the annealing of direct and reverse primers for the missing gene fragments in the mutants under study; 5) In the presence of primer annealing at the next stage of molecular analysis, Long-PCR is planned to detect the insertion of the mobile element, which determines the absence of this fragment during standard PCR; 6) For mutants without insertions of large mobile or other genomic DNA, conduct in situ fish hybridization on polythene chromosomes using labeled DNA probes from gene regions flanking the missing fragment to detect molecular inversions or translocations of the gene. It is planned to study at least 50 mutants of five studied genes with the above-mentioned intragenic structural changes of different types.

Analysis of gene mutations associated with aberration break

To study the genetic nature of gene mutations associated with an inversion or translocation break, the following stages of work are planned: 1) From a general database of radiation-induced mutations of five studied genes, select a sample of them for which a gene mutation is associated with structural changes in the genome; 2) By crossbreeding with a known large deletion of the gene under study, obtain the corresponding hemizygotes as the initial genetic material for subsequent molecular studies; 3) To carry out work on the isolation of high molecular DNA and create a base of genomic DNA of the mutants under study; 4) Based on the data obtained earlier by the PCR method, to classify mutants according to the localization and size of the fragments missing on the gene map; 5) For mutants with the absence of one fragment, carry out work corresponding to steps 4-6 for “point-like” mutants; 6) For mutants with extended intragenic deletions, carry out work on *in situ* fish hybridization on polythene chromosomes; 7) For mutants with supposedly large insertions of genomic DNA, to perform long-PCR to determine the exact localization and size of the inserted fragment. In this part of the project it is planned to study more than 50 mutants of this kind.

Genomic analysis of inherited DNA changes

To achieve the first goal of the project in terms of establishing the spectrum and frequency of DNA changes inherited by offspring at the genome level after irradiation of D. melanogaster males-parents, the following tasks must be solved: 1) To obtain the isogenic D. melanogaster lines, which males will be irradiated by ^{60}Co γ-rays (10-40 Gy); 2) Irradiated males are individually crossed with five females of the same isogenic line to produce offspring (females and males); 3) To isolate high-molecular-weight genomic DNA from 20-30 individual offspring by the phenol-chloroform method; 4) To create a genomic DNA bank with radiation-induced changes in the offspring from males irradiated with different doses of γ-radiation; 5) To carry out a genome wide shotgun sequencing according to Sanger on the Illumina setup based on Evrogen (Moscow); 6) to analyze the obtained sequencing results using bioinformatics programs at Ksivelue (Moscow).

Analysis of transcriptome (RNA) *D. melanogaster*

To achieve the second goal of the planned project to study the genetic control of the radiosensitivity of the D. melanogaster genome at the transcriptome level (RNA) using lines that contrast in radiosensitivity (Fig. 6), the following tasks need to be solved: 1) To check

previously established differences in radiosensitivity for high inbred lines maintaining in our Drosophila collection; 2) When confirming the genotypic purity of these lines, to obtain transcriptome from the first instar larvae irradiated at this and later stages of ontogenesis.

In the process of performing the above works, it is planned to use the following equipment in all parts of the project: Eppendorf microcentrifuge, BioRad T100 amplifier, horizontal electrophoresis chamber SE-1, Elf-8 current source, IMPLEN spectrophotometer, OHAUS scale, light-optical microscope, Carl Ceis, Sequencer SeqStudio, GeneChip Scanner 3000 7G System (Affymetrix), Evos Fluorescence Microscope, UVP HB-1000 Hybridizer.

Expected results

Implementation of the Project will provide the first new basic data, first of all, on the nature of structural gene changes in point and chromosomal mutations induced by γ-rays and neutrons. At the same time, the molecular analysis of “hot sites” of radiation mutagenesis at the DNA context level will allow to determine the organization and sequence of “hot sites”. These data will have fundamental and great predictive value.

For the first time, priority results of sequencing the offspring genome-wide of γ-irradiated male parents will allow assessing at the DNA level the genetic effects of radiation on the germline cell genome of the studied Drosophila and compare them with similar literature data obtained in mice that significantly increase the validity of using this experimental data for extrapolation to humans.

