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Relationship between viability and genotoxic effect of gamma rays delivered at different dose rates in somatic cells of *Drosophila melanogaster*

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ABSTRACT
The role of dose rate (DR) on biological effects of ionizing radiation is an area of significant research focus and relevant to environmental exposures. The present investigation was aimed to examine the direct relationship between viability and genotoxicity in *Drosophila melanogaster*, induced by gamma rays in a range of doses from 2 to 35 Gy administered at three different DR. Results indicated that larval-adult viability was reduced in relation to dose but not DR. No marked differences were found in the LD_{50} produced by differing DR tested. Frequencies of somatic mutation and recombination increased in direct correlation with dose and DR. Data demonstrate the importance of determination of the relationship between viability and genotoxicity induced by DR in *in vivo* systems for toxicological and radioprotection studies.

KEYWORDS
Somatic mutation; D. melanogaster; Radiation dose rate; larvae-viability; Gamma rays

Introduction

The growing use of nuclear power increases occupational exposures and risk of industrial accidents. It is well known that ionizing radiation produces lesions in DNA, diminished reproductive capacity, reduced somatic growth, inhibition of bone marrow stromal cells and genotoxic effects correlated with an increasing dose rate (DR) (Lecomte-Pradines et al. 2017; Zhang et al. 2010; Zuo et al. 2012). Based upon these observations there is a growing interest examining the role of different factors such as DR in the biological consequences attributed to ionizing radiation, particularly when delivery of a low-DR occurs, a situation which is environmentally relevant (Brenner et al. 2003). As with other organisms such as *Caenorhabditis elegans* or mouse (Lecomte-Pradines et al. 2017; Zhang et al. 2010), radiation may exert either acute or lethal and sublethal effects on Drosophila induced by high or low doses of radiation, respectively (Hall and Giaccia 2012).

Ionizing radiation might induce direct breakage on the chemical bonds of biological macromolecules when it is absorbed by cells (Kam and Banati 2013). Ionizing radiation might also affect proteins, nucleic acids, and complex lipids as a result of the generation of reactive oxygen species (ROS) via radiolysis of water or alterations in mitochondrial functions (Kam and Banati 2013). Numerous studies showed that the amount of genetic damage produced by radiation, increases in direct relation to DR (Bedford and Mitchell 1973; Brooks, Hoel, and Preston 2016; Hall and Giaccia 2012; Tanarro and Tanarro 2008). This expected effect of DR was observed in different systems and assays. In rat fibroblasts irradiated at 0.0, 3.9, 7.4 and 11.3 Gy for 4 or 67 hr, the number of chromosomal aberrations rose in relation to dose and DR (Brooks et al. 1995). When DNA damage was measured after exposing human fibroblasts at different doses (0–5 Gy) at 0.3 or 1.8 Gy/min from a ^{137}Cs source, Ishizaki et al. (2004) found that high DR enhanced the formation of phosphorylated histone complex (γH2AX). Further, at sites of double strand breaks, this effect increased in relation to dose; however, no marked response was noted with low DR (Ishizaki et al. 2004). In another study, mice of strain C57BL6/FYDR/FYDR were exposed to a cumulative dose 400-fold greater than background level (approximately 10.5 cGy) at a low DR of 0.00017 cGy/min, no marked alteration in DNA damage was detected, but at a DR of 7.1 cGy/min the number of oxidized bases,

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micronucleus (MN) formation, homologous recombination, and gene expression increased (Olipitz et al. 2012). Brooks, Hoel, and Preston (2016) also reported that control of the cell cycle becomes dysfunctional when cells are exposed to high doses and DR that induce alterations in cell replacement which constitutes an important mechanism that has been linked to the incidence of cancer development.

