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Biological effects of low-dose γ-ray irradiation on chromosomes and DNA of *Drosophila melanogaster*

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ABSTRACT

While the damage to chromosomes and genes induced by high-dose radiation (HDR) has been well researched in many organisms, the effects of low-dose radiation (LDR), defined as a radiation dose of ≤100 mSv, are still being debated. Recent research has suggested that the biological effects of LDR differ from those observed in HDR. To detect the effect of LDR on genes, we selected a gene of *Drosophila melanogaster*, known as the *multiple wing hair (mwh)* gene. The hatched heterozygous larvae with genotype *mwh*/+ were irradiated by γ-rays of a ^{60}Co source. After eclosion, the wing hairs of the heterozygous flies were observed. The area of only one or two *mwh* cells (small spot) and that of more than three *mwh* cells (large spot) were counted. The ratio of the two kinds of spots were compared between groups irradiated by different doses including a non-irradiated control group. For the small spot in females, the eruption frequency increased in the groups irradiated with 20–75 mGy, indicating hypersensitivity (HRS) to LDR, while in the groups irradiated with 200 and 300 mGy, the frequency decreased, indicating induced radioresistance (IRR), while in males, 50 and 100 mGy conferred HRS and 75 and 200 mGy conferred IRR. For the large spot in females, 75 mGy conferred HRS and 100–800 mGy conferred IRR. In conclusion, HRS and IRR to LDR was found in *Drosophila* wing cells by delimiting the dose of γ-rays finely, except in the male large spot.

Keywords: low-dose radiation; *Drosophila*; chromosome; gene; mutation; somatic recombination

INTRODUCTION

There have been controversies over the biological effects of low-dose radiation (LDR, also known as low-dose ionizing radiation, LDIR) for a long time, despite LDR being ubiquitous in our environment, ranging from medical exposure for diagnosis and radiotherapy to exposure to radioactive substance leakage following an accident at a nuclear facility and exposure to space radiation during a flight. Considerable evidence suggests that the biological effects of LDR differ from those of high-dose radiation (HDR) [1–4].

Animal experimental studies indicate that exposure to LDR (typically doses of <200 mSv according to United Nations Scientific Committee on the Effects of Atomic Radiation [5]) may be harmful. It induces genetic and epigenetic changes and is more frequently associated with a range of physiological disturbances for the immune system, circulatory system, visual system, embryonic development, brain development, tumorigenesis and DNA molecules than the eruption rate inferred by the linear no-threshold (LNT) model [2, 3, 6, 7]. These phenomena are known as 'hypersensitivity (HRS),' which means that harmful effects are greater than expected from those existing in the LNT model. One of the mechanisms of HRS is known as the 'bystander effect' [2, 8]. Meanwhile, induced radioresistance (IRR), such as radio-adaptive response to LDR, has been reported [9–11]. However, the most effective doses causing HRS and the boundary of doses for IRR are varied and depend on animal species, genetic background, age, gender, way of radiation exposure (e.g. acute, fractionated or chronic) and type of radiation [2, 4, 12–15].

Ionizing radiation can induce genomic lesions, such as DNA double-strand breaks, whose incomplete or faulty repair can result in gene mutations, which, in turn, can influence the cellular functions and alter the fate of the affected cells and organ systems [15].

Ionizing radiation-induced sequence alterations/mutations occur stochastically, contributing to increased cancer risk in irradiated individuals for higher animals like humans [6]. Thus, both LDR and HDR have the potential to initiate cancer development in organisms because most cancers are caused by the initial alterations of oncogenes, anticancer genes, DNA repair-relevant genes, cell attachment genes and cell migration genes.

In contrast, some experimental studies have indicated that LDR can benefit organisms. For example, it can reduce tumorigenesis, prolong lifespan and enhance fertility, known as radiation hormesis, and the adaptive response by LDR to subsequent HDR [3, 4, 10, 11, 16–21].

The most useful assessment of the stochastic effects of radiation must be able to detect any genetic alterations or epigenetic changes that remain following DNA repair. However, with LDR, the probability of influence on biochemistry, physiology and DNA is usually very weak and is sometimes hindered by the background influence, to the extent that it becomes hard to distinguish the LDR influence from the background influence.

Only a few hypersensitive detection procedures can identify the DNA damage induced by radiation, ranging from doses of a few micrograys (μGy) to ∼10 mGy [7, 22]. In addition, only a few studies have investigated the effect of LDR on particular genes. A heredity study of gene analysis used a fruit fly, *Drosophila melanogaster*, with low breeding expenses, short generation, high reproducibility and many available mutants such as gene mutations, chromosomal recombination and easy-to-obtain pure strains.

The fruit fly has mechanisms of DNA replication, gene mutation and DNA repair that are important and in common with humans for the propagation of essential genes to the next generations.

This work evaluated the biological effects of LDR from a ^{60}Co source on a single gene of *Drosophila melanogaster*. To handle as many species as possible, we had to await the development of a system that can yield quick and accurate evaluations.