For the first time, the transcriptome analysis will allow to identify the gene sets responsible for differences in Drosophila genome radiosensitivity.

Timetable on Project

"The molecular genetics of radiation-induced changes at the gene, genome and transcriptome level in Drosophila melanogaster"
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Stages	Content	Institute
2020 г.	To perform works on PCR analysis of structural intragenic changes in γ- and neutron-induced mutants for five genes studied. To obtain isogenic lines for genomic analysis of mutations inherited in the offspring. To conduct preliminary work on the assessment of the radiosensitivity of the D. melanogaster lines from the LNP genetic collection. To work out the method of *in situ* FISH hybridization on Drosophila polytene chromosomes Perform sample work at Afimetrix	JINR
2021г.	Sequencing of the intragenic deletion ends of γ-ray- and neutron-induced mutations of the genes studied Sequencing of the genomic DNA of the offspring from isogenic males irradiated by 20-40 Gy γ rays ^{60}Co. To conduct a comparative computer analysis of the obtained sequencing results using the program from the resource EMBL-EBI. To begin work on in situ hybridization using mutants with chromosomal changes. To test the transcriptome analysis technique on Drosophila control lines using the Afimetrix.	JINR
2022 г.	To complete work on in situ FISH hybridization and sequencing of introgenic structural changes for γ- and neutron-indused mutants Conduct a comparative statistical analysis of the results obtained. To carry out the sequencing of the offstring genome from the males irradiated by 10 Gy γ rays To assess the nature of the dose dependence for inherited DNA micro changes. Compare these results with data available in the literature in mice. To carry out a comparative analysis of transcriptomes from Drosophila lines with different radiosensitivity. To identify the gene sets which are responsible for different radiation response.	JINR VIGG, RAS, (Moscow) SFU Rostov-on – Don

Head of Project **I.D. Alexandrov**

Human Resource Assessment

The team of the project participants includes 10 JINR staff members, among which 4 are highly qualified specialists (1 Ph.D., 3 Ph.D.) in the field of molecular and radiation genetics, who are proficient in genetic, cytogenetic and molecular methods of studying radiation-induced mutations *D. melanogaster* and having many years of experience in this field. At the same time participants of the project are Corr. RAS, prof. I.A. Zakharov-Gezikhus (IOGen, Moscow) and Dr. Sc., Head. Laboratory of experimental mutagenesis of SFU V.A. Chistyakov (Rostov-on-Don)

List of journal publications

1. E.V. Kravchenko, A.N. Rusokovich et al. Radiation biology of structurally different genes of Drosophila melanogaster. Message 8. White gene: general characteristics of radio-mutability and PCR analysis of “point” mutations // Rad. biology. Radioecology, 2019 (in print)

2. E.V. Kravchenko, S.V. Dubovik et al., Message 7: gene yellow. General characteristics of mutability and PCR analysis of “point” mutations // Rad. biology. Radioecology, 2018, t. 58, No. 4, p. 341-351

3. I.D.Alexandrov, M.V. Alexandrova Radiation biology of structurally different genes of Drosophila melanogaster. Message 6: The cinnabar gene: sequencing of γ- and neutron-induced "point" mutations // Rad. biology. Radioecology, 2018, t. 58, No. 1, p.15-25

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List of presentations at conferences

1. I.D. Alexandrov, M.V. Alexandrova, K.P. Afanasyeva, et al. Radiation biology of Drosophila melanogaster structural genes / Abstracts of the All-Russian Conference "Drosophila in Genetics and Medicine", p.8, Gatchina, October, 2017.

2. Afanasyeva KP, Alexandrova MV, Alexandrov I.D. Molecular genetic analysis of γ- and neutron-induced "point" and structural mutations of the vestigial *D. melanogaster* gene / theses of the All-Russian conference "Drosophila in genetics and medicine", p.11, Gatchina, October, 2017.

