

PROJECT REPORT

1. General information on the project

Scientific field

Condensed Matter Physics, Radiation and Radiobiological Research

Title of the project / LRIP subproject

“Molecular genetics of radiation-induced gene and genome changes in *Drosophila melanogaster*”.

Project RADIOGEN

Theme / LRIP code

04-2-1132-2017/2023

Actual duration of the project

04.22-04.23

Project Leads

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2. Annotation

The report presents the results of research in two main directions of *Drosophila* radiation genetics performed in the period 04.2022-04.2023:

Within the framework of the first direction, the spectra of inherited gene mutations and DNA changes underlying these mutations after exposure of germline cells to γ -rays and neutrons were studied and presented.

Within the framework of the second direction, complex research was carried out to study inherited γ -ray-induced DNA changes in the entire genome of progeny from irradiated (^{60}Co , 40 Gy) males using high-tech whole-genome sequencing and bioinformatic processing. The results obtained provide the first insight into the multiplicity of genomic DNA changes, in the spectrum of which deletions of various sizes are predominant occurring inside or outside of the coding genes.

3. A detailed scientific report

Within the first direction, the results of genetic and cytogenetic analysis of γ - and neutron-induced heritable mutations at the five studied genes of *D. melanogaster* were summarized.

A common pattern of genetic changes for these genes and two radiations was detected. These changes included the following four mutation types (Fig. 1):

- non-inherited gene mutations as a result of concomitant genomic alterations leading to sterility of F_1 offspring;
- multilocus deletions leading to the loss of the studied gene;
- gene mutations associated with an inversion or translocation breakpoint in the gene region under study;
- inherited recessive Mendelian "point" mutations with intragenic DNA changes.

