

# The Nature of Radiation-induced Inherited Recessive Gene Mutations in *Drosophila Melanogaster*

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## Abstract

The nature of gene mutations induced by ionizing radiation in germ cells and transmitted to offspring remains one of the most important problems in radiation genetics of higher eukaryotes. The data accumulated in this field were obtained by different authors under different experimental conditions which does not give a complete insight about the nature of radiation-induced inherited mutations at different genome levels (chromosome, gene, DNA). We obtained new data in this field under the same experimental conditions for five different genes. This first allow us to get a fairly complete picture of the spectrum and frequency of inherited recessive gene mutations in *Drosophila melanogaster* sperm cells exposed to  $\gamma$ -rays and neutrons. The spectra of genetic alterations underlying inherited recessive gene mutations for  $\gamma$ -rays and neutrons closely coincide and can be divided into two main classes: (i) mutations associated with chromosomal aberrations of different types (so-called gene/structural mutations) and (ii) point intragenic mutations (gene/point mutations) having DNA changes of different nature. Substantially, neutrons are 2.5 times more efficient than  $\gamma$ -rays in induction of the gene/structural mutations (4.1 and 1.6 E-0.6 / locus / Gy, respectively). In the gene/point mutations induction, the efficiency of two radiation studied is almost the same (1.2 and 1.1 E-0.6 /locus/Gy for neutrons and  $\gamma$ -ray, respectively). Precise PCR assay of the gene/point mutations allows us to identify two main classes of these mutations: (i) mutations with DNA changes not detected by this method (PCR<sup>+</sup> mutants) and (ii) a group of mutants with intragenic deletions of different sizes and localization, including mutants with clusters of such independent deletions. Further sequence analysis of  $\gamma$ -ray-induced PCR<sup>+</sup> *black* mutations made it possible to identify variety of DNA changes among which base substitutions are predominant mutation lesions (45%) while vast majority (90%) of neutron-induced mutations had changes in the form of the gene conversion event. The significance of a new molecular data obtained (clusters of DNA changes within the gene and in one turn of the DNA double-stranded helix, gene conversion) for a better understanding of the fundamental mechanisms of radiation mutagenesis and for assessment of genetic hazard (risk) of ionizing radiation at the molecular level is discussed.

**Keywords:** Neutrons,  $\gamma$ -rays, *Drosophila melanogaster*, Locus-specific mutations, Chromosomal aberrations, Point mutations, PCR assay, Sequencing

## Introduction

Analysis of early [1] and current [2] data on epidemiology and genetics of inherited developmental anomalies and other disorders allows us to note an interesting and important fact that among the various detected genetic changes, point mutations underlie almost one-half of the recessive Mendelian diseases [1] circulating in modern human populations. At the same time, the results of molecular analysis showed that the DNA changes underlying these mutations are represented mainly by base substitutions, indels, extended deletions or

insertions and duplications [3-5]. Taking into account the well-known and important fact that the most dangerous mutagen for human is ionizing radiation with which humans are increasingly exposed on Earth (nuclear power station, radiotherapy, neutron research, nuclear disaster etc.) and in outer space. Therefore, it is important to know: (i) does ionizing radiation induces point mutations in germ cells in general, (ii) if so, what is the efficiency of sparsely and densely ionizing radiation in induction of such mutations, and (iii) what DNA changes underlie these mutations.

Fundamental concept of classical (pre-molecular) radiation genetics is that sparsely ionizing radiation regularly induces two classes of genetic alterations [6,7] - chromosomal aberrations and point mutations (either Müller's "intragenic mutations" [8], or Lünig's "apparent gene mutations" [9], or de Serres's "gene/point mutations" [10]). Wherein, all recessive sex-linked lethal mutations and recessive locus-specific visible mutations in *Drosophila* and mice were considered as point mutations only by biophysical criteria (linear dose-response, no fractionation, dose rate and density ionization effects) without analysis of their genetic nature. However, precise cytogenetic studies on *Drosophila melanogaster* showed that a significant part of X-ray-induced recessive sex-linked lethal mutations were represented by chromosomal aberrations of various types, the frequency of which increased with increasing dose [11]. Additionally, it was found that neutrons are more efficient than X-rays in induction of recessive sex-linked lethal mutations [12]. Moreover, the frequency of minute chromosomal deletions increases linearly with the dose of  $\gamma$ -rays [13]. In the light of these contradictory data, one cannot but agree with S. Wolf's conclusion that "many of the radiation-induced true mutations that have been studied may in reality be the result of chromosome breakage and rejoining" [6].