3. Afanasyeva K.P., I.D. Alexandrov, M.V. Alexandrova Comparative characteristics of the damaging effects of γ-quanta, fission neutrons and 12C ions at different levels of the genome of *D. melanogaster* generative cells. Abstracts on the materials of the First All-Russian Scientific Conference "Toxicology and Radiobiology of the XXI Century", St. Petersburg, May, 2017

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Форма № 26

The proposed timetable and resources needed for the Project

The molecular genetics of radiation-induced changes at the gene, genome and transcriptome level in Drosophila melanogaster
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Required equipment, funding sources		Cost (ths.$)	The 1st year 2020	The 2nd year 2021	The 3rd year 2022
Equipment	1. Microcentrifuge* 2. PH meter * 3. Autoclave*	5 0.6 5.6	5 0.6	 5.6	
sources funding	The costs from the budget	11.2	5.6	5.6	

*- replacement of obsolete equipment with more high-performance and functional

Head of Topic G.V. Mytsin

Head of Project I.D. Alexandrov

Форма № 29

Estimated Project Cost

The molecular genetics of radiation-induced changes at the gene, genome and transcriptome level in Drosophila melanogaster
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Name of cost items	full cost	2020 year	2021 year	2022 year
Direct project costs				
Materials (ths. $)	117,8	39	39,8	39,0
Equipment (ths.$)	11,2	5,6	5,6	-
Travel expenses (ths. $)	15.0	5.0	5.0	5.0
Total direct costs:	144	49,6	50,4	44,0
Including from the LNP budget	144	49,6	50,4	44,0

Head of Project I.D. Alexandrov

Director of LNP V.A. Bedniyakov

Assistant director of LNP for economic and financial affairs G. A. Usova

CCTV analysis

Project strengths are:

1. Long-term experience of the main implementers of the project in the field of general, radiation and molecular genetics, their wide popularity in the scientific circles of our country and abroad.
2. The presence of a unique collection of radiation-induced mutations of five different D. melanogaster genes, which are the subject of study in both the first and the planned project Radiogene.
3. Great experience in the first project Radiogene
4. The combination of advanced scientific ideas and the most current DNA technology
5. Scientific support of the planned research by a member of the Russian Academy of Sciences, which has great authority and wide recognition in science.

Project weaknesses:

1. The small number of young professionals (3 employees)

Opportunities:

1. High scientific potential and modern scientific base open up broad prospects for attracting young specialists from JINR Member States
2. Increasing the scientific authority of JINR and the LNP as an organization where modern fundamental molecular-radiation genomic researches are conducted
3. The availability of the most advanced equipment such as Afimetrrix may become attractive for joint research of JINR and other research institutes.

Threats:

As part of the planned project no threats have been identified.

Appendices (figures, reviews and an extract from the minutes of the STC meeting)