Although it is well known that the biological consequences increase at higher radiation doses the influence of low dose and low-DR radiation exposures on health is not well understood. The definition of low dose is controversial Mosse, 2012, because there is no universal agreement to this categorization. It is now well-known that extrapolating data generated from cell lines cultured as monolayers to health risks in humans may be unrealistic. At present extrapolation of the effects of high-dose exposures based upon the linear no-threshold (LNT) model are employed to determine low dose and low-DR radiation exposures (Brenner et al. 2003). The linear non-threshold (LNT) model has been widely accepted as a basis for the estimation of radiation risks to humans (Koana et al. 2007). However, Mitchell, Bedford, and Bailey (1979) found that reducing the DR from 1.54 to 0.37 Gy/hr in HeLa cells resulted in more cell death for a given absorbed dose. In contrast, several investigators noted that low doses and DR induce beneficial biological effects. de Toledo et al. (2006) exposed acute or chronically fibroblasts at doses between 1 and 10 cGy at 6 cGy/min or 3.3 Gy/min and reported that the frequency of MN increased in direct relation to dose at higher DR, whereas the highest dose at the lowest DR reduced MN frequency to a level similar or lower than baseline, yet lymphocyte viability was the same when exposed at both DR. With myeloid cells (ML-1), Amundson et al. (2003) demonstrated that radiation doses at a range of 0.28–290 cGy/min, there were changes in the genome correlated with DR. It is of interest that inhibition of apoptotic induction detected with the lowest radiation dose was accompanied by enhancement in expression of genes regulated by P53, involved in the control of the cell cycle and apoptosis (Amundson et al. 2003).

The genome-wide expression techniques of mouse and human cells enabled measurement of gene expression patterns after exposure to a net dose of radiation at low or high DR. It is well-known shown that the gene expression pattern are markedly altered *in vitro* or *in vivo* (Abend et al. 2016) There are genes that response to a high DR exposures, (Amundson et al. 2003) while others are altered by a low DR (Bong et al. 2013). The induced genes that have been detected are involved in apoptosis pathways, protein synthesis, heat shock, immune response, DNA repair, cell cycle control and oxidative stress response (Paul et al. 2015; Ghandhi et al. 2015). These genes conduced to beneficial effects (Tang, Loke, and Khoo 2016) such as reduction of tumorigenesis (Sakai, Nomura, and Ina 2006), increasing longevity and improving immune responses (Ina and Sakai 2004).

Experiments in *in vivo* systems are fundamental in estimating the risk of exposure to artificial sources of ionizing radiation due to the increase use of sources for medical and industrial purposes. As human studies are not feasible there has been a shift to biological systems such as invertebrates or animal models to extrapolate observations in these models to correlate to humans with respect the role of radiation dose and DR (Morgan and Bair 2013). *Drosophila melanogaster*, the fruit fly, is an excellent *in vivo* model, essential to provide a mechanistic basis for understanding the radiation biological effects in areas of significant research focus such as dose (Gy) and DR (Gy/hr) (Zhikrevetskaya et al. 2015)

The dose–response relationship between ionizing radiation and induced mutation frequency was reported using a sex-linked recessive lethal assay in mature sperm of *D. melanogaster* and confirmed by successive studies using bacteria, yeast, mammalian cells cultures and mice (Koana et al. 2007). The use of *D. melanogaster* in scientific investigations has several advantages: this species is inexpensive, provides information on processes such as mutagenesis, somatic recombination and lethal gene induction (Würgler, Sobels, and Vogel 1977; Vogel et al. 1999; Pandey and Nichols 2011; Alaraby et al. 2016). Previously *Drosophila* was employed to study radiation-induced oxidative stress and the role of radioprotective agents (Cruces, Pimentel, and Zimmering 2003; Pimentel, Cruces, and Zimmering 2000; Zimmering et al. 1990). It is now known that 75% of the genes that produce diseases in humans have homologs in the fruit fly (Adams et al. 2000). These genes are associated with the development of human diseases such as cancer (Pandey and Nichols

2011; Vogel et al. 1999; Würgler, Sobels, and Vogel 1977). To contribute with testable and predictive models of human health effects following exposure to ionizing radiation, the purpose of this study was to (1) examine the direct relationship between viability and genotoxicity induced by three different DR of gamma rays *in vivo* and (2) provide potential reference information for genotoxic and radioprotection investigations.

Materials and methods

Biological material

Strains from *Drosophila melanogaster* with genotype *mwh* +/*mwh* + and *flr*^{3}/*In* (3LR) TM3, *Ser* were used. Both strains possess genetic markers that modify wing trichomes. The *mwh* marker (multiple wing hair) is located on the left arm of chromosome 3 in position 3–0.3. The *flr*^{3}/TM3 marker, *Ser* is situated on the left arm of chromosome 3 in position 3–39. The TM3 balancer is necessary since the *flr*^{3} allele is lethal in a homozygous condition.