The mutation of *multiple wing hair* (*mwh*) of *Drosophila* has been used for mutagenic testing for a long time as well as *flr* [23–25] and *white ivory* [26]. The formation mechanism of the phenotype of many feathers per hair cell appearing in the homozygote or hemizygote of the *mwh* mutant was determined [27]. The *mwh* gene encodes a G protein binding domain-formin homology 3 (GBD-FH3) domain-containing protein that functions both before and after wing hair initiation [27].

In this study, the heterozygote of the +/*mwh* larva was exposed to γ-rays from LDR to HDR. After the emergence of adult flies, their wings were dissected and observed under a microscope to count the rate of deletion of the normal gene (mwh^+), the mutation of the *mwh* gene, and the somatic chromosomal recombination between the *mwh* gene and the centrosome.

Modifying the traditional method, we used a microscope to experiment with several irradiated samples. We carried out the experiment to detect injury to DNA or chromosome aberration with low-incidence LDR without treating the samples as specimens. In addition, we also recovered the wings, including the *mwh* cells, to be able to further analyze the gene at the molecular level to determine the commonality or difference between the features of lesions caused by HDR and LDR. A report indicated that the genomic variations existing in Nature and those caused by LDR (<100 mGy) are hardly different [7]. In contrast, the variation rate caused by HDR (>0.15 Gy) is considerably higher than that existing in Nature [7]. Moreover, the study indicated that the properties of mutations in the human hypoxanthine phosphoribosyltransferase (*HPRT*) gene are different [7].

Therefore, it is necessary to accurately determine the dose level at which HRS or IRR occurs in organisms and to delimit the dose more finely to be able to handle LDR properly. We examined the accurate dose of γ-rays for HRS and the border of HRS/IRR using the *Drosophila melanogaster* gene, *mwh.* To this end, we developed and utilized a speedy and sensitive method for detecting low-frequency mutations.

MATERIALS AND METHODS

Culturing the fly strains

The fly strains were fed with a standard medium, comprising 5% dry yeast, 7% glucose, 5% cornmeal, 1.2% agar and 0.35% propionic acid at 24°C, as described by Tanaka *et al.* [28].

Principles of detecting the effects on the *mwh* gene by wing spot method

The detection system of DNA and chromosomal aberration mutation in *Drosophila melanogaster* using the heterozygote for *mwh* was originally developed by Graf *et al.* [23] and was explained by drawings [29].

Briefly, trans-heterozygous larvae for the recessive marker mutation *mwh* were produced. Normal cell division in the wing disc cells of the genotype is +/*mwh*, resulting in heterozygous descendant cells with normal wing hairs in which only one large hair exists per cell because the *mwh* mutation is recessive. In the cell division process of the wing disc cells, deletion of the terminal short chromosome containing the normal *mwh* gene happens spontaneously. The deletion causes 'multiple wing hair'. However, the cells can divide at most once due to the existence of other important genes, thus causing one or two small cell clones, known as the 'small single spot (small spot)'. The small spot can be produced by other means, e.g. an additional mutation in the wild-type *mwh* gene, resulting in the homozygote cell(s) of the *mwh* gene mutation and chromosomal mal-disjunction [26, 30]. During cell division, somatic chromosomal recombination between the *mwh* gene and centromere rarely happens. The recombination causes regions of more than three cells with *mwh*, known as the 'large single spot (large spot)'. The large spot is also considered to emerge through *mwh* gene mutation [24]. These events are enhanced by many factors such as incomplete DNA replication, carcinogenic compounds, microwaves and irradiation [23, 29–31].

DNA analysis

In our study, males of the mutant strain mwh^1/mwh^1 obtained from *Drosophila* (Genetic Resource Center, DGRC No.101704) were mated with virgin females of a wild-type strain known as *Canton-S*. To confirm that the mwh^1 homozygote does not have the *mwh* gene, since the *mwh* mutant has been reported to be the null allele [27], a part of the seventh exon of the *mwh* gene was amplified by forward primer #555 (5′-TTGTCGAGGAGGATGAGGAC-3′) and reverse primer #727R (5′-TACGATGTTAACCGGCACAA-3′), which were determined using Primer3 (https://primer3plus.com/cgi-bin/dev/primer3plus.cgi). Approximately 100 wings were dissected, and genomic DNA was prepared using the Kaneka DNA purification kit for animals (Kaneka

Co. Ltd., Osaka, Japan). The gene encoding ribosomal protein 49 (RP49) was also amplified as an internal positive control by forward primer #444 (5′-TGCTAAGCTGTCGGTGAGTG-3′) and reverse primer #611R (5′-GTTCGATCCGTAACCGATGT-3′).

Polymerase chain reaction (PCR) was performed by mixing 25 μL of Fast Multiplex PCR Kit (KK5801, Kapa Biosystems, Wilmington, USA), 1 μL of genomic DNA, 1.25 μL of both primers and 21.5 μL of distilled water. The amplification conditions were 94°C, 1 min, 30 cycles of 94°C, 1 min → 57°C, 1 min 1 mi°C, 3 min, and 72°C, 2 min. The sequencing reaction was obtained from Microgen (Kyoto, Japan).