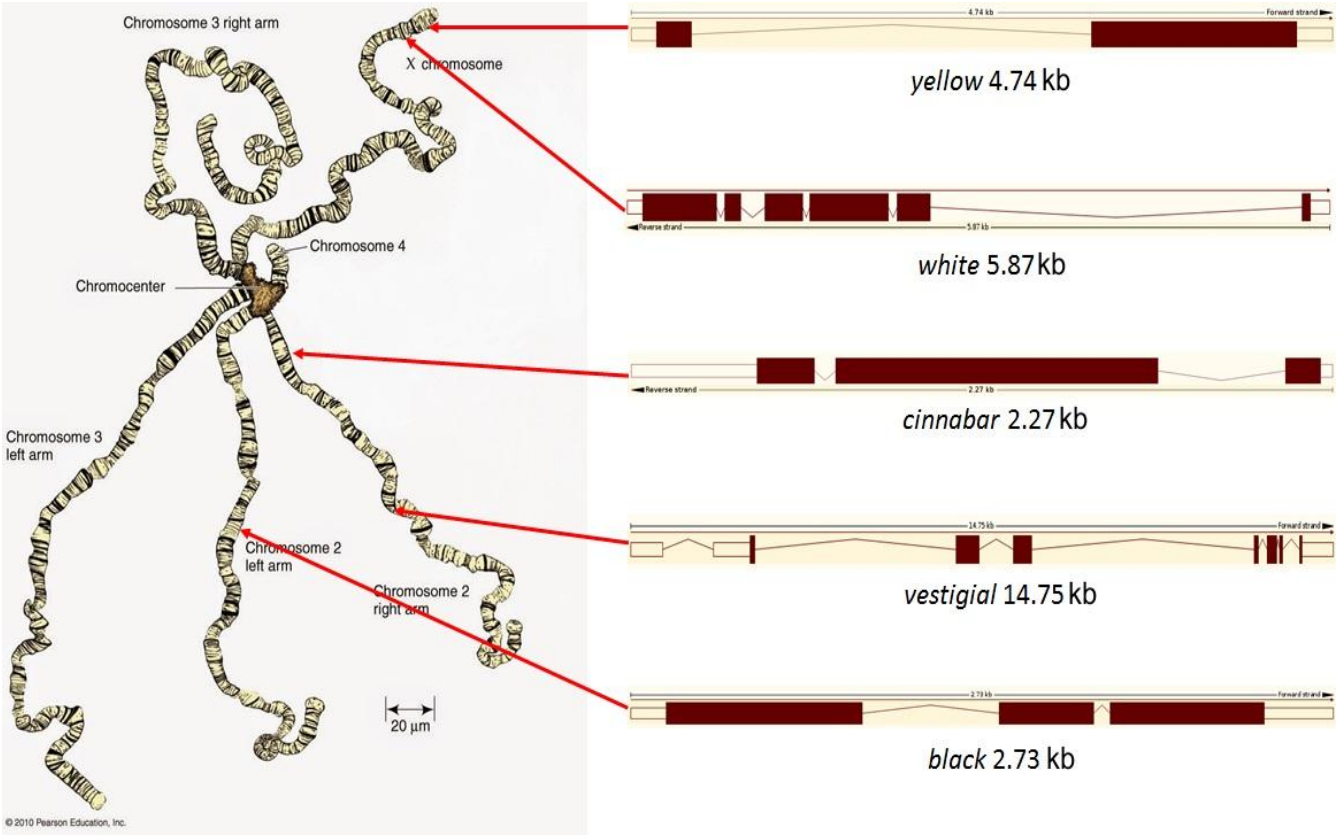

Fig. 1. The size, molecular organization and location on the polytene chromosomes of the five *Drosophila melanogaster* genes radiation-induced mutations of which are studding.