Larvae collection

For this purpose, virgin females *mwh* +/+ *mwh* were crossed with males *flr*^{3}/*In* (3LR), TM3, *Ser*, from 4 to 5 days old. It was necessary to homogenize larvae at a specific age (48-hr-old), the parents were crossed for 2 hr, immediately after placed to oviposit in 250 ml flasks with regular culture medium consisting of agar, corn flour, sucrose, dextrose, yeast and propionic acid and nipagine as antibiotics, in groups of 100 couples per bottle. Eggs laid were restricted to a two hr period to obtain more homogeneous samples and then allowed to develop in a culture room at 25 ± 1°C and 60% relative humidity for 3 days to obtain 48-hr-old larvae. Larvae possess groups of undifferentiated dividing cells which transform during metamorphosis into the adult fly structures: legs, antennae, and wings (Klebes et al. 2002). These larvae were collected by density difference with a 20% sucrose solution (Graf et al. 1984).

Radiation treatment

The collected larvae were divided into 9 groups where 7 were irradiated with sublethal doses: 5, 10, 15, 20, 25, 30 or 35 Gy of gamma rays, one without radiation as negative control and the last one with 1 mM CrO_3 as a positive control. Larvae were irradiated in glass tubes (2.5 cm diameter and 3 cm height) containing a wet filter paper. The doses were delivered using three different DR: 5.1, 32.9 and 860.9 Gy/hr. Three independent experiments were performed with three replicates each and for each DR in a span of 6 months and each experiment for each DR was conducted simultaneously. After irradiation, between 900 and 1500 larvae were tested per dose and DR, and were placed in groups of 100 per homeopathic vial with 0.8 g of synthetic medium (Formula 4–24 *Drosophila* Medium® Carolina Biological Supply, Co. USA) hydrated with 2.5 ml distilled water. Larvae were irradiated with a Co^{60} source: 5.1 Gy/hr in a Vick-Rad 2000 (Vickers Radiation Company, Swindon, England); 32.9 Gy/hr in a Gammacell (MDS Nordion, Canada) and 860.9 Gy/hr in a Transelektro LGI-01 irradiator.

Larval-adult viability test

The treated larvae terminated development in a culture room under optimal conditions of temperature and humidity. The number of emerged adults, females and males from each of the treatments was counted daily, separately. The larval-adult viability was calculated and plotted from the number of emerged individuals to determine the LD_{50} and dose–response relationship for each DR tested.

Genotoxicity test

The SMART test (somatic mutation and recombination test) was used in the wing of *D. melanogaster* (Graf et al. 1984). Briefly, the SMART employed with *D. melanogaster* is an efficient and versatile short-term eukaryotic *in vivo* assay which detects several types of mutation as well as somatic recombination in cells of the imaginal disc of the larvae. This test is mainly based upon induction of loss of heterozygosity of two recessive markers that code for the shape of the trichome in *D. melanogaster* wing cells: i.e. multiple wing hair (*mwh*) and flare (*flr*^{3}). These enable the formation of mutant cell groups (clones) which are expressed as mutant cell spots on the wing

(Graf et al. 1984). The wing somatic mutation assay was employed in numerous studies to assess genotoxicity of various agents (physical, chemical), extracts and complex mixtures (de Andrade, Reguly, and Lehmann 2004). After treatment, the viable individuals were fixed in alcohol at 70% to make permanent slices with the wings of the *mwh +/+ flr*[3] genotype organisms. Subsequently, the number of spots – genetic damage – induced was analyzed at 400X using a compound microscope from 40 wings (20 females and 20 males) from each experiment.

Statistical analysis

To establish differences between treatments, the larval-adult viability results were analyzed with a Student's *t-test* at 0.05 probability level. The toxicity was obtained dividing the total of viable adults by the number of larvae tested. The LD_{50} was calculated for different DR using the PROBIT method (Log probit analysis by maximum likelihood at CI 95%). The linear regression fitted was undertaken by least squares in order to obtain the dose relationship for each DR tested. Data of mutations and recombination were analyzed using the SMART computer program which is based upon the multiple-decision procedure of Frei and Würgler (1988), which enables to obtain four different diagnoses: negative (-), weakly positive (w), positive (+) and inconclusive (i). The procedure was based upon two hypotheses: (1) there is no difference in the mutation frequency between control and treatment series; (2) radiation treatment results have an increasing mutation frequency n times the induced in negative controls. Because small single spots and total spots have a comparatively high spontaneous frequency, m is fixed at a value of 2 (testing for a doubling of the spontaneous frequency to define a negative results). For the large single spots and the twin spots, which have a lower spontaneous frequency, m = 5 is used. Both hypotheses are tested at 5% significance level. To test against the hypotheses, the conditional binomial test according to Kastenbaum and Bowman or Chi-Square test for proportions may be applied (Frei and Würgler 1988).