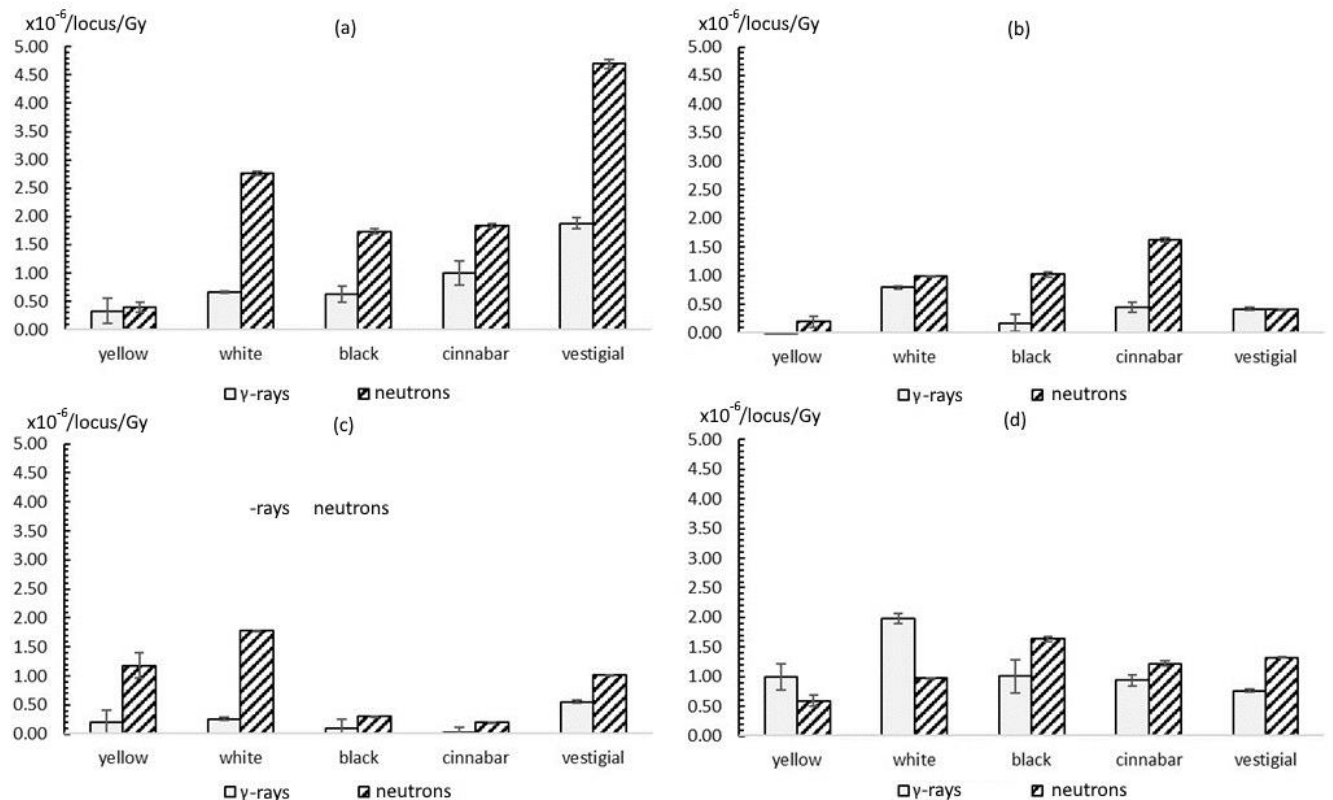


Fig. 1. Comparative frequency of γ - and neutron-induced gene-specific mutations of different types per unit dose (1 Gy) for five studied genes: a) non-inherited gene mutations; b) multilocus deletions; c) gene mutations associated with an inversion or translocation breakpoint in the gene region; d) inherited recessive Mendelian "point" mutations.

The induction mutation rate per 1 Gy was established for each mutation type and for both radiation studied. It is important to note that these mutation rates are gene-specific and highly dependent on the radiation quality (Fig. 1). In particular, neutrons are much more efficient than γ -rays in induction of (a)-(c) gene mutations which associated with a gross chromosomal aberrations.

Interestingly, the frequency of neutron-induced “point” mutations at the autosomal genes (*black*, *cinnabar*, *vestigial*) is somewhat higher than that at the sex-linked genes (*yellow*, *white*) (Fig.1, d).

As the molecular analysis shown, a higher frequency of “point” mutations at the autosomal genes is due to the contribution of intragenic changes generated by recombination-dependent repair pathway in the form of gene conversion, leading to the replacement of irradiated and damage paternal gene to normal unirradiated maternal one in early zygote after syngamy. This recombination-dependent repair pathway is unlikely in F₁ female heterozygotes having sex-linked inversions.

To study the nature of DNA changes in *cinnabar* “point” mutants induced by γ -rays and neutrons, the sequence analysis of 47 mutants was performed. The data obtained were compared with the results for the *black* mutants studied earlier (Table 1.)

Table 1. DNA changes underlying γ - and neutron-induced *cinnabar* and *black* “point” mutants of *D. melanogaster*

| DNA change | <i>cinnabar</i> | | <i>black</i> | |
|-------------------------------|-----------------|-----------|----------------|------------|
| | γ -rays | neutrons | γ -rays | neutrons |
| Base substitutions | 5* (16,1%)** | 7 (46,6%) | 20 (46,5%) | |
| Indels | 5 (16,1%) | 0 | 7 (16,2%) | 0 |
| Deletion extended | 11 (35,5%) | 4 (26,7%) | 6 (14,0%) | 2 (9,5) |
| Insertion extended | 2(6,4%) | 1 (6,6%) | 3 (7,1%) | |
| Gene conversion event | 8 (25,8%) | 3 (20%) | 6 (14,0%) | 18 (85,7%) |
| Insertion instead of deletion | 0 | 0 | 1 (2,3%) | 1 (4,8%) |
| Total | 31 | 15 | 43 | 21 |

*- The number of changes; **- Relative rate of changes

As these data show, six different DNA changes can underlie the "point" mutations in both studied genes. It is important to note that frequency of individual DNA changes is unique for each gene studied and for γ -rays and neutrons.

Within the framework of the second direction, the genomic sequencing and IT-analysis were performed for three control and nine experimental female offspring. According to the results obtained (Fig. 2) 44.8% of total DNA changes were difficult to interpret, 16.1% of DNA changes were the mosaics and 39.1% of DNA changes were identified as complete deletions of different sizes, duplications, insertions, inversions or translocations.