Determination of the developmental stage of *Drosophila* with the highest sensitivities to γ-rays

After the female flies laid eggs on the food, the eggs were collected using a brush and 20% sucrose. They were then transferred into 1.5 mL microtubes to be exposed to 15 000 mGy γ-rays from a ^{60}Co source at the Radiation Research Center of Osaka Prefecture University, Japan. The dose rate at a defined distance of each dish from the ^{60}Co source was calculated considering the attenuation of the ^{60}Co radioactivity. In the irradiation institute, when exchanging the radiation source, the reference dose rate distribution curve (the relationship between the distance from the radiation source and the absorbed dose rate place) was prepared using a traceable-ionization chamber-type irradiation dose-rate measuring device 550-6A-T (FLUKE-Biomedical, Everett, USA) which was already calibrated at the national standard gamma radiation field (National Institute of Advanced Industrial Science and Technology).

It was reported that at the pupal stage, the development of all wing hairs had already been completed. Thus, no more phenotypic change even after gene mutation or chromosomal aberration occurred. Among all the developmental stages from the egg to the third instar larva, the third instar larva exhibited the highest sensitivity to radiation [30, 32]. Our strains and cross patterns should be confirmed the same phenomenon.

For the collection of the first, second and third instar larvae, larvae from l, 2 and 3–4 days after laying eggs were collected by inserting 20% sucrose into the culture vials. After 5 min, the floating larvae were put in a glass dish. Based on size difference, the first, second and third instar larvae were discriminated, inserted into 1.5-mL microtubes, and exposed to γ-rays.

The dose of γ-rays was determined as 15 000 mGy based on our preliminary experiment [29]. For the 0-mGy group, the tubes were kept at the staff waiting room as the background control. After irradiation, all the larvae were grown in new culture vials. As it has been reported that in *Drosophila melanogaster* as well as other animals, females are generally more vulnerable to DNA damage and mutation rates than males [33, 34], wings of females and males were separately counted and analyzed. The wings were separated from the body using tweezers in ethanol and were inserted into microtubes. Ethanol was then replaced by pure water and finally by 0.5% sodium dodecyl sulfate (SDS). Using a syringe, the wings were transferred to a slide glass with a drop of 0.5% of SDS. The wing hairs were observed using a ZOE™ Fluorescent Cell Imager (Bio-Rad Laboratories, Inc., Hercules, USA). The small and large spots were counted separately.

Determination of the dose showing HRS and IRR to γ-rays in *Drosophila*

The third larvae were collected and inserted into 1.5-mL tubes with a cap. Larvae were irradiated at room temperature (24°C) with doses of 20, 50, 75, 100, 200, 300, 500, 800, 1500, 3000 and 7000 mGy with dose rates of 400, 1000, 1500, 2000, 4000, 6000, 10 000, 16 000, 30 000, 60 000 and 140 000 mGy/h, respectively. At the place of 20 and 50 mGy irradiation, actual doses were measured using glass dosimeters, Dose Ace (Chiyoda Technol Corp., Tokyo, Japan). The dosimeters read within 10 % errors from the calculated doses. After that, the process was the same as in the previous section.

Statistical analysis

Examination of the increase or decrease in spot eruption frequency by irradiation of different doses was performed using the conditional binominal test [25, 35] because the event of DNA lesion and chromosomal recombination occurs stochastically at a low rate, and there were some cases of a scarce number (<five) of mutant spot emergences. The significance level was set at 0.05.

RESULTS

Examination of the fly mutant strain *mwh*^{1} using phenotype and gene analysis

The *mwh*^{1} allele is an autosome recessive mutant of the *mwh* gene [27]. The wings of a wild-type *mwh*^{1} homozygote (i.e. *N378–32*) and F_1 hybrid were prepared by crossing *N378–32* and *mwh*^{1}.

Subsequently, PCR was performed following the preparation of genomic DNA from *N378–32* flies, *mwh*^{1} heterozygous flies and *mwh*^{1} homozygote flies.

Agarose electrophoresis of the purified PCR product showed that *N378–32* flies had a band of nearly 200 bp, whereas *mwh*^{1} flies had no band (Fig. 1A). The results also showed that both of them had an RP49 band. F_1 heterozygous flies also had a band of almost 200 bp and an RP49 band (data not shown). After purifying the 200-bp PCR product using a Nalgene Kit, sequencing confirmed it to be a part of the target seventh exon of the *mwh* gene (data not shown). Using a ZOE™ Fluorescent Cell Imager (Bio-Rad Laboratories, Inc., Hercules, USA), most wing hairs were observed to be normal in the *N378–32* and F_1 hybrid. In addition, almost all of the *mwh*^{1} wing hairs showed a mutant phenotype with two to three wing hairs per wing cell (Fig. 1B).

Even the irradiated +/*mwh*-type heterozygous flies were observed to have normal wing cells with one hair per cell. The small spots comprising one or two cells with one long hair and one to three surrounding short hairs per cell (Fig. 2A) emerged like islands among the normal wing cells (marked by white dotted lines). The large spots comprising more than three cells with one long hair and one to three short hairs per cell (Fig. 2B) also emerged like islands among the normal wing cells (marked by white dotted lines).

