## Results of whole-genome analysis of mahogany mutant

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Heavy-ion-beam mutagenesis is generally recognized as an effective method for mutation breeding.<sup>1,2)</sup> 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 the suitable condition for the large-scale screening of mutant lines of heavy-ion-beam mutagenesis.<sup>3)</sup> To elucidate the biological effect of heavy-ion-beam irradiation on the genome, we established several mutants that expressed typical phenotypes on eyes, wings, bodies, and bristles by a carbon-ion beam irradiation.

In this report, we show the analysis of data obtained from the whole-genome sequence of the mahogany (mah) mutant. The mutant eye color is darker than that of the wild type (Figs. 1a, b). This mutant line was established by the condition with 50 keV/ $\mu$ m linear energy transfer at 10 Gy dose level. Whole genome analysis revealed that the causal mutation of mah was a large deletion (Fig. 1c). An open reading frame of mah is 1,581 bp and a 387 bp deletion was observed in the first exon of a mah gene (Fig. 1c). An in-frame deletion produces a mutated Mahogany protein from which 129 amino acid residues were removed (Fig. 1c).

The role of the Mahogany protein (Mah) has an unknown function. InterPro is a freely available database



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

a) Wild type eye color is vivid red. b) The mutant eye color becomes darker than that of the wild type. c) The alignments of genome and protein sequences at start and end sites of the deletion. The top line indicates the cDNA sequence. The middle line indicates the numbers of amino acid residues. The bottom line indicates amino acid sequence. The red texts represent deleted regions.





Fig. 2. Diagrams of protein domain analysis using InterPro and deleted Mahogany protein.

a) A result data of domain analysis of Mahogany protein using InterPro database. The red box highlights the deleted three transmembrane domains. b) A diagram of wild type and deleted proteins of Mahogany. Full length Mahogany protein consist of 527 amino acid residues. The numbers indicate the numbers of amino acid residues of the proteins. The deleted protein lacks 129 amino acid residues (87–215).

used to classify protein sequences into families and to predict the presence of important domains and sites.<sup>4)</sup> InterPro analysis of the predicted protein Mah identified 11 transmembrane helices and a conserved domain found in amino acid transporters (Fig. 2a). The deleted protein lacks three putative transmembrane domains (Figs. 2a, b). Both the amounts of ommochrome pigments and pteridine pigments are decreased in classical *mah* mutants.<sup>5)</sup> These results indicate that Mah function is related to both pigments transport in eye ommatidia.

Chemical mutagen mostly introduces point mutations into genomic DNA. In this report, we confirmed that heavy-ion-beam mutagenesis has diverse mutations, for example small insertions, point mutations, small deletions, and large deletions, in the animal genome. Currently, we are analyzing other mutants. The data will be helpful for the elucidation of the biological effect of heavy-ion-beam irradiation to the animal genome.

## References

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