# Development of the mutant isolation system in fruit flies

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Heavy-ion beam mutagenesis is an effective mutation breeding method [1, 2]. Although this method has been highly successful with plants, its use for animals has been limited. To extend its application to animals, we plan to acquire more basic data to determine optimal conditions for heavy-ion-beam irradiation using *Drosophila melanogaster* (fruit fly) as a useful model system.

Over the past century, several unique genetics tools have been developed using the fruit fly. A balancer chromosome is one such popular tool and is known to prevent homologous recombination during meiosis. A single balancer line was previously found to be more suitable for stabilizing the mutant isolation system than a double balancer line[3]. Therefore, we focused only on third-chromosome events and re-established a third chromosome balancer line before starting the irradiation experiment.

To overcome the instability problem of a graphic record at previous data[3], we decided to use commercial cuvettes with a plane surface [Fig. 1a]. Because most of the vials commonly used for fly maintenance have a curved surface, heavy-ion-beam irradiation condition is uneven in the vials depending on the thickness of curved plastic. To decrease the opportunity of the energy loss in heavy-ion beam by flies overlapping, only two flies were put into each cuvette. Then, six cuvettes were arranged in a commercial container in order to use a uniform irradiation range with an automatic sample changer [Fig. 1b]. To evaluate the stability of the improved mutant isolation system, we subjected the fruit flies to a carbon-ion beam with linear energy transfer (LET) values of [80keV/μm] at several dose levels (1.0, 3.0, 10.0, 30.0, and 60.0 Gy).

To estimate the effect of heavy-ion-beam irradiation, we measured the number of F1 progeny as a biological effect. In this study, males and females were immediately separated after eclosion and were bred for 3 days. Then every two males were put into each cuvette for irradiation [Fig. 1b]. It was performed in the females equally. After irradiation with different heavy-ion-beam doses, the males were provided fresh harems of virgin females every 2–3 days. The females were provided with males and the medium was replaced every 2–3 days. The oviposited eggs were bred, and the number of progeny that survived to the adult stage was determined.

Decrease in the reproductive ability of males was found to be caused by aging and not irradiation. In contrast, the reproductive ability of females did not change during the observation period [Fig.2]. The results for the males showed a linear correlation between the number of progeny and irradiation dose. In contrast, a non-linear curve was observed for the females [Fig. 2]. These data suggest that a radiosensitivity is different between males and females.

We have developed a stable mutant isolation system using fruit flies by using heavy-ion-beam irradiation. Currently, we are establishing various mutants and will be analyzing DNA damage in homozygotes. These data will be helpful for optimizing the irradiation system in the future.

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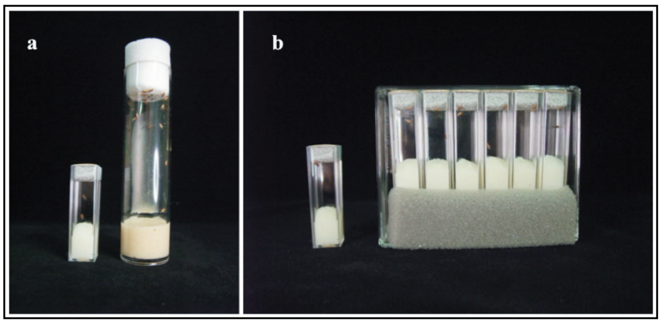


Fig. 1. a) A photograph of a cuvette and a breeding vial. b) A photograph of sample cuvettes for irradiation using an automatic sample changer.

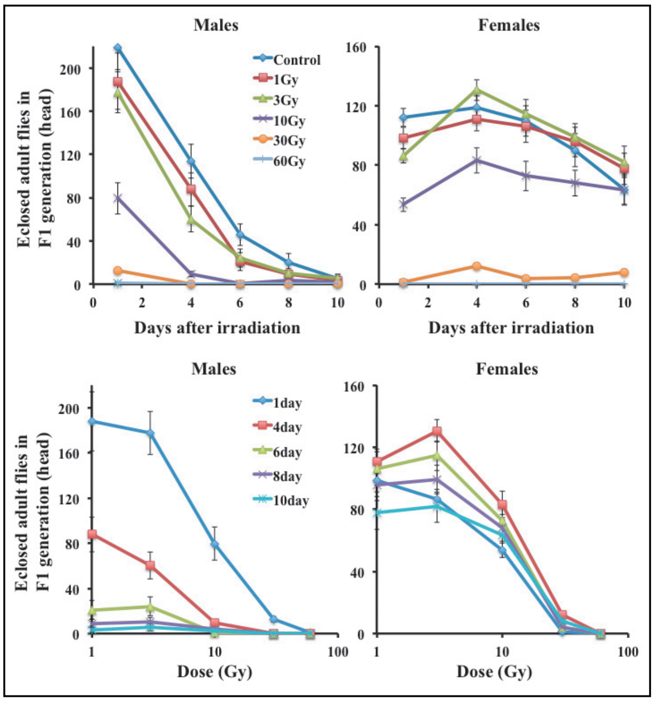


Fig. 2. Upper panels show the correlation between the number of F1 progeny and the days after heavy-ion-beam irradiation. Lower panels show the correlation between the number of F1 progeny and the irradiation dose. F: filial generation.

References

1) T. Abe et al.: Gamma Field Symp. **39**, 45 (2000).
2) A. Tanaka et al.: J. Radiat. Res. **51**, 223 (2010).
3) K. Tsuneizumi et al.: RIKEN Accel. Prog. Rep. **46**, 255 (2013).