Determination of the developmental stage of *Drosophila* with the highest sensitivities to γ-rays

First, the emergence rates of the small and large spots at four larval stages (i.e. egg, first instar, second instar and third instar) of the

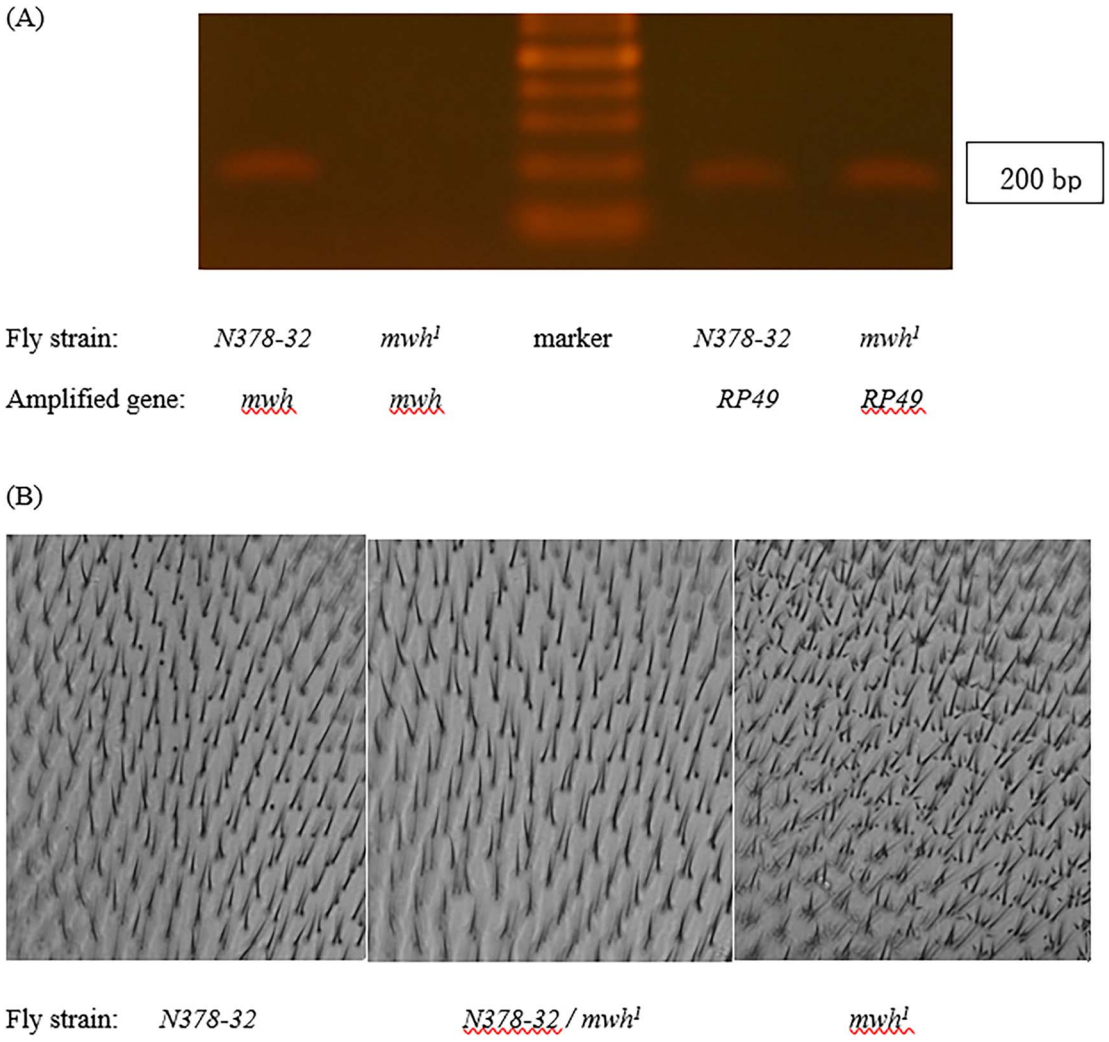


Fig. 1. Confirmation of the *mwh*^{1} mutant flies by PCR and phenotype. (A) Using *mwh* primers, a 200-bp band was observed in the genomic DNA of *N378–32*, whereas no band was observed in the genomic DNA of *mwh*^{1}. Using RP49 primers, a 200-bp band was observed in the genomic DNA of both *N378–32* and *mwh*^{1}. (B) Most wing hairs were normal in *N378–32* (left) and F_1 hybrid (middle), and almost all the wing hairs of *mwh*^{1} exhibited a mutant phenotype with two to four wing hairs per wing cell (right).

Drosophila +/*mwh*-type heterozygote irradiated by 15 000 mGy γ-rays were detected (Table 1), confirming that the most sensitive stage to γ-rays was the third instar for both small and large spots.