Results

Larval-adult viability

Figure 1 illustrates the larval-adult viability plotted from the mean number of emerged individuals from three independent experiments. The curves indicated that larval-adult viability of irradiated individuals decreased indirect relationship with net dose but not with DR. The LD_{50} calculated from the dose–

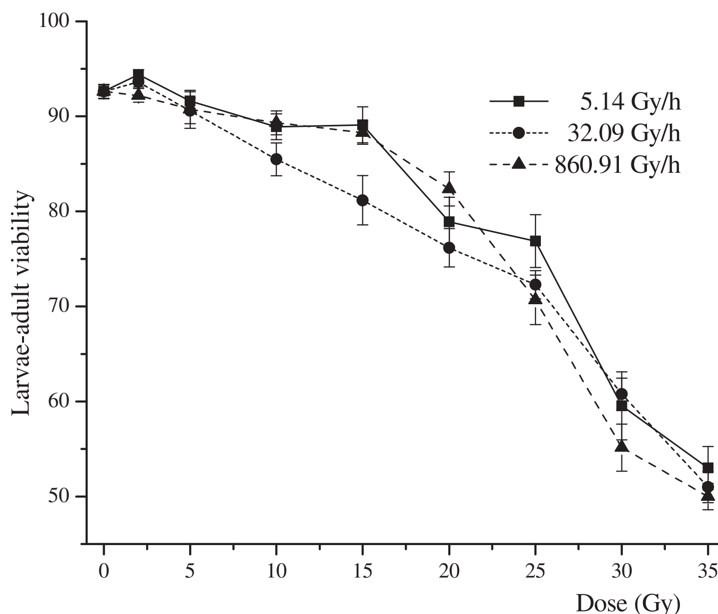

Figure 1. Larval-adult viability of *mwh +/+ flr*[3] individuals of *Drosophila*, after being irradiated with different doses and DR of gamma rays. Data represent the results from three independent experiments performed with three replicates each one for each DR.

response relationship curves for each DR tested were: 41.83 ± 1.4, 38.92 ± 1.5, 40.51 ± 2.6 Gy with 5.1, 32.9 y 860.9 Gy/hr, respectively, with no significant differences between them. Although 10 and 15 Gy to the intermediate DR (32.9 Gy/hr) resulted in a significant fall in viability of the larvae, the dose of LD_{50} did not change markedly.

Genotoxicity

Wing cells blades were analyzed to identify small single spots (one to two cells) of either *mwh* or *flr*, large single spots (>2 cells) of either *mwh* or *flr*, and *mwh–flr* twin spots. Briefly (a) single *mwh* spots are inferred to arise from a separation between *mwh* and *flr* as from an interchange or from mutation/deletion at the *mwh+* locus; (b) single *flr* spots from mutation/deletion at the *flr+* locus or double exchange; and (c) twin spots following interchange between *flr* and the centromere. Since *flr* may behave as a cell semi-lethal in some tissues, the possibility cannot be excluded that some fraction of large *mwh* spots originated as twin spots, which then lost *flr*-bearing cells.

Table 1 provides results from three independent experiments for the frequency of somatic mutations and recombination induced in *D. melanogaster* after exposure to different doses and DR of gamma radiation. A significant rise in the three categories of spots: small, large and twin over the all tested dose range (+) and for each DR was detected. It is important to note that with 2 and 5 Gy, the frequencies of total-induced spots was similar for the DR of 5.1 and 32.9 Gy/hr and was increased significantly with respect to control with 5 Gy at the three DR tested.

The size of the induced clone showed a relationship between dose and DR. The slopes calculated by the regression analysis for twin spots frequency (Table 1) induced by each RD tested, were: 5.1 and 860.9 Gy/hr = 0.002

Table 1. Somatic mutation frequency induced in the *mwh* +/+ *flr*^{3} flies of *D. melanogaster* after treatment in larvae stage of 48 h age with different net doses of gamma rays at three DR.

