Results of whole-genome analysis of *pink* and *ebony* mutant

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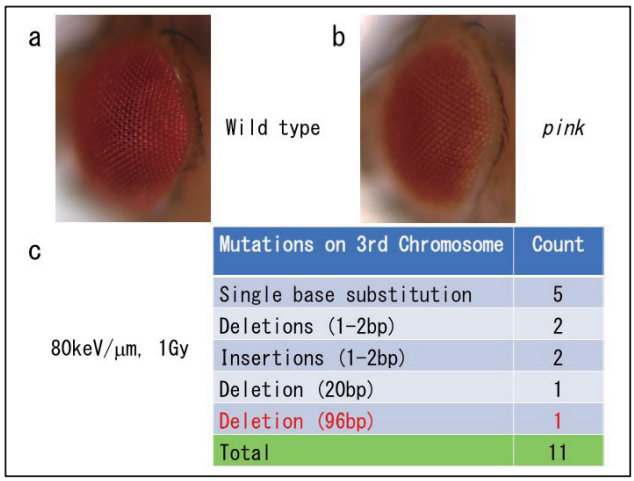
Heavy-ion-beam mutagenesis is generally recognized as an effective method for mutation breeding.[1, 2] Although this method was greatly successful with plants, its application is limited for animals. Therefore, we plan to acquire more basic data to set up optimal conditions for the heavy-ion-beam irradiation system by using *Drosophila melanogaster* (fruit fly) as the model.

In our previous study, we determined that 1-3 Gy irradiation using a carbon-ion beam is suitable for the large-scale screening of mutant lines.[3] To elucidate the biological effect of heavy-ion-beam irradiation to the genome, we analyzed the whole-genome sequencing of several mutants established by the condition of 1-3 Gy irradiation. In this report, we applied the high-performance bioinformatics pipeline, which was developed for analyzing rice exome sequencing results obtained in our laboratory[4], to fly genome analysis. Mutants are established using the third chromosome balancer, which is a unique genetic tool for fruit fly. Because the third chromosome balancer is known to prevent homologous recombination only on the third chromosome, the following results are limited to third chromosome events.

In this report, we show the result of *pink* [Fig. 1] and *ebony* [Fig. 2] mutants. Each mutation is summarized in Fig. 1c and Fig. 2c. These data suggest that 1-3 Gy dose is sufficient for several mutations such as single base substitution, deletion, insertion, rearrangement, and large deletion. These diversities of mutations indicate an advantage in screening by the heavy-ion-beam mutagenesis. Both mutants contain 11 or 12 mutations, and then the third chromosome consists of 5.2 x 10^7 base pairs (bp). This means that the heavy-ion-beam irradiation introduces a mutation into the genome at a frequency of approximately 4.5 x 10^6 bp.

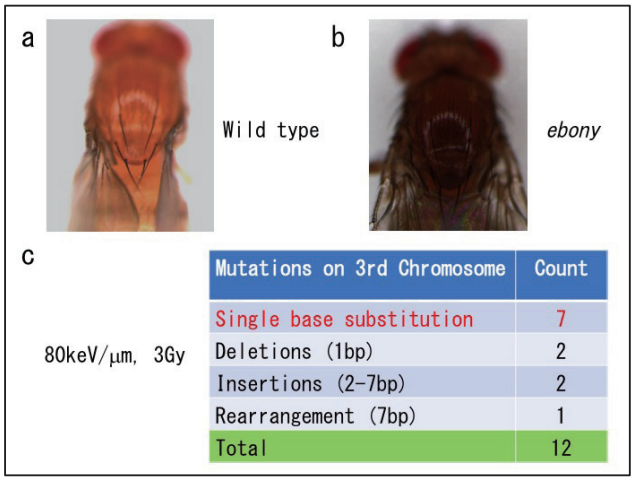
Among 11 or 12 mutations, an alteration in protein sequence was observed in only one gene. We purchased several mutants of the same gene from the *Drosophila* stock center. Through the complementation test known as a genetic technique for fruit fly to determine whether two mutants belong to the same gene, we determined that the causal genes are *pink* and *ebony*. Though it is thought that the fruit fly has about 15000 genes, we could easily identify the causal genes of mutants by using whole-genome sequencing and the pipeline. These results suggest that the pipeline technique is a powerful tool for mutation analysis.



Mutations on 3rd Chromosome	Count
Single base substitution	5
Deletions (1-2bp)	2
Insertions (1-2bp)	2
Deletion (20bp)	1
Deletion (96bp)	1
Total	11

Fig. 1. Phenotype of the *pink* mutant and the result of whole-genome analysis.

a) Wildtype eye color is vivid red. b) The mutant eye color becomes lighter than wildtype. c) The mutant is established by the condition with 80 keV/μm linear energy transfer at 1 Gy dose level. Whole-genome analysis revealed 11 mutations caused by heavy-ion-beam irradiations. Each mutation was categorized into substitution, deletion and insertion. The causal mutation of *pink* was classified into large deletion with red text.



Mutations on 3rd Chromosome	Count
Single base substitution	7
Deletions (1bp)	2
Insertions (2-7bp)	2
Rearrangement (7bp)	1
Total	12

Fig. 2. Phenotype of the *ebony* mutant and the result of whole-genome analysis.

a) Wildtype body color is yellowish brown. b) The mutant body color becomes darker than wildtype. c) The mutant is established by the condition with 80 keV/μm linear energy transfer at 3 Gy dose level. Whole-genome analysis revealed 12 mutations caused by heavy-ion-beam irradiations. Each mutation was categorized into substitution, deletion, insertion and rearrangement. The causal mutation of *ebony* was classified into single base substitution with red text.

References

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2) A. Tanaka et al., J. Radiat. Res. **51**, 223 (2010).
3) K. Tsuneizumi et al., RIKEN Accel. Prog. Rep. **49**, 253 (2016).
4) H. Ichida et al., RIKEN Accel. Prog. Rep. **49**, 254 (2016).

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