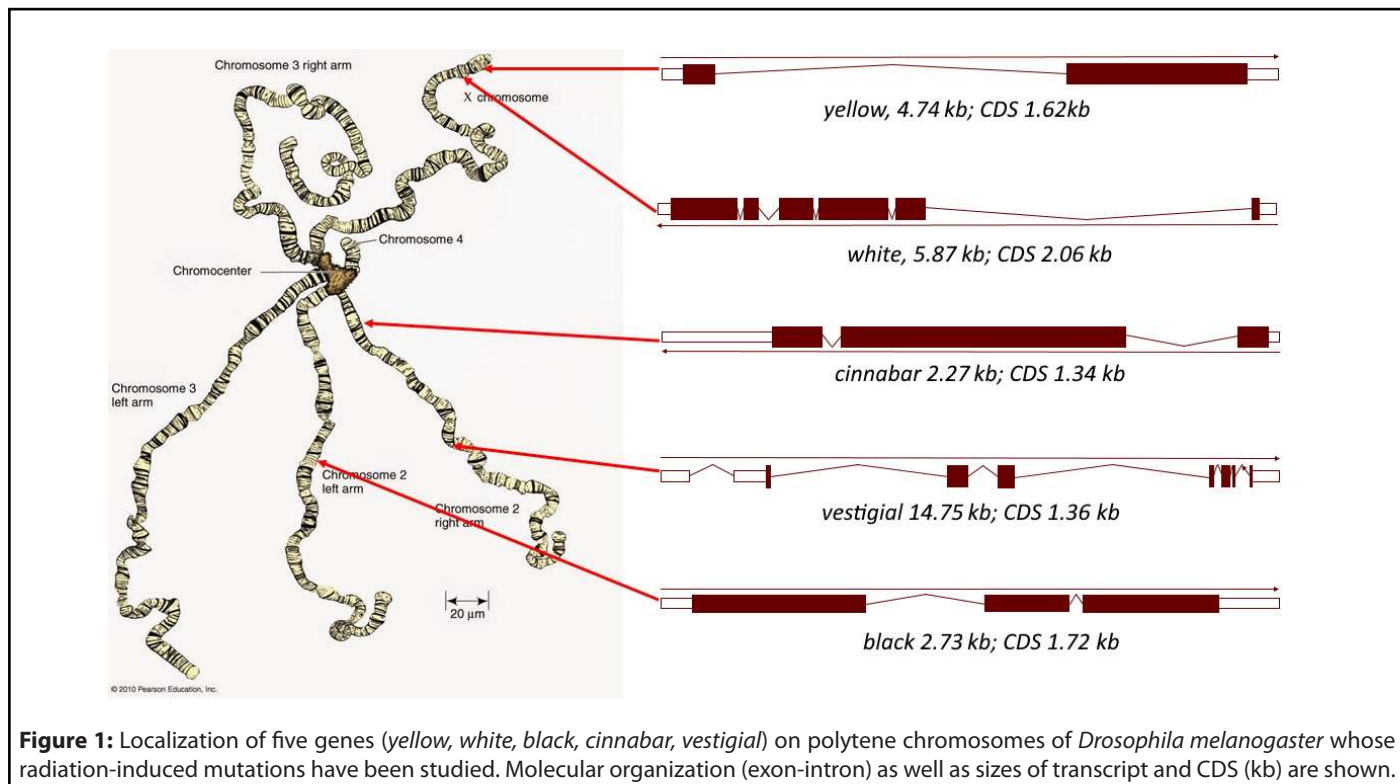
## Results and Discussion

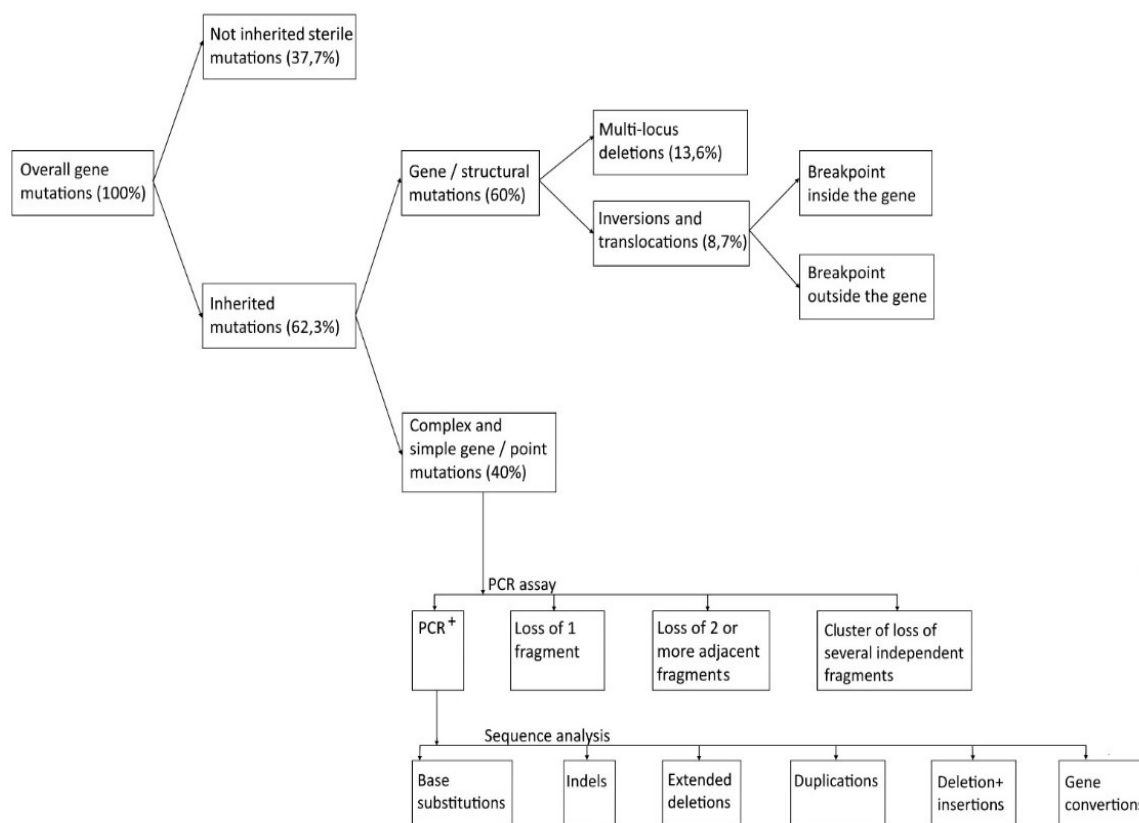
Given the ambiguity in the issue of whether ionizing radiation induces true point (intragenic) mutations in germ cells, large-scale and long-term research on induction and analysis of

$\gamma$ -ray- and neutron-induced recessive locus-specific visible mutations at five genes of *Drosophila melanogaster* have been carried out. All experiments used the same methodology of comparative analysis and the same complex of genetic, cytogenetic and molecular methods, the details of which were described earlier [14,15]. Briefly, each irradiated male was mated with five females of genotype *Ins (1) sc<sup>a</sup> +dl-49, y<sup>31d</sup> sc<sup>S1</sup> sc<sup>8</sup> w<sup>a</sup> b<sup>1</sup> cn<sup>1</sup> vg<sup>1</sup>*. After 24 h, the irradiated males were discarded. Then, inseminated females laid eggs for two consecutive three-day periods. Among the regular F<sub>1</sub> progeny, mutant male or female with putative *de novo* mutation was isolated and tested for fertility by mating with flies of appropriate sex from the balancer strains. Further steps of mutation analysis included genetic (obtaining mutant homozygotes or heterozygotes if recessive lethality was observed, test for allelism, intergenic recombination), cytogenetic on polytene chromosomes, and molecular (PCR, sequencing) researches.

Five studied genes (sex-linked *yellow*, *white*, and autosomal *black*, *cinnabar*, *vestigial* at chromosome 2), their localization on polytene chromosomes and exon-intron organization are shown in Figure 1.