Determination of the dose showing HRS and IRR to γ-rays in *Drosophila*

The third instar larvae of the +/*mwh*-type heterozygote were irradiated by different doses of γ-rays from 20 to 7000 mGy. Fig. 3 shows the rates of small and large spots, as well as major differences between adjacent γ-ray doses. The small and large spots of the wings were then examined using the conditional binominal test and the significance test of the eruption frequencies under all combinations of different doses of γ-rays in females and males, and small spots and large spots (Table 2).

Because one of the important objectives of this study was to determine boundary doses causing HRS from IRR, histograms are shown describing the small and large spot rates, as well as the major differences between adjacent doses based on the results from Table 2.

For the small spot in females, indicating the mutation or deletion of the *mwh* gene or chromosomal disjunction, the eruption frequency increased in the groups irradiated with 20, 50 and 75 mGy compared with the adjacent doses (0 and 100 mGy), exhibiting HRS to LDR, while in the groups irradiated with 200 and 300 mGy, the frequency decreased, illustrating IRR (Fig. 3A). However, in males, the eruption frequency increased in the groups irradiated with 50 and 100 mGy compared with the adjacent doses (20, 75 and 200 mGy), indicating

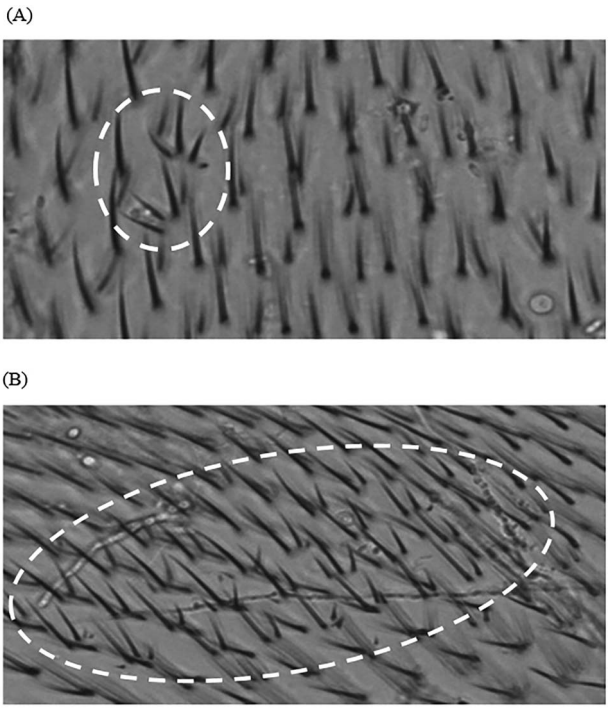


Fig. 2. Representative photographs of the small and large spots. The small spots comprising one or two cells with one long hair and one to three surrounding short hairs per cell (A) emerged like islands among the normal wing cells (marked by white dotted lines). This picture presents an example of a small spot with two *mwh* cells of a 20-mGy irradiated fly. The large spots comprising more than three cells with one long hair and one to three short hairs per cell (B) also emerged like islands among the normal wing cells (marked by white dotted lines). This picture presents an example of a large spot with 26 *mwh* cells of a 50-mGy irradiated fly.

Table 1. Emergence rates of small and large spots at four stages of Drosophila

No. of spots/No. of wings[a]	Rate of small spot	Rate of large spot
Egg	0.13 (0.38)	0 (0.08)
First instar	0.22 (0.40)	0.13 (0.07)
Second instar	0.03 (0)	0.31 (0)
Third instar	1.60 (0.02)	1.10 (0.05)

[a]Emergence rates of small and large spots are shown as the number of mwh spots divided by the total wing number with 15 000-mGy γ-irradiation. The parentheses show the rates of spots without irradiation.

HRS to LDR, while in the irradiated groups of 75 and 200 mGy, the frequency decreased, illustrating IRR (Fig. 3B). For the large spot in females indicating mutation of the *mwh* gene or chromosomal recombination, the eruption frequency increased in the 75-mGy irradiated group compared with the adjacent doses (50 and 100 mGy), indicating HRS to LDR, while the eruption frequency decreased in the group irradiated with 100–800 mGy, illustrating IRR (Fig. 3C). However, in males, neither HRS nor IRR was observed (Fig. 3D). Thus, HRS to LDR was found in *Drosophila* wing cells by delimiting the dose of γ-rays finely, except for the male large spots. In addition, sex difference of sensitivity to low-dose γ-rays and peak dose for HRS was observed.