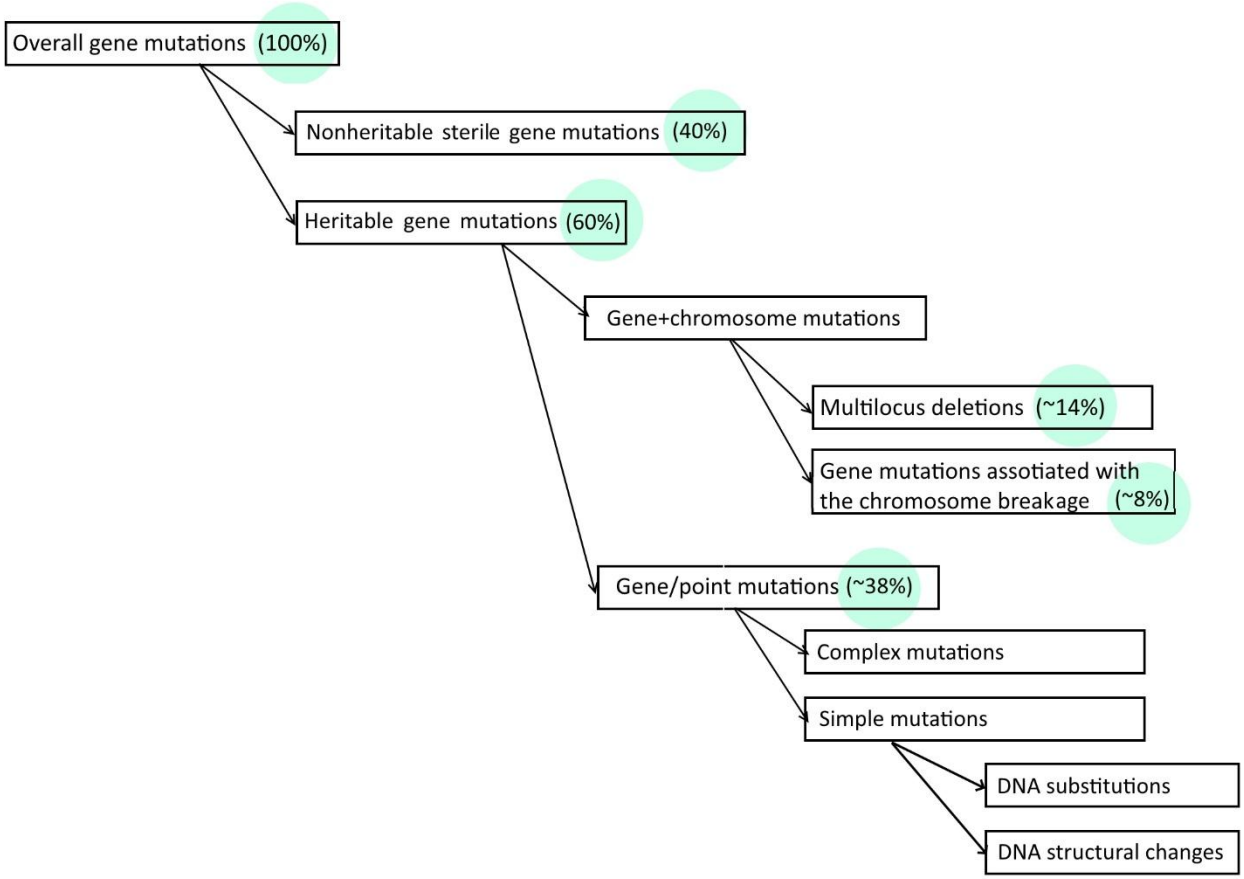

Fig. 2. Spectrum of γ-ray- or neutron-induced locus-specific mutations in the sperm cells of *Drosophila melanogaster* recovered by the genetic, cytogenetic and PCR-analysis.