As can be seen, the position of the studied gene-targets on polytene chromosomes is different. So, the *yellow* and *white* genes are located in the peritelomeric region of the X chromosome, the *cinnabar* is mapped in the pericentromeric region of the chromosome 2R, whereas the *black* and *vestigial* are located almost in the middle of the autosomes 2L and 2R, respectively. The sizes of the transcripts of these genes





**Figure 2:** Summarized spectrum of  $\gamma$ -ray- and neutron-induced recessive locus-specific mutations in the mature sperm cells of *Drosophila melanogaster* identified by genetic, cytogenetic and molecular (PCR, sequencing) methods. The relative rates of mutations (in parentheses) are shown for  $\gamma$ -rays only.

also differ greatly, varying from 2.27 (*cinnabar*) to 14.75 kb (*vestigial*), while the length of their CDSs vary insignificantly (average is 1,62 kb).

### Genetic and Cytogenetic Nature of Radiation-Induced Inherited Recessive Gene Mutations

The results of a complex (genetic, cytogenetic) analysis of radiation-induced visible mutations of five genes have been described in earlier for each gene [16-21] and generalized spectrum of mutations for  $\gamma$ -rays and neutrons is shown in Figure 2. Different types of visible mutations are shown in Figure 2 in the order of their identification, first by classical methods of genetics and cytogenetics, and then, for point mutations, by modern molecular methods (PCR, sequencing). Since the spectra of mutations for two types of radiation closely coincide it allows us to distinguish two main classes of mutations: mutations associated with chromosomal aberrations of different types (so-called gene/structural mutations) and point intragenic mutations (gene/point mutations) of different nature.

### Gene/structural mutations

**Sterility of  $F_1$  mutant flies:** As in other papers [22], here the term sterility means the inability of  $F_1$  mutants to give progeny and the precise nature of such sterility is unknown so far. In our case, sterile  $F_1$  mutants are observed in the spectrum of genetic changes of each of the five studied genes and for both radiation types (Table 1). Wherein, neutrons are 2.5 times more efficient than  $\gamma$ -rays in induction of such mutants (2.5 and 1.0 E-06 / locus / Gy, respectively for an average of five genes). Taking into account the high efficiency of densely ionizing radiation in comparison with sparsely ionizing radiation in induction of structural chromosomal alterations [7,8], it can be assumed that the dominant sterility observed is the result of radiation-induced large chromosomal alterations. This assumption is confirmed too by our data showing that neutrons are more effective than  $\gamma$ -rays in induction of gene mutations associated with visible chromosomal aberrations (multilocus deletions, inversions, translocations) (Table 1). This assumption is supported also by the data showing that large deletions underlie the sterility of X-ray-induced

Table 1: Number, absolute (E-06/locus/ Gy) and relative (%) rates of $\gamma$ -ray- and neutron-induced mutations of different types for five genes of <i>Drosophila melanogaster</i> .													
Loci studied	yellow		white		black		cinnabar		vestigial		Total for 5 genes		
	$\gamma$ -rays	neutrons	$\gamma$ -rays	neutrons	$\gamma$ -rays	neutrons	$\gamma$ -rays	neutrons	$\gamma$ -rays	neutrons	$\gamma$ -rays	neutrons	
Number of males irradiated	3393												
Number of F <sub>1</sub> alleles+ studied	201989	135227	201989	135227	383986	261184	383986	261184	383986	261184	1555936	1054006	
All F <sub>1</sub> mutations	(23)* 1.5**	(12) 1.0	(56) 3.7	(33) 6.5	(55) 1.9	(46) 4.7	(70) 2.4	(48) 4.9	(104) 3.6	(73) 7.5	(308) 2.6	(212) 5.4	
Gene/ structural mutations													
Sterile F <sub>1</sub> mutants	(5)	(2)	(10)	(14)	(18)	(17)	(29)	(18)	(54)	(46)	(116)	(97)	
Multilocus deletions	(0)	(1)	(12)	(5)	(5)	(10)	(13)	(16)	(12)	(4)	(42)	(36)	
Inversions	(3)	(3)	(2)	(7)	(3)	(3)	(1)	(2)	(12)	(8)	(21)	(23)	
Translocations	(0)	(3)	(2)	(2)	(0)	(0)	(0)	(0)	(4)	(2)	(6)	(8)	
Total	(8) 0.5	(9) 1.8	(26) 1.7	(28) 5.5	(26) 0.9	(30) 6.1	(43) 1.5	(36) 3.7	(82) 2.8	(60) 3.1	(185) 1.6	(164) 4.1	
Gene /point mutants													
Simple	(10)	(3)	(27)	(4)	(17)	(11)	(12)	(4)	(15)	(9)	(81)	(31)	
Complex	(5)	(0)	(3)	(1)	(12)	(5)	(15)	(8)	(7)	(4)	(42)	(18)	
Total	(15) 1.0	(3) 0.6	(30) 1.9	(5) 1.0	(29) 1.0	(16) 1.6	(27) 0.9	(12) 1.2	(22) 0.8	(13) 1.3	(123) 1.1	(49) 1.2	
*In parentheses, the number of mutations recovered is shown as the sum of mutations detected at doses of 5, 10, 20 and 40 Gy for $\gamma$ -rays and 2.5, 5, 10 and 20 Gy for neutrons. **Absolute mutations rate (E-06/locus/Gy) is given as a weighted average frequency for doses of 5,10,20 and 40 Gy for $\gamma$ -rays (total 75Gy) and 2.5, 5, 10 and 20 Gy for neutrons (total 37,5Gy) on a linear dose-response curve [17,19-22]. For example, for $\gamma$ -rays absolute frequency of point mutations induction for 5 genes total is 123 / 1555936 / 75Gy = 1.1 E-06 / locus / Gy. #The relative frequency of mutations of this type among all recovered ones is given for two types of radiation studied.													