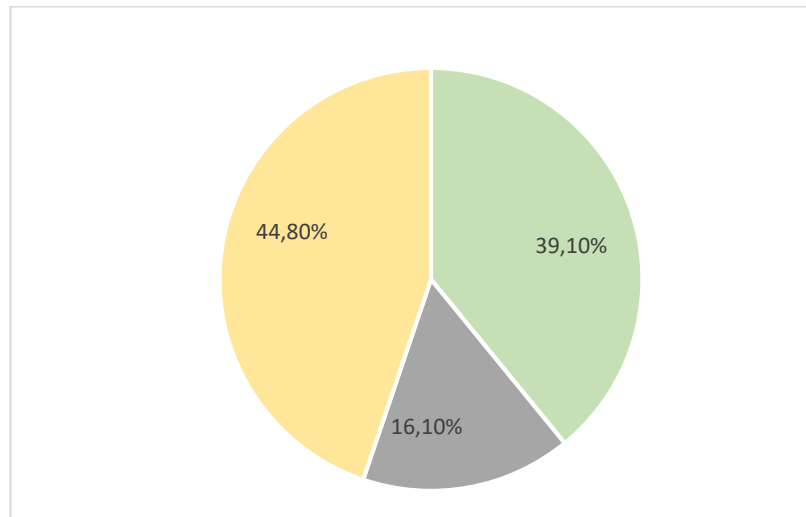


Fig. 2. Complete (green), mosaic (grey), and difficult to interpret (yellow) DNA changes detected by whole-genome sequencing and IT-analysis of progeny from γ -irradiated (40 Gy) males of *D. melanogaster*.

Complete mutations represent heterozygous altered region containing 50% of a normal maternal DNA and 50% mutant paternal DNA (ratio 1:1). In mosaic mutations, this ratio of normal and mutant DNA is 3:1. The presence of such mosaic mutational changes indicates that they can be result of induced single-strand DNA damage in the sperm genome of irradiated males. These damages can lead to formation of mosaic cell clones in the process of subsequent replications and cell divisions.

All complete and mosaic mutations are unevenly distributed among the 9 studied offspring from irradiated male parents, varying from 1 to 12 changes per genome. These changes include 36 deletions of various sizes (17–2280 bp and one deletion of 71460 bp), 5 duplications, 7 inversions, and 3 translocations. In three control progeny observed two deletions only (Fig. 3). The predominance of deletions among other changes shows that the offspring of irradiated males most often inherit this type of genomic DNA changes.