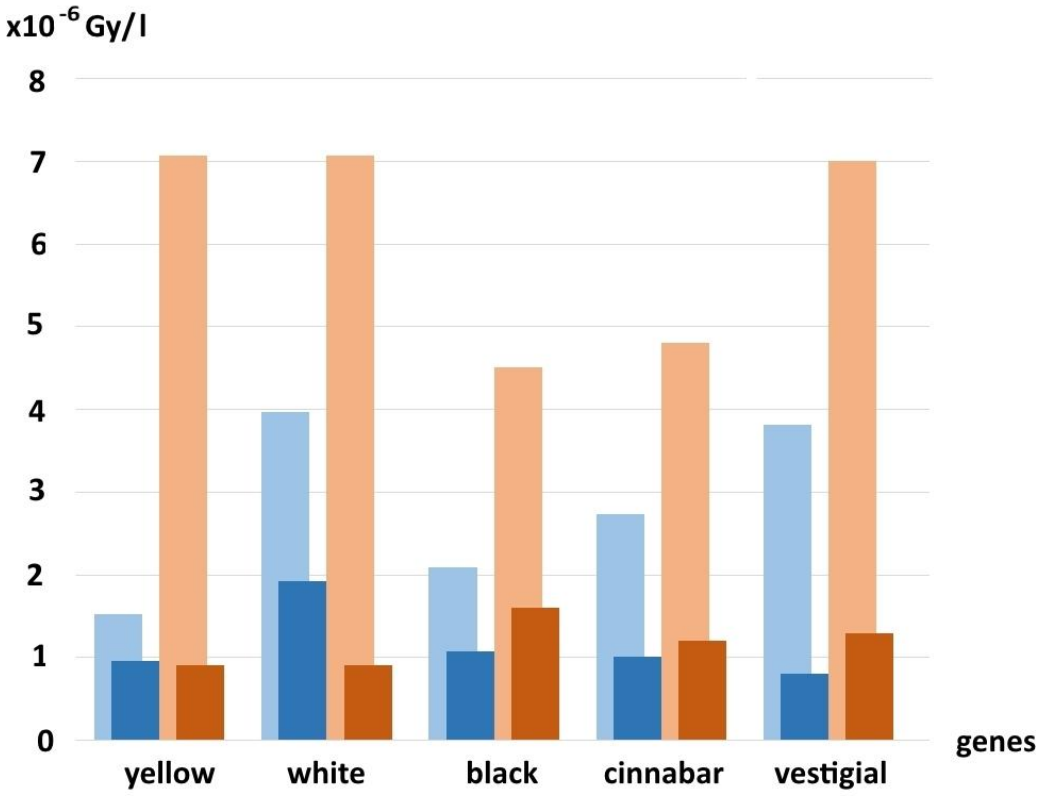

Fig. 3 The frequency (x10^{-6}/Gy/l) of the gene/point ( ) and gene+chromosomal ( ) mutations induced by γ-ray ( ) and neutrons ( ) in the *Drosophila melanogaster* sperm cells.

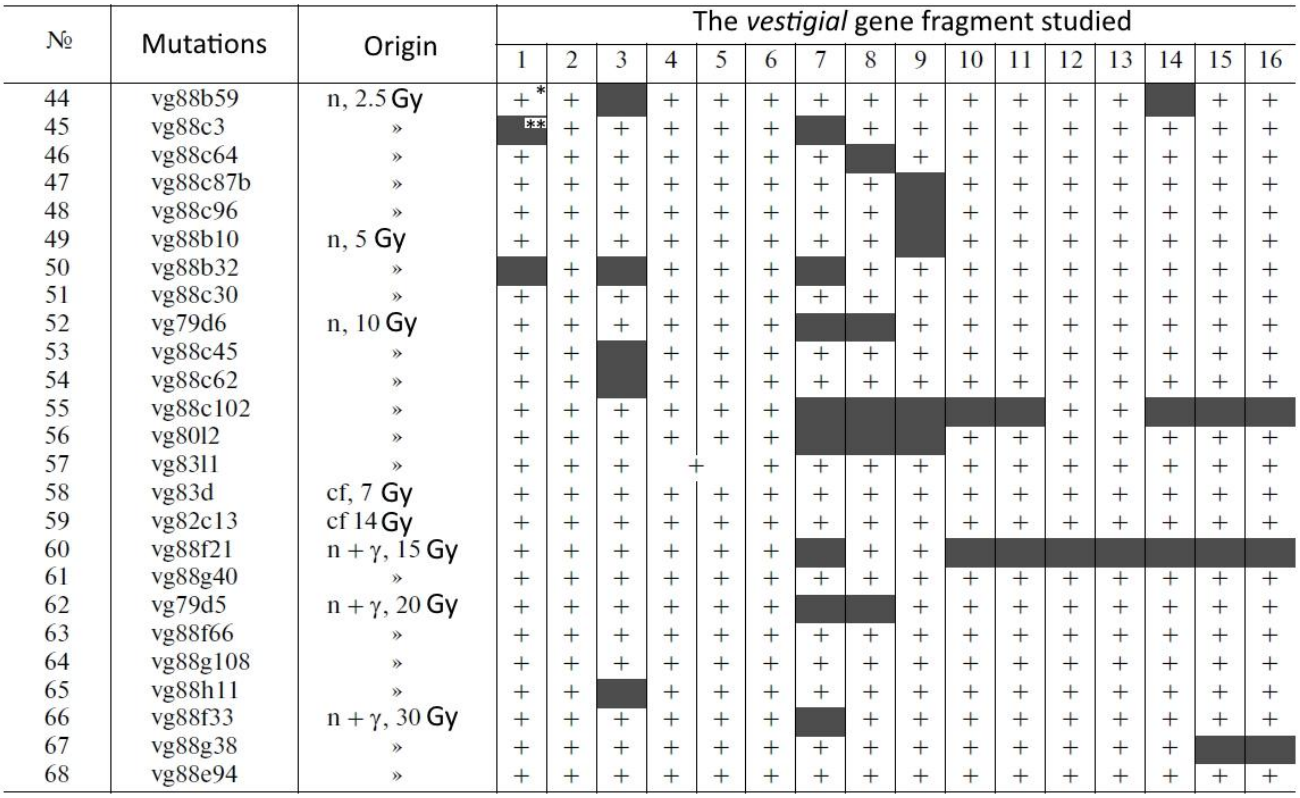

Fig. 4. The size and location of intragenic deletions induced by different doses of neutrons at the *vestigial* gene of *Drosophila melanogaster* recovered by the PCR technique. *-a normal fragment, **- lack of fragment