*vermillion* mutants [23]. Genetic analysis of fertile F<sub>1</sub> mutants showed that many of them had recessive lethality along with a locus-specific phenotype. Such mutants were maintained as heterozygotes with balancer chromosomes. Their cytogenetic analysis revealed that some of them were multilocus deletions while others were the gene/structural mutations with inversion or translocation breakpoint within the region of the gene under study.

**Multilocus deletions:** Precise cytogenetic analysis of multilocus deletions involving *black*, *cinnabar* or *vestigial* loci showed that their sizes vary significantly from 1 to 10 or more bands of polytene chromosomes [24]. Wherein, neutrons are more efficient than  $\gamma$ -rays in induction of such chromosomal deletions for five genes under study (Table 1).

**Locus-specific inversions and translocations:** A certain part of locus-specific mutants with recessive lethality had an inversion or translocation exchanges with one of the breakpoints within the region of the studied gene, and the second breakpoints were not randomly distributed over autosomes 2 and 3 [25,26]. Precise mapping of these breakpoints using the method of hybridization *in situ* has shown that the breakpoint can be located within the gene as well as distal or proximal to the gene regardless of radiation type [27,28]. Neutrons are also more efficient than  $\gamma$ -rays in induction of locus-specific inversions and translocations (Table 1).

Thus, a significant part (the relative rates are 60 and 77% for  $\gamma$ -rays and neutrons, respectively) of radiation-induced recessive mutations at five genes are chromosomal aberrations of one type or another (so-called de Serre's "gene/structural" mutations).

### Point mutations

A certain part of the putative point mutations without visible changes in the region of gene location had a complex phenotype: mutation of the studied gene and recessive lethality. Recombination analysis of such mutations showed that most of them had lethal point mutations independently induced at the same chromosome [15]. In some cases, mutants had independently occurred and cytologically visible chromosomal aberrations. The share of such "complex" mutations is about a third of all point mutations induced by  $\gamma$ -rays and neutrons (Table 1). The share of point mutations in their total spectrum after neutrons is almost two times less (23%) compared to that after exposure to  $\gamma$ -rays (40%). But the absolute rates of point mutations induction for  $\gamma$ -rays and neutrons are almost the same (1.1 and 1.2 E-0.6 / locus / Gy, respectively) (Table 1). It is important to note that these average of the mutation rates for the gene studied well correlate with the average CDS length (Figure 1) of the same genes (1.62 kb) showing that true point mutations seem to be located mainly in the coding parts of the exons.