DISCUSSION

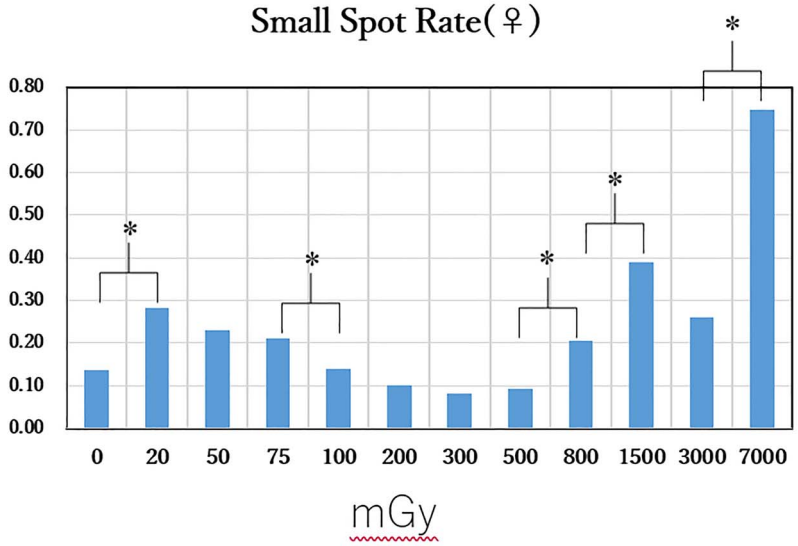
LDR is generally defined as a radiation dose of $\leq$100 mSv. However, even if it is limited to research on human cell cultures, the doses causing HRS by LDR and the boundary discriminating HRS/IRR varies from 10 to 200 mGy, depending on the cell line and the quality of radiation [2, 4, 12–15]. Most of these studies utilized wide intervals of doses. Before undertaking research on HRS for novel experimental systems, it is necessary to determine the doses contributing to HRS and IRR, as well as the accurate border of doses distinguishing HRS and IRR. This is essential because IRR occurs in doses near those that elicit HRS; hence, it is particularly important for the analysis of the mechanisms underlying HRS/IRR events. This is achieved by observing the responses using irradiation of gradually increasing doses.

However, the frequency of stochastic effects, induced especially by low-dose rate exposures or LDR, displayed a wide scatter. This scatter precluded distinguishing radiation-induced events from spontaneous events. Hence, the dose limit for radiation safety recommended by the International Commission on Radiological Protection (ICRP) is based on the LNT model, a model in which ionizing radiation always produces detrimental effects. Notably, carcinogenic changes induced by radiation are thought to fit the LNT model [1]. This hypothesis argues that there is an elevation in the frequency of stochastic or random effects, regardless of the exposure dose and even at doses of $<$100 mSv. Nonetheless, the model is still hypothetical due to the scatter in the frequency of events in the low-dose region. Only a limited number of experimental systems are available for the analysis of the biological effects of extremely low doses or LDR [7, 22, 36].

Most human cell lines exhibit HRS to LDR, which is not predicted by back-extrapolating the cell-survival response from HDR. As the dose is increased, it begins to exhibit increased IRR. Hence, cell survival follows the usual downward-bending curve with a further increasing dose. One explanation is that the DNA repair mechanisms do not engage sufficiently with LDR, hypersensitizing the cells. In contrast, at high doses, the repair mechanisms can be employed, rendering the cells resistant [22, 37].

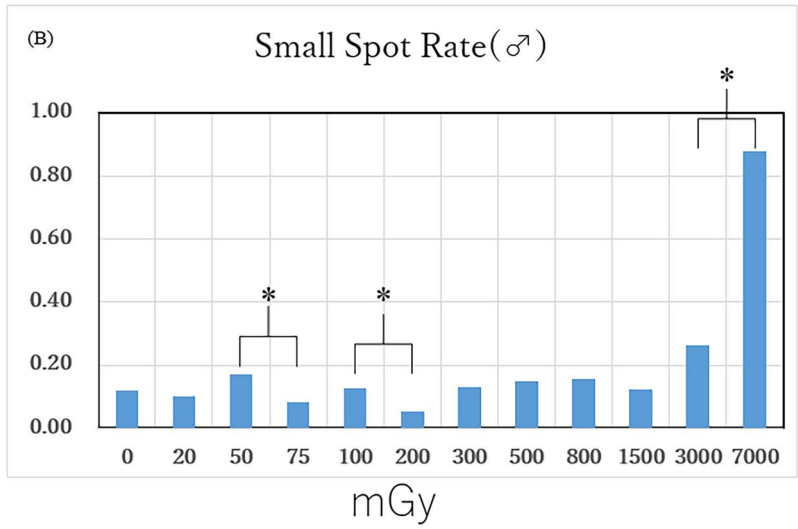
With LDR, the probability of damaging mutations on genes is minimal; therefore, the detection of the damage is very hard. In the case of microbes (e.g. bacteria) and mutations on genes involved in drug resistance, rare mutants can be selected easily in a huge population by culturing millions of cells. To apply the same method to eukaryotic cells, the *HPRT* gene was used to analyze mutation by LDR. This was because if any mutation occurs in the *HPRT* gene, the cells can survive in 6-thioguanine-containing culture medium, while cells with a normal gene cannot survive, hence their easy selection [38]. The cloning of the *HPRT* gene helped in analyzing the rate of each type of mutation using LDR and HDR [7, 39]. However, because no *HPRT* gene was identified in *Drosophila*, this method would not be applicable for detecting rare mutations.