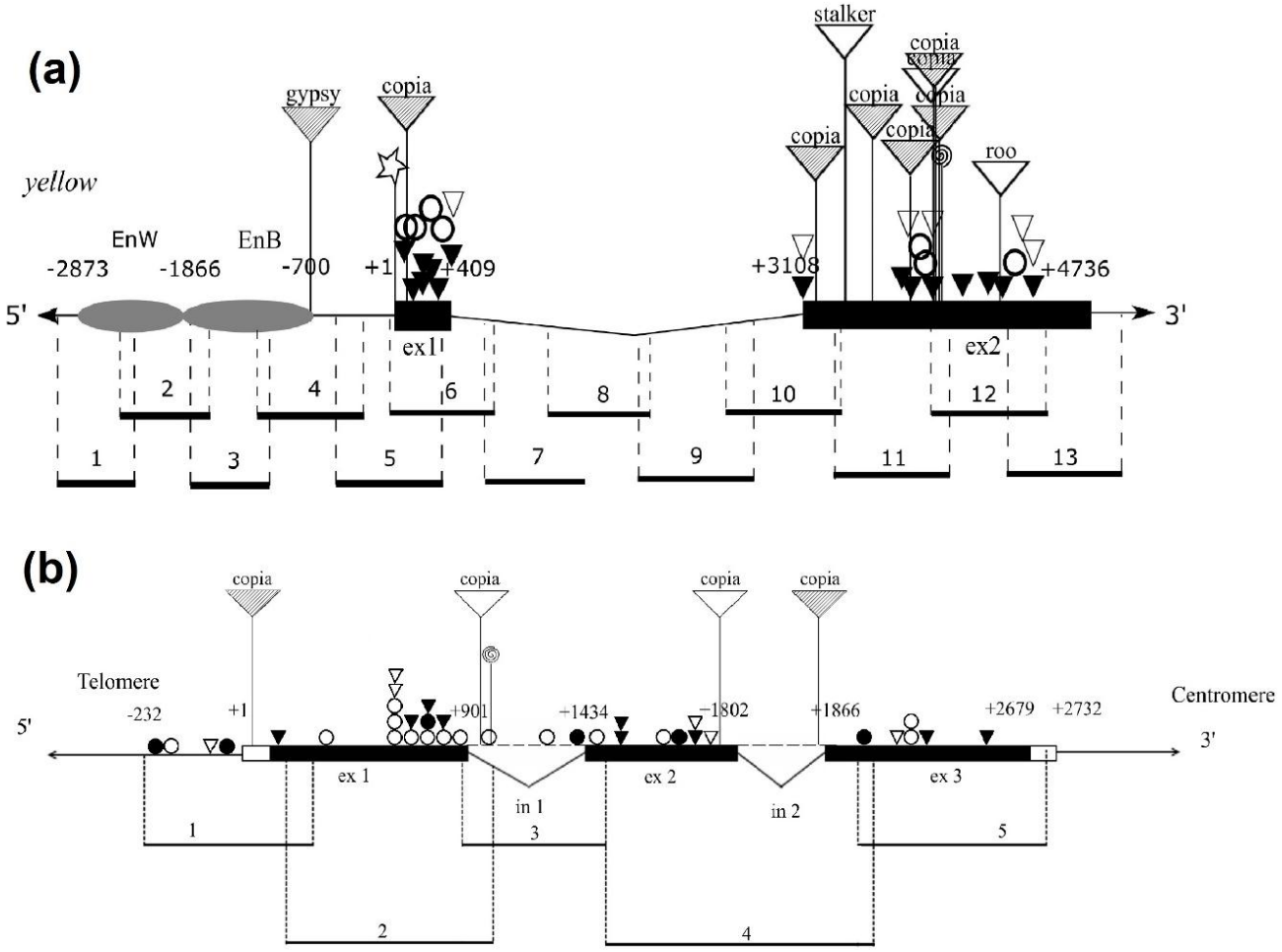

Fig. 5. Distribution of γ-ray-induced DNA changes on the linear map of the *yellow* (a) and *black* (b) genes of *Drosophila melanogaster*. ○ - base substitution, ● - substitution of > 2 bases ▼ -microdeletion, ▽ - microinsertion, , - spontaneous insertion of a large retrotransposons