## Molecular Nature of Gene/Point Mutations

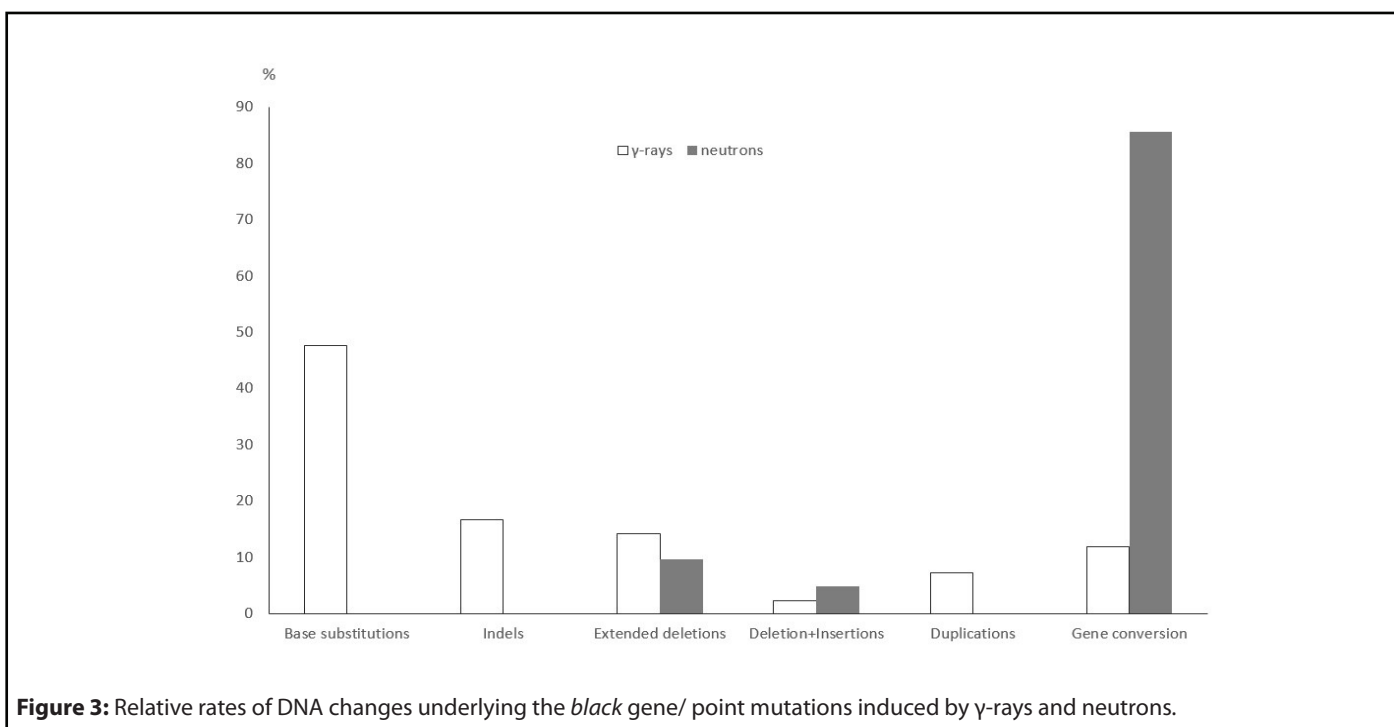
### PCR assay

To exclude mutations with total loss of the gene, PCR analysis of 25 *yellow*, 39 *white*, 51 *black*, 49 *cinnabar* and 34 *vestigial* point mutations induced by  $\gamma$ -rays as well as 1, 15, 13, 11 and 25 mutations of the same genes, respectively, induced by neutrons was carried out [17-21]. It is interesting to note that no total loss of the gene was found in all studied samples, and the observed intragenic changes could be grouped into four different types: (i) mutants with changes not detected by PCR (PCR+ mutants); (ii) mutants that did not have one fragment among others, into which all genes were subdivided; (iii) mutants with the loss of two or more adjacent fragments, and (iv) mutants with the loss of two or more fragments in different parts of the gene (cluster of the mutational lesions) (Figure 2). Additionally, PCR analysis of 25  $\gamma$ -ray-induced and 27 neutron-induced inversion or translocation *vestigial* mutations was carried out [30]. According to the results obtained, all four types listed above were also observed among aberrational mutants induced by the studied types of radiation.