(A)



γ-rays (mGy)	0	20	50	75	100	200	300	500	800	1500	3000	7000
Female Wings	308	146	161	156	275	89	110	86	157	85	203	122
Small spot No.	42	41	37	33	38	9	9	8	32	33	53	91
Small spot Rate(♀)	0.14	0.28	0.23	0.21	0.14	0.10	0.08	0.09	0.20	0.39	0.26	0.75

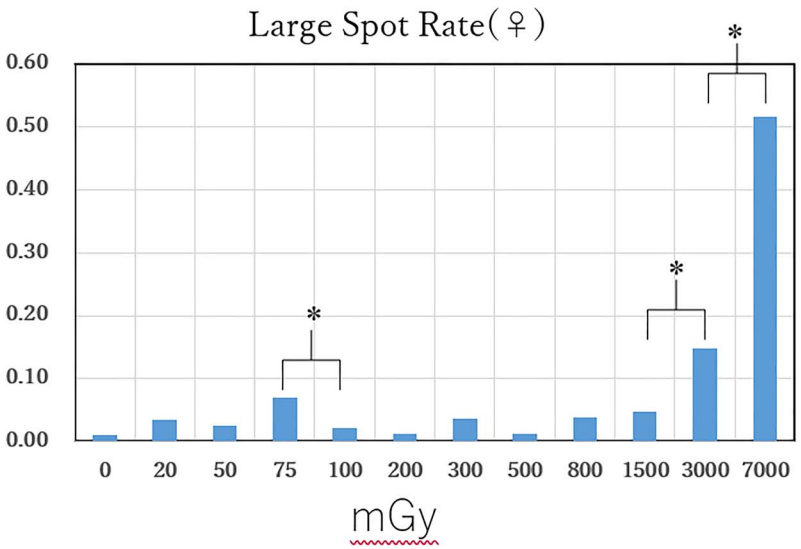
(B)



γ-rays (mGy)	0	20	50	75	100	200	300	500	800	1500	3000	7000
Male Wings	183	88	133	110	214	76	62	81	128	41	216	106
Small spot No.	22	9	23	9	27	4	8	12	20	5	57	93
Small spot Rate(♂)	0.12	0.10	0.17	0.08	0.13	0.05	0.13	0.15	0.16	0.12	0.26	0.88

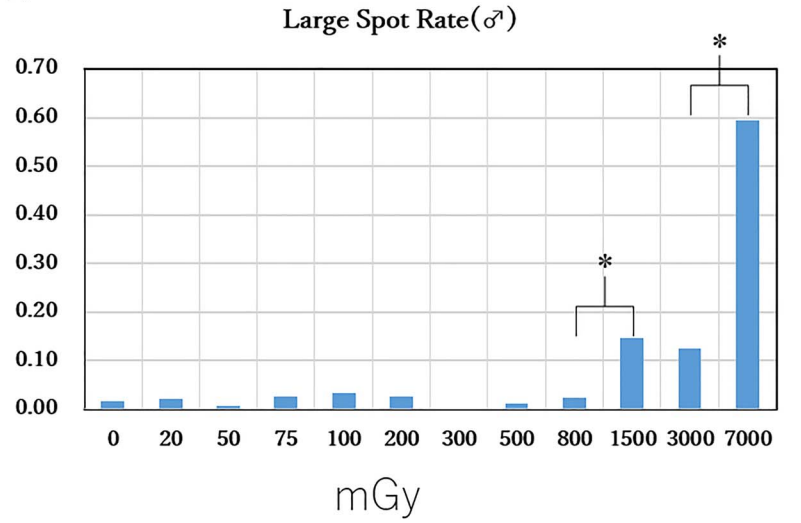
Fig. 3. Histograms of the rates of small and large spots, as well as major differences between the adjacent γ-ray doses. Histograms of the rates of female small (A), male small (B), female large (C), and male large (D) spots are shown. The horizontal axis displays the γ-ray doses described by mGy, while the vertical axis shows the spot rate per wing. Asterisks denote significant differences in the rates observed between the adjacent doses (see also Table 2). The raw data of the wing numbers and spot rate (spot numbers/wing numbers) are posted under each histogram.

(C)



γ-rays (mGy)	0	20	50	75	100	200	300	500	800	1500	3000	7000
Female Wings	308	146	161	156	275	89	110	86	157	85	203	122
Large spot No.	3	5	4	11	6	1	4	1	6	4	30	63
Large spot Rate(♀)	0.01	0.03	0.02	0.07	0.02	0.01	0.04	0.01	0.04	0.05	0.15	0.52

(D)



γ-rays (mGy)	0	20	50	75	100	200	300	500	800	1500	3000	7000
Male Wings	183	88	133	110	214	76	62	81	128	41	216	106
Large spot No.	3	2	1	3	7	2	0	1	3	6	27	63
Large spot Rate(♂)	0.02	0.02	0.01	0.03	0.03	0.03	0.00	0.01	0.02	0.15	0.13	0.59

Fig. 3. Continued.

Table 2. Examination of major differences between the rates of spot emergence by different γ ray doses.