DR Gy/h	Net dose Gy	No. of wings	Spots: Small n	Small s/w	Large n	Large s/w	Twin n	Twin s/w	Total n	Total s/w	Frequency of clone formation x 10^{-5} cells observed	corrected
			(1–2 cells), m = 2		(>2 cells), m = 5		m = 5		m = 2			
1mMCrO_3		120	95	0.79	273	2.28	182	1.52	550	4.58	12.9	4.8
Control	**0**	120	27	0.22	6	0.05	3	0.03	36	0.30	1.6	1.7
5.1	**2**	120	44	0.37 -	17	0.14 i	2	0.02 i	63	0.52 -	2.3	2.1
	5	120	58	0.48 +	50	0.42 +	6	0.05 i	114	0.95 +	8.9	9.6
	10	120	56	0.47 +	94	0.78 +	5	0.04 i	155	1.29 +	17.6	20.0
	15	120	63	0.52 +	96	0.80 +	4	0.03 i	163	1.36 +	26.5	32.8
	20	120	83	0.69 +	91	0.76 +	11	0.09 +	185	1.54 +	20.5	22.5
	25	120	63	0.52 +	174	1.45 +	15	0.12 +	252	2.10 +	33.1	36.4
	30	120	105	0.88 +	141	1.20 +	11	0.09 +	257	2.14 +	37.7	47.3
	35	120	72	0.60 +	230	1.92 +	7	0.06 i	309	2.58 +	82.7	96.4
32.9	**2**	120	43	0.36 -	12	0.10 -	5	0.04 i	60	0.50 -	2.4	2.1
	5	120	56	0.47 +	92	0.77 +	6	0.05 i	154	1.28 +	21.5	26.3
	10	120	73	0.61 +	121	1.01 +	12	0.10 +	206	1.72 +	26.0	29.5
	15	120	83	0.69 +	174	1.45 +	10	0.08 +	267	2.23 +	49.0	55.8
	20	120	128	1.07 +	168	1.40 +	13	0.12 +	314	2.62 +	36.1	38.6
	25	120	56	0.47 +	294	2.45 +	20	0.17 +	370	3.08 +	77.1	84.0
	30	120	130	1.08 +	256	2.13 +	15	0.12 +	401	3.34 +	107.2	115.9
	35	120	123	1.02 +	355	2.96 +	16	0.13 +	494	4.12 +	108.4	126.0
860.9	**2**	120	60	0.50 i	44	0.37 +	4	0.03 i	108	0.90 +	3.2	3.7
	5	120	105	0.87 +	119	0.99 +	22	0.18 +	246	2.05 +	26.9	28.5
	10	120	85	0.71 +	161	1.34 +	12	0.10 +	258	2.15 +	38.9	43.6
	15	120	75	0.62 +	236	1.97 +	26	0.22 +	337	2.81 +	83.7	96.3
	20	120	66	0.55 +	341	2.84 +	11	0.09 +	418	3.48 +	112.8	126.5
	25	80	66	0.83 +	218	2.73 +	11	0.11 +	295	3.68 +	146.0	168.2
	30	80	53	0.66 +	258	3.23 +	11	0.11 +	322	4.02 +	151.3	168.6
	35	120	76	0.63 +	519	4.32 +	23	0.19 +	618	5.15 +	242.3	266.6

Statistical diagnoses according to Frei and Würgler (1988): +: positive; -: negative; w: weak positive; i: inconclusive, respect to control; m: multiplication factor. Probability levels: alpha = beta = 0.05. One-side statistical test. DR: Doses rate; s/w: spot per wing. Data represent the results from three independent experiments performed with three replicates each one for each DR.

Table 2. Somatic mutation frequency induced in the *mwh*/TM3, *Ser* flies of *D. melanogaster* after treatment in larvae stage of 48 h age with different net doses of gamma rays at three DR.