Wherein, only three inversion and one translocation mutants showed a total gene loss [30]. If to unit (ii-iv) types of DNA changes in one group, then all detected PCR changes can be divided into two main classes: (i) PCR + mutants and (ii) deletion mutants. It is important to note that among the gene/point mutants, a share of  $\gamma$ -ray-induced mutants of the first class (72.2%) is almost two times higher than that of neutron-induced mutants (43.1%), showing that  $\gamma$ -irradiation is more efficient than neutrons in induction of DNA changes that are not detected by this method. A somewhat different picture is observed for gene/structural *vestigial* mutants, where a share of PCR+ mutants is approximately the same for  $\gamma$ - and neutron-irradiation (36.0 and 37.0%, respectively). Bearing in mind the significant proportion of changes of the first class and their still unclear nature, we carried out a sequence analysis of a large samples of  $\gamma$ -ray- and neutron-induced *black* point mutations.

### Sequence analysis

Sequencing of *black* [15,30] and *cinnabar* [31] genomic regions in  $\gamma$ -ray- and neutron-induced PCR+ mutants revealed a wide spectrum of DNA changes. Base substitutions prevail in  $\gamma$ -ray-induced mutants whereas vast majority of neutron-induced mutants had DNA sequence of the maternal allele *b*<sup>1</sup> instead of paternal allele *b*<sup>+ D32</sup> (Figure 3), which can only be the result of gene conversion events. On the whole, two new, interesting and important findings were established [15]: (i) three  $\gamma$ -ray-induced mutants had a cluster of base substitutions, insertions and deletions of a small sizes within one turn of the double-stranded DNA helix and (ii) among the  $\gamma$ -ray- and neutron-induced PCR + autosomal *black*, *cinnabar*, *vestigial* mutations, there were mutants with gene conversion events [32]. Their appearance is possible only after the uniting



**Figure 3:** Relative rates of DNA changes underlying the *black* gene/ point mutations induced by  $\gamma$ -rays and neutrons.

of the maternal and paternal genomes at the stage of the first mitotic cycle of early embryogenesis and, therefore, initial putative complex DNA damage could not be repaired before this stage. These findings are obviously of great importance for understanding the biophysical and repair processes underlying radiation mutagenesis.

## Conclusion

The results of our experiments presented above convincingly show that mutants with the same visible phenotype may be determined by variety (up to fourteen, Figure 2) of genetic alterations at the chromosomal and intragenic levels. Thus, the fundamental statement of classical radiation genetics about two main classes of radiation mutations is confirmed by our results at the gene level. These results also indicate that precise knowledge of genetic nature of an inherited gene mutation requires a complex of genetic, cytogenetic and molecular methods. Only this approach makes it possible to identify true point (intragenic) mutations among the changes at the chromosomal level, the molecular nature of which is finally established with the help of PCR and sequencing. The molecular approach allows us to obtain new interesting and important findings: for the first time we found that unlike neutrons,  $\gamma$ -rays regularly induce clusters of radiation-induced DNA changes even within one turn of a double-stranded helix but neutrons unlike  $\gamma$ -rays induce extremely high efficiency gene conversion, triggers for which could be complex DNA damages. The last phenomenon was discovered due to the fact that the maternal allele *black*<sup>1</sup> was marked with base substitutions. Such a high efficiency of neutrons in induction of gene conversion seems to be able to explain the negative

results of studies [33] to identify the genetic consequences for humans and their progeny after the atomic bombing in Japan. In scientific term, sequencing-based data open up a prospect for solving an important and still open issue about which initial radiation-induced DNA damage (single- and double-strand breaks of different types, complex DNA damage) underlie inherited DNA changes. This issue can apparently be solved by simulating the yield of primary DNA changes under the same conditions of the radiation experiment under which the genetic experiment was carried out. In practical terms, the results of sequencing of radiation-induced *de novo* mutations in germline cells opens up the prospect for assessment of hazard (risk) of different quality radiation at the molecular level, where, as we have shown [15], these assessments can significantly differ from those at the phenotypic level.

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MELANOGASTER. СООБЩЕНИЕ 6. ГЕН CINNABAR: СЕКВЕНИРОВАНИЕ Г-И НЕЙТРОН-ИНДУЦИРОВАННЫХ "ТОЧКОВЫХ" МУТАЦИЙ. Радиационная биология. Радиоэкология. 2018;58(1):15-25.

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