Effects of heavy-ion beam irradiation on non-model fruit fly, *Drosophila miranda*

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Irradiation has been a useful tool for making deletion mutants and establishing strains with balancer chromosomes in *Drosophila melanogaster*.[1] These flies have been fundamental resources in the fields of genetics, development, evolutionary biology, and so on. However, irradiation has so far been applied primarily only to this model species of *Drosophila*. If the same resources become available for other *Drosophila* species, researchers working on other fly species can utilize these resources for a variety of studies. For example, *D. miranda* acquired the so-called neo-sex chromosomes by fusing an autosome with the ordinary Y chromosome approximately a million years ago, and it has a high potential for elucidating the early evolutionary phase of sex chromosomes.[2,3] The purpose of this study is, therefore, to examine the effects of heavy-ion beam irradiation on the fertility and the genome of *D. miranda*.

We irradiated the iron (Fe)-ion beam (806 keV/μm; 0.5, 1, or 2 Gy) to the flies with 3–4 days after eclosion. For irradiation, 15-mL tubes were used, in which 3 mL of the medium consisting of 1% agar and 50% grape juice was poured at an angle of 30°. Five males with the irradiation of the Fe-ion beam were then crossed with five virgin females on the same (zeroth) day of the irradiation in a vial containing a normal corn medium (Fig. 1). On the 1st, 4th, and 7th days after irradiation, only the males were transferred to a new vial and crossed with another five virgin females. After 3 days of crossing, all the females were discarded from each vial. All the males were also discarded with females on the 10th day. The number of F_1 flies eclosed in each vial was counted. To consider variations, we prepared four vials (that is, 5 males × 4 vials) for each irradiation condition as replicates. Among the F_1 individuals (Fig. 2), we extracted genomic DNA from each of the five males from the 1–3 days vials derived from the males with irradiation of 2 Gy indicating a lower fertility, and resequenced the genomes using Illumina HiSeq X. Following sequencing, the regions with mapping depth of zero in the F_1 individual but of at least 1 in the wildtype individuals were regarded as the candidate regions of deletions. The same experiments were also conducted for the argon-ion beam (Ar, 189 keV/μm; 1 Gy) and the carbon-ion beam (C, 30 keV/μm; 5 Gy) and the genomes of two F_1 males were sequenced for each ion beam.

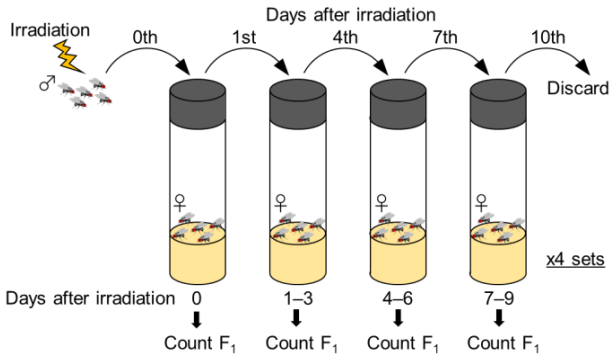

Fig. 1. Crossing experiments following irradiation.

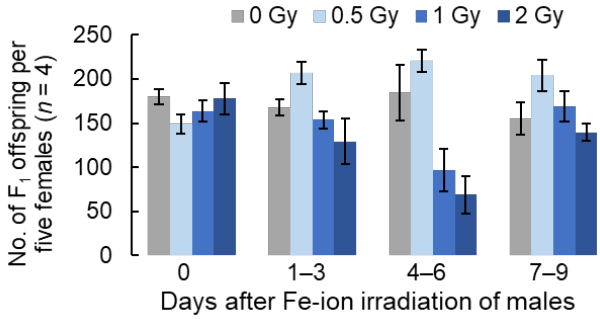

Fig. 2. Transition of male fertility after Fe-ion irradiation.

Using the strategy described, we successfully detected a number of genes containing the candidate regions of deletions on the Y/neo-Y chromosomes (Table 1). However, deletions were also detected on the X/neo-X chromosomes, which was not expected because the X/neo-X chromosomes were inherited only

Table 1. Number of genes in which deletions were detected.

Chr.	Assembly size (Mb)	Average no. of genes* in which deletions were detected		
		Fe, 2 Gy	Ar, 1 Gy	C, 5 Gy
Y/Neo-Y	101.5	6.5	6.5	36.0
X/Neo-X	103.0	7.7	4.0	25.5

* The average number of genes with deletions per individual is shown. Only the genes in which deletions were detected within the exons were counted.

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from the non-irradiated females. We speculate that sequence polymorphism between each F_1 male and reference genomes caused such erroneous identifications. We would like to optimize the conditions for making mutants with large deletions in future studies by repeating the irradiations under various conditions.

This research was supported by the KAKENHI (21H02539), granted to MN.

References

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