or other genomic DNA, copia - the same insertions in radiation-induced mutants.

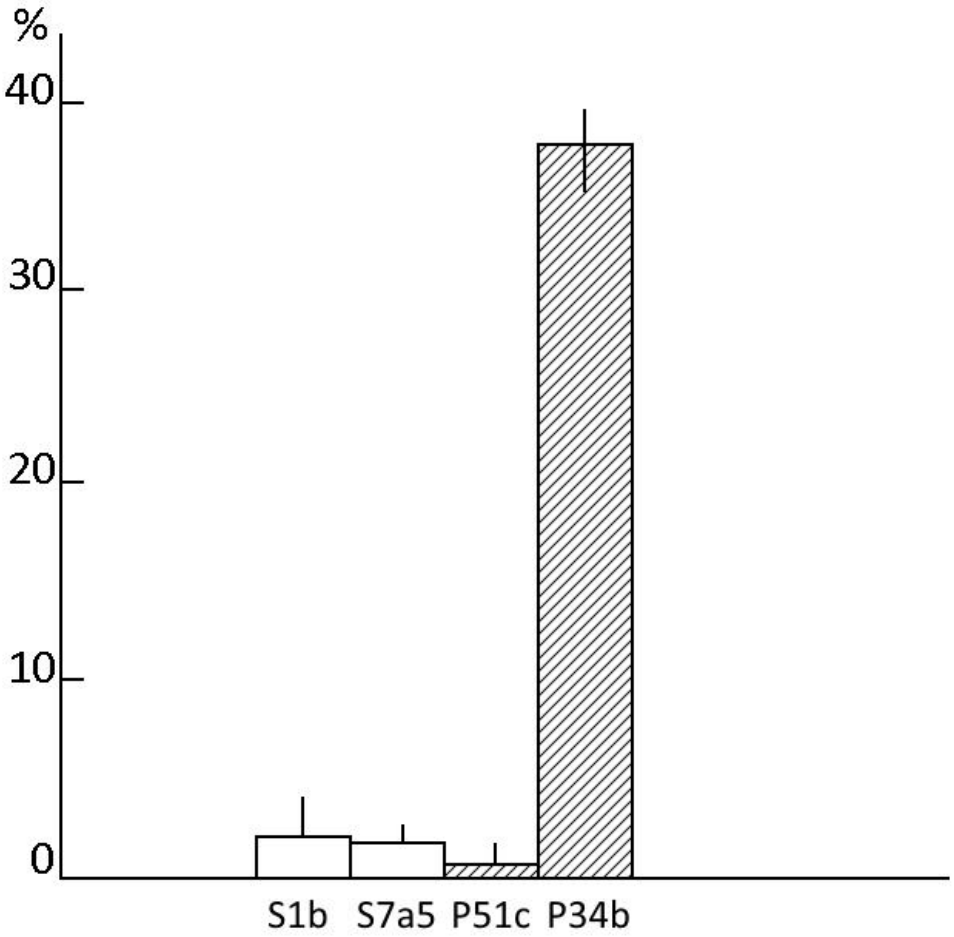

Fig. 6. Well-marked differences in the response (survival) to γ-ray- exposure (LD 80=12Gy) of four high-inbred lines of *Drosophila melanogaster* at the first instar larvae stage of ontogenesis.