Effects of heavy-ion beam irradiation on non-model fruit fly, *Drosophila miranda*: part II. Inducing large deletions to males

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Dosage compensation is a mechanism to mitigate the copy number difference of X-linked genes between females and males (two copies and one copy, respectively). In *Drosophila*, the X-linked genes are upregulated by two times in males to equalize the dosage of X-linked genes between sexes at the transcriptional level.[1)] The X and Y chromosomes are originally a pair of autosomes in many organisms. Therefore, dosage compensation is unlikely to be required at the initial stage of sex chromosome evolution, because the Y chromosome should have almost exactly the same genes as the X chromosome. Yet, as the genes on the Y chromosome are non-functionalized due to cessation of meiotic recombination with the X chromosome,[2)] dosage compensation should necessarily be established on the X chromosome. However, how fast the dosage compensation is established after non-functionalization of Y-linked genes largely remains unknown.

To tackle this question, we have utilized *Drosophila miranda*. This species acquired the so-called neo-sex chromosomes by fusing an autosome with the ordinary Y chromosome approximately a million years ago.[3)] Since the neo-Y chromosome (neo-Y) still contains many functional genes due to its young age, this species is an ideal platform to study the evolution of dosage compensation.[4–6)] In particular, the irradiation of heavy-ion beams to *D. miranda* is a promising approach, because irradiation may induce deletions on neo-Y, which mimics the degeneration of the Y chromosome. Then, we can test whether the X-linked gametologs corresponding to the Y-linked genes located within the deletion are upregulated in the F_1 male. We also aim to examine the effect of irradiation on the male fertility.

We previously irradiated *D. miranda* males with iron-ion (Fe, 806 keV/μm; 0.5, 1, or 2 Gy), argon-ion (Ar, 189 keV/μm; 1 or 2 Gy), and carbon-ion (C, 30 keV/μm; 5 or 10 Gy) beams. However, the amount of irradiation was possibly not sufficient to significantly decrease the male fertility and induce large deletions on the genome.[7)] To obtain males with larger deletions on neo-Y, we irradiated males with 10 and 50 Gy of Ar-ion beams 3–4 days after eclosion. For irradiation, 15-mL tubes were used, in which 3-mL of the medium comprising 1% agar and 50% grape juice was poured at an angle of 30°. Five males irradiated with the Ar-ion beam were then crossed with five virgin females on the same (zeroth) day of irradiation in a vial containing a normal corn medium. On the 1st, 4th, 7th, 10th, and 13th days after irradiation, only the males were transferred to a new vial and crossed with five other virgin females. All males were discarded on the 16th day. After seven days of crossing, all females were discarded from each vial. 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.

The results showed that the fertility clearly drops in the irradiated males (Fig. 1). In particular, only 24 F_1 males including all replicates were obtained derived from the males with the 50-Gy irradiation of the Ar-ion beam. Similarly, the 10-Gy irradiation also significantly weakened the male fertility. We extracted genomic DNA and total RNA separately from each of the 21 and nine F1 males derived from males with the 50- and 10-Gy irradiation, respectively. Genome sequencing of these males detected nine deletions from six males ranging from 100 kbp to 13 Mbp. Seven of them were on the neo-Y. We are conducting RNA-seq for the six males to test if dosage compensation becomes effective immediately. Specifically, we will examine if the neo-X-linked gametologs of the deleted neo-Y-linked genes are upregulated.

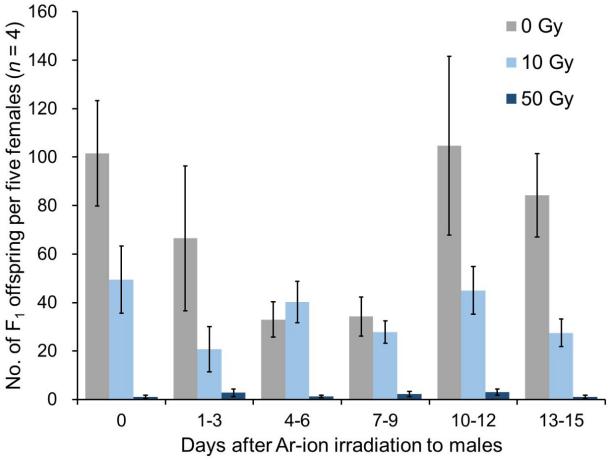

Fig. 1. Transition of male fertility after Ar-ion irradiation.

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