DR	Net	No. of	Spots: Small		Large		Total		Mutation	M	R
Gy/h	Gy	wings	n	s/w	n	s/w	n	s/w	frequency	%	%
			(1–2 cells), m = 2		(>2 cells), m = 5		m = 2		Corrected[a]		
1mMCrO$_3$		120	68	0.56	8	0.06	76	0.63	0.72	15.7	82.3
Control	0	120	35	0.29	2	0.02	37	0.31	0.35	96.7	3.3
5.1	5	120	26	0.22-	7	0.06i	33	0.27-	0.31	32.6	67.4
	15	120	36	0.30-	7	0.06i	43	0.36-	0.41	30.1	69.8
	25	120	56	0.46+	15	0.12+	71	0.59+	0.59	28.1	71.9
	35	120	42	0.35-	20	0.17+	62	0.52+	0.58	22.5	77.5
32.9	**5**	**120**	**25**	**0.21-**	**7**	**0.06i**	**32**	**0.27-**	**0.30**	**23.4**	**76.6**
	15	**120**	**38**	**0.32-**	**18**	**0.15+**	**56**	**0.47+**	**0.53**	**23.8**	**76.2**
	25	**120**	**39**	**0.33-**	**17**	**0.14+**	**56**	**0.47+**	**0.51**	**16.6**	**83.4**
	35	**120**	**32**	**0.32-**	**14**	**0.12+**	**53**	**0.44i**	**0.50**	**12.1**	**87.9**
861.9	5	120	37	0.32-	4	0.03i	41	0.34-	0.39	19.0	81.0
	15	120	48	0.40i	10	0.08+	58	0.48+	0.63	22.4	77.6
	25	120	55	0.46+	28	0.23+	83	0.69+	0.85	23.1	76.9
	35	160	53	0.44+	29	0.24+	82	0.68+	0.76	14.8	85.2

Statistical diagnoses according to Frei and Würgler (1988): +: positive; -: negative; w: weak positive; i: inconclusive, respect to control; m: multiplication factor. Probability levels: alpha = beta = 0.05. One-side statistical test. DR: Doses rate; s/w: spot per wing; M: mutation and R: recombination. Data represent the results from three independent experiments performed with three replicates each one for each DR.

and 32.9 Gy/hr = 0.004 and correlation coefficients (r^2) 5.1 Gy/hr = 0.49, and 860.9 Gy/hr = 0.06 which were low with the exception of intermediate DR (32.9 Gy/hr = 0.79). However, to obtain recombination frequency that includes exchanges occurred between *mwh* and *flr* markers, producing two daughter cells with mwh phenotype, the spot frequencies induced in the *mwh*/TM3, *Ser* wings irradiated with 5, 15, 25 or 35 Gy were counted (Table 2), and subtracted from the total frequency of mutation and recombination induced in the *mwh* +/+ *flr*^{3} wings. The frequencies were corrected taking into account the 14% decrease in the area of the *mwh*/TM3, *Ser* wing (Table 3) provoked by serratia character (Table 2 and Figure 3), the total area of the wings was measured (Vidal et al. 2017) using the Image program J 1.46 (Collins 2007). The recombination data were plotted (Figure 4). The linear regression results demonstrated slope significant to 5.1 DR ($F < 0.02$); for 32.9 Gy/hr ($F = 0.058$) and regression coefficient (r^2) for 5.1 and 32.9 Gy/hr showed good linear correlation but not for 860.9 Gy/hr (−0.25) (Figure 4).

Table 3. Wing area of *mwh* +/+ *flr*^{3} and *mwh*/TM3, *Ser* individuals treated with 15 Gy of gamma rays.

	♀		♂	
Genotype	*n*	Wing zise (mm^2) ± *SEM*	*n*	Wing zise (mm^2) ± *SEM*
mwh +/+ *flr*^{3}	40	3.76 ± 0.04	40	2.92 ± 0.02
mwh/TM3, *Ser*	40	3.16 ± 0.03	40	2.59 ± 0.03
Reduction in area in *mwh*/TM3, *Ser*		0.61		0.33

n: represents 20 wings from flies treated with 15 at 5.14 Gy/h plus 20 wings from individuals treated with 15 Gy at 860.91 Gy/h, from three independent experiments.

Discussion

Health risks associated with exposure to low-DR were estimated by extrapolating empirical linear fits for data on humans exposed to relatively high doses (BEIR, National Research Council 2006; Kim et al. 2015); however, the biological effects of radiation depend upon several factors such as DR. Although radiation effects were examined extensively, the biological consequences of low-DR exposure are controversial (Kim et al. 2015). In this study, *D. melanogaster* was employed as a multicellular model organism to investigate the correlation between viability and genetic damage induced directly in the same individuals treated with different DR gamma rays. Recently González et al. (2018) showed that ascorbic acid (Aa) reduced the genetic damage induced by 20 Gy of gamma rays and effectiveness was dependent upon the DR with which the 20 Gy were administered. Only the lowest dose of Aa decreased the frequency of somatic mutations with the high-DR tested

(González et al. 2018). Vidal et al. (2018) also demonstrated that 0.5 or 1 Gy of gamma rays at 5.4 or 36 Gy/hr induced radio- and chemo-protection against damage induced by 20 Gy or 1 mM of chromium trioxide. Both studies support the theory of the direct effects of DR on *D. melanogaster.*

The fact that the larval-adult viability decreased in direct correlation with dose but not with DR (Figure 1) contrasts with the postulation of a direct effect of DR, which indicated that if radiation dose is administered at a high-DR the biological effect is expected to be higher, and if it is exposure to low-DR the biological effect is expected to be lower (Brooks, Hoel, and Preston 2016; Tanarro and Tanarro 2008). This result puts this study in another perspective if one considers that cell toxicity mediated by radiation doses are an important criterion for cancer treatments with radiotherapy; however genetic damage, induced directly in the same exposed individuals, demonstrated that the induction was associated with the dose and DR. Our findings are in agreement with the phenomenon termed "expected effect of the dose rate" (Bedford and Mitchell 1973; Hall and Giaccia 2012) and this was revealed by slope (m) and the regression coefficient r^2 calculated by linear regression from plotting total frequency of spots induced by the three different DRs (Figure 2). It is feasible

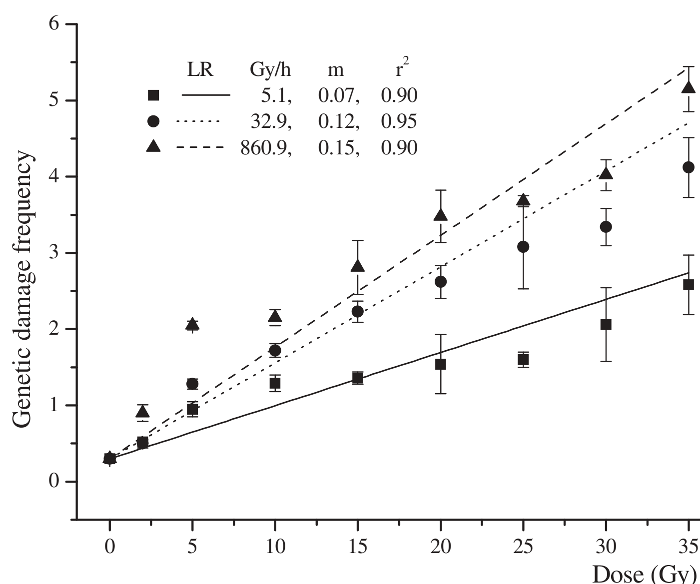

Figure 2. Frequency of genetic damage of individuals *mwh +/+ flr*^{3} of *Drosophila*, after being irradiated with different doses and DR of gamma rays. The slop (m) and regression coefficient (r^2) are results of the linear regression fitted by least-square (regression equation). Data represent the results from three independent experiments performed with three replicates each one for each DR.

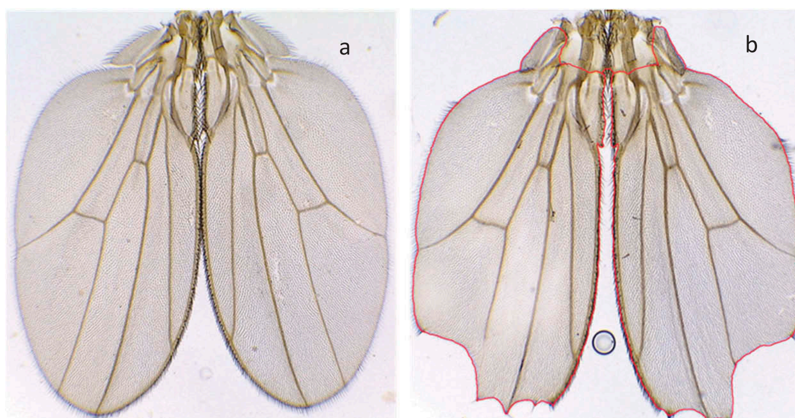

Figure 3. Shows the wings form and size: (a) *mwh/flr*^{3} and (b) *mwh*/TM3, *Ser*, where the line shows size 14% less average area between females and males than the *mwh/flr*^{3} wings.

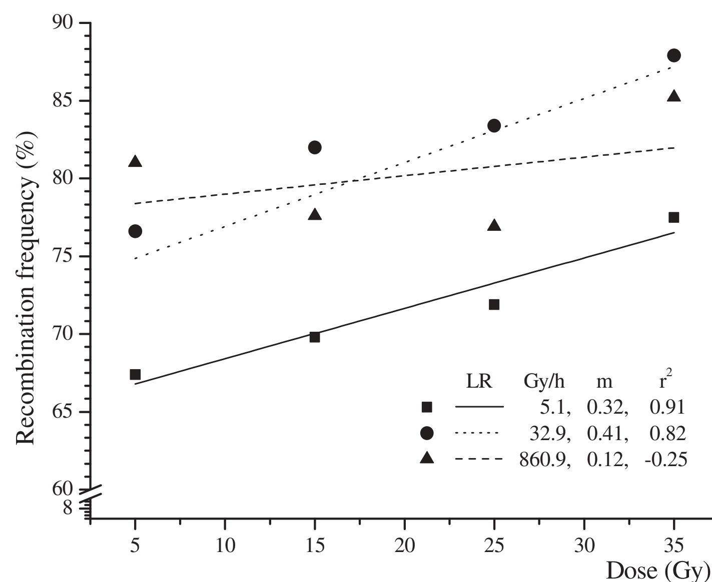

Figure 4. Dose relationship of the recombination induced by the different DR in a dose range from 5 to 35 Gy. The slop (*m*) and regression coefficient (r^2) are results of the linear regression (LR) fitted by least-square (regression equation). Data represent the results from three independent experiments performed with three replicates each one for each DR.

that the results of genetic damage induced by low-DR in this study may be due to repair mechanisms which reverse the damage produced by radiation. This was previously seen using *Drosophila* strains with defects in repair mechanisms such as *mei* or *mus*, where these strains even at low doses at low-DR generated a higher level of genetic damage, compared to that in wild type strains (Iushkova, Zaĭnullin, and Startseva 2011). Kim et al. (2015) found that the sex-linked recessive lethal mutation frequency in *D. melanogaster* with a low dose at low-DR exposure was significantly lower in immature spermatocytes and spermatogonia compared to sham-irradiated group, whereas irradiation with a higher dose resulted in a significant elevation in mutation frequency. If low-DR produced induction of repair enzymes activities as reported by Ishizaki et al. (2004), it is conceivable that a dose administered to low-DR may exert cell toxicity equivalent to a high dose. However, less genetic damage induction was found.

Somatic recombination has important implications in the estimation of the risk of developing cancer (Ramel et al. 1996). In the SMART test, if recombination takes place in the region of the chromosome between *mwh* and *flr* markers, two daughter cells arise: one expressing mwh phenotype and the other expressing the wild type phenotype are formed, these mwh spots are indistinguishable from those originating from point mutation or deletions. The method to estimate the proportion of induced mwh singles ascribable to mutation *vs*. recombination, is to compare the frequency of mwh singles in the *mwh* +/+ *flr* with *mwh* +/+ TM3, *Ser* individuals. The latter is taken as a measure of mutations, because, the products of mitotic recombination between the TM3 chromosome bearing multiple inversions and its structurally normal homolog are not viable (Zimmering et al. 1997). For this reason and in order to exclude the % of *mwh* spots resulting from mutation events, the wings of the *mwh*/TM3, *Ser* individuals were analyzed. Results from linear regression analysis (Figure 3), showed that the rate of somatic recombination-induction was significant dose-dependent increases with 5.1 and 32.9 Gy/hr. However somatic recombination-induction by 860.9 Gy/hr was not. Some investigators using doses at low DR found -reverse effects (Marin et al. 1991; Russell and Kelly 1981); however, results obtained in this investigation were contradictory.

Conclusions

Data obtained in this study demonstrated directly the relationship between viability and genotoxicity induced by three dose rates (DR) of gamma rays *in vivo* and indicated that larval-adult viability was reduced in relation to dose but not DR; however,

somatic mutation frequencies and recombination increased in direct association with both dose and DR. The applied radiation, within the tested DR, confirmed a direct effect of DR of gamma rays.

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