(A) Small spot

♂＼♀	0	20	50	75	100	200	300	500	800	1500	3000	7000
0		*	*	*						*	*	*
20					*	*	*	*				*
50					*	*	*	*		*		*
75			*		*	*	*	*		*		*
100										*	*	*
200			*		*				*	*	*	*
300									*	*	*	*
500						*			*	*	*	*
800						*	*			*		*
1500												*
3000	*	*		*	*	*	*	*	*			*
7000	*	*	*	*	*	*	*	*	*	*	*	

Continued

In *Drosophila*, most reports concerning the effects of LDR show that compared with background radiation, LDR inhibits the mutation rates [22, 30, 37, 40]. The authors discovered that using low-dose X-ray irradiation (<200 mGy), the DNA repair mechanisms cannot be recruited. Conversely, by increasing the exposure dose over the threshold level, the DNA repair mechanisms can recognize the impaired DNA and be activated with a result of a decrease in the mutation rate. Therefore, the dose dependence of the mutation rate is expected to yield a U-shaped curve.

The number of wing cells comprising one wing of *Drosophila* is ~30 000. In addition, if one cell causes the deletion or mutation of the normal *mwh* gene or chromosomal recombination around the *mwh* gene, the cell can be easily detected by *mwh*, usually composed of one long hair and one to three surrounding small hairs instead of one [27]. Therefore, the sensitivity of detection is extremely high (i.e. 0.0033%), and it is probably the most sensitive and accurate way to detect the effects of LDR.

Previous works exploring the effects of LDR on *Drosophila* examined only one to three doses of LDR, namely, 200 mGy [30, 37], 50, 200 and 500 mGy [40]. In all cases, 200 mGy exhibited the sensitivity forming the bottom of the U shape, illustrating IRR. By dividing doses more finely, we found that for the small spot in females 20, 50 and 75 mGy conferred HRS to LDR, while 200 and 300 mGy conferred IRR, and in males, 50 and 100 mGy conferred HRS, whereas 75 and 200 mGy conferred IRR. The reason for the decrease in the small spot rate at 75 mGy and increase at 100 mGy is unclear. Because at most doses the small spot rates in males are smaller than those in females (Fig. 3A and B), the error became large in this LDR range, and it may happen that the spot ratio at 75 mGy was small in males. As for the large spot in females, 75 mGy conferred HRS while 100–800 mGy conferred

Table 2. Continue

(B) Large spot

♂＼♀	0	20	50	75	100	200	300	500	800	1500	3000	7000
0				*					*	*	*	*
20											*	*
50											*	*
75					*	*		*			*	*
100											*	*
200											*	*
300											*	*
500											*	*
800											*	*
1500	*	*	*	*	*	*	*	*	*		*	*
3000	*	*	*	*	*	*	*	*	*			*
7000	*	*	*	*	*	*	*	*	*	*	*	

Considerable differences between the rates of small spot emergence and those of large spot emergence by different γ-ray doses were examined. The upper-right squares and the lower-left squares are results of females and males, respectively. The asterisk illustrates the significant differences. Doses of γ-rays are described as a number with mGy. *Significant differences were observed between two doses of γ-rays.

IRR, however in males, neither HRS nor IRR was observed. These findings reinforce the evidence of previous work [37, 40], illustrating that for doses of <200 mGy, the repair system cannot be employed; hence an increase in mutation rates compared with the background rates is observed. Koana *et al*. hypothesized that the mutation at background (0 mGy) irradiation from a single-strand DNA break could be repaired by 200 mGy irradiation, thus reducing the mutation rate at 200 mGy [30]. However, our results showed insignificant difference in the spot rate between 0 and 200 mGy irradiation in both females and males, and for small and large spots (Table 2). One reason for the discrepancy might be the difference in the quality of the radiation sources. Moreover, the peak doses of HRS measured using the small and large spots are different. As mentioned previously, the small spot can be produced by the deletion of the terminal short chromosome which contains the normal *mwh* gene, an additional mutation in the wild-type *mwh* gene, and chromosomal mal-disjunction. The large spot can be produced by chromosomal recombination between the *mwh* and the centrosome and an additional mutation in the wild-type *mwh* gene. Deletion of the terminal short chromosome and chromosomal recombination are both initially induced by the DNA double-strand

break. Moreover, mutation of the *mwh* gene is caused by base deletion and chemical modification of the bases. Organisms such as fruit flies have repair mechanisms that can cope with these DNA impairments [41, 42]. The reason behind the dose difference of HRS and IRR between the small and large spots is that the sensitivities of these repair mechanisms are varied; however, no research has to date affirmed the sensitivity of the individual repair mechanism of *Drosophila*.

Future research recommends investigating the alterations of the *mwh* gene of each small and large spot at the DNA structure and sequence level. In addition, it is advised that researchers probe the influence of different kinds of radiation that can be measured the frequency of the visible phenotype to the one-cell level; it is also advised that chromosome deletion and base sequence abnormality be detected. Similar to mutation by ethyl methanesulfonate, next-generation sequencing needs to determine DNA alterations specific to LDR and HDR and common to both LDR and HDR [43].

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CONFLICT OF INTEREST

None declared.

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