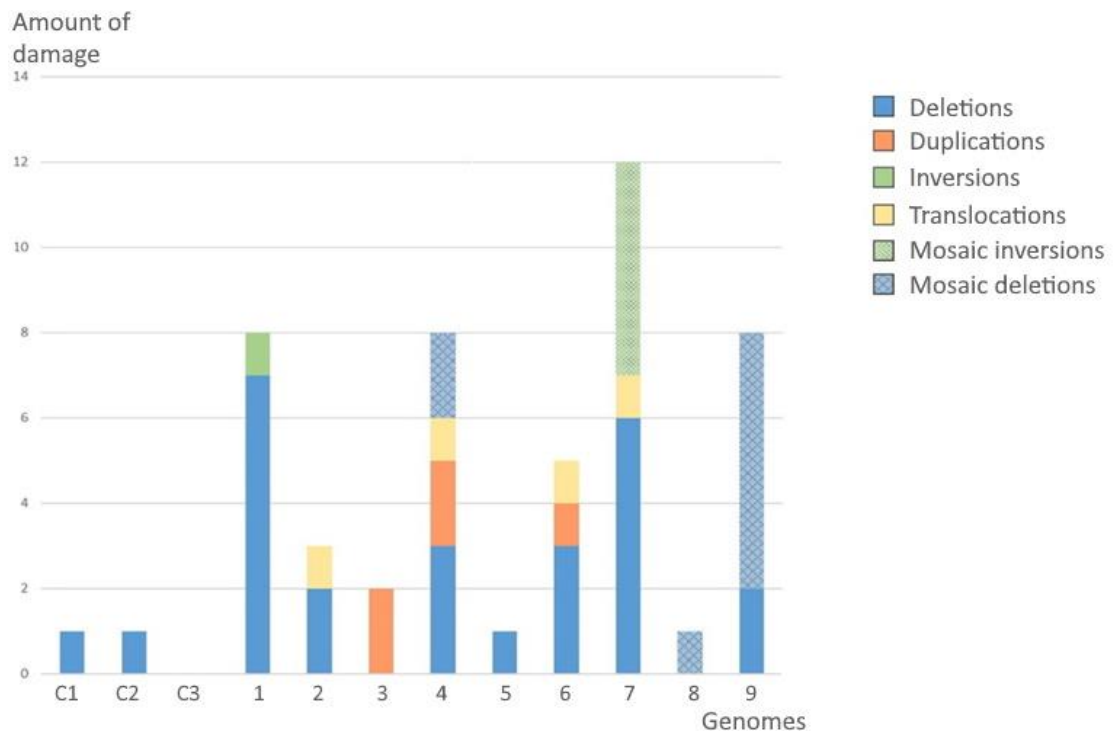


Fig. 3. The types and number of genomic DNA changes in 9 offspring from γ -irradiated ^{60}Co , 40 Gy males and in 3 control (k1-k3) males.

Attention is drawn to the high multiplicity of DNA changes in 5 offspring (1, 4, 6, 7, 9 genomes) in which the number of DNA alterations varies from 5 to 12 changes. These data indicate multiple hits of γ -rays on the sperm genome and low efficiency of error-free repair of primary damage in the egg after fertilization. The presence of two genomic deletions in the three control offspring suggests that these changes are the result of a spontaneous mutational mutagenesis in the Iso 9.1.

4. List of publications and talks given at international conferences and meetings

1. I. D. Alexandrov, M. V. Alexandrova, K. P. Afanas'eva, A. N. Rusakovich, N. E. Kharchenko "Radiation biology of structurally different genes of *Drosophila melanogaster*. Message 9. General patterns and locus-specific features of radiomutability of sex-linked and autosomal genes. Radiation biology. Radioecology. (Impact factor (RSCI): 0.685) (in press).
2. Afanas'eva K.P., Alexandrov I.D. et al., "Genomic changes in the offspring of *D. melanogaster* males irradiated with ^{60}Co γ -quanta". Scientific publication "Sakharov Readings 2022: Environmental Problems of the 21st Century", Proceedings of the International Scientific Conference May 19-20, Minsk, p. 328.
3. "Radiation genetics: yesterday, today, tomorrow" Plenary report, Alexandrov I.D. 22nd International Scientific Conference "Sakharov Readings 2022: Environmental Problems of the 21st Century" May 19-20, 2022, Minsk

5. Results of related activities

1. Scientific and educational activities: Afanasyeva K.P., project curator in International Student Practices, March, 2022.

2. Scientific and popularization activities:

1. Popular science lecture by Artem Rusakovich "From an elephant fly and not only", JINR House of Scientists, March, 2023

2. Popular science lecture by Anastasia Rusakovich "Genealogy and genes", JINR Universal Library named after D.I. Blokhintsev, November, 2022.

6. Conclusion

The planned studies were completed, unfortunately, only by 50% (only one dose of 40 Gy was used and genome analysis was carried out only for 12 offspring) due to underfunding of the project. Nevertheless new fundamental data have been obtained that reveal the general patterns of mutation induction for 5 genes and two different quality radiation (γ -rays and neutrons). Among the 4 observed types of gene mutations, a class of "point" mutations and these rates per unit dose (1 Gy) were detected for the first time. The molecular analysis of "point" mutations was continued and spectra of DNA changes for two different genes were characterized after the action of γ -rays and neutrons. The first results of a pilot experiment on genomic research have been obtained, which showed the multiple nature of DNA damage throughout the genome in the offspring of γ -irradiated *D. melanogaster* males. Experiments have been carried out to obtain the reference genomic DNA of the Iso.9.1 line.

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