Establishment of large mutant lines on rotifer using heavy-ion-beam irradiation

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In larviculture, rotifers (*Brachionus plicatilis* sense strict) are generally used as the initial feed for hatched larvae,[1] while larger preys (*e.g.*, *Artemia*) are needed with larval growth. The improper management of feed size and density results in mass mortality, a high incidence of morphologically abnormal development, and a substantial depletion of fish larvae.[2] To improve the survival and growth of fish larvae, prey of appropriate size between those of rotifer and *Artemia* is needed. The aim of this study was to apply heavy-ion-beam-based mutation breeding techniques[3,4] for the selection of rotifer mutants with larger body sizes.

The size distributions of the lorica length of rotifers are 170–320 μm in individuals carrying amictic eggs. The next food item *Artemia* nauplii has a body length of 400–1,000 μm, and thus, there is a large size gap (320–400 μm) between rotifer and *Artemia* nauplii. The rotifer strain is widely divided into two types, obligate and cyclically parthenogenesis, based on their life cycle.[5] In aquaculture facilities, the intensive mass culture of live food rotifers is performed via parthenogenetic reproduction to induce their rapid proliferation. The Notojima strain is known as the largest rotifer strain used in Japan and has obligate parthenogenesis. Based on these characteristics, this study used the Notojima strain as the raw material for ion-beam irradiation.

In our previous study, we measured the biological effect of heavy-ion-beam irradiation on rotifers under different conditions.[6] In this study, a large-scale screening method was developed, and mutant lines with increased body sizes were selected using the length of the lorica as indicators. Heavy-ion-beam irradiation was performed using carbon (C) (1.62 GeV, LET = 23 keV/μm) at six irradiation doses of 100, 150, 200, 300, 400, and 600 Gy and argon (Ar) (3.8 GeV, LET = 312 keV/μm) at six irradiation doses of 25, 50, 75, 100, 150, and 200 Gy. After irradiation, morphometric characteristics were compared with control groups without irradiation.

The results of categorising lorica lengths of the control and the 56 selected larger mutant strains are shown in Fig. 1. Strains were classified according to their average lorica length: Class I for 340–350 μm, Class II for 350–360 μm, and Class III for 360–370 μm. Class I corresponds to over 11% elongation, Class II to 15%, and Class III to 18% compared to the lorica length of the controls. The large mutant lines from 1968 rotifers irradiated with C-ion beams were categorized as follows: 30 lines to Class I, 19 lines to Class II, and 3 lines to Class III. In the large mutant lines from 1080 rotifers irradiated with Ar-ion beams, there were 3 lines in Class I and 1 line in Class II (Fig. 1). Steel's multiple comparison tests showed a difference at 0.001 significance level in lorica lengths of all larger mutant strains from Class I to Class III compared with the control group (305.6 ± 3.0 μm).

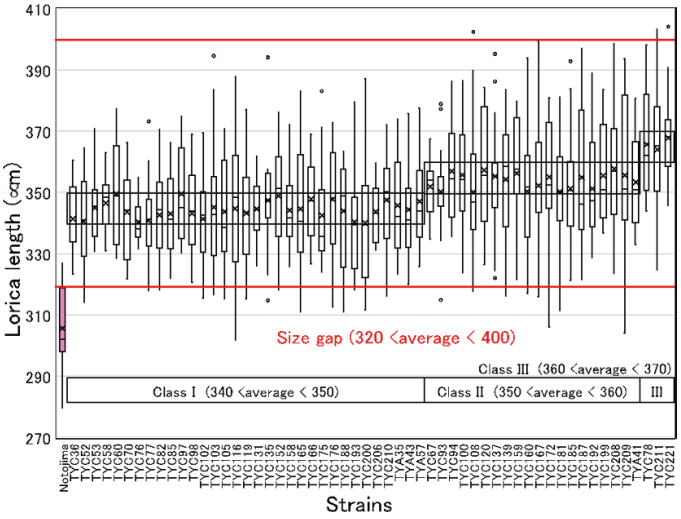

Fig. 1. Comparison of lorica lengths between large mutant strains and the control.

The size range of lorica length of the larger mutant strains was 320–400 μm, which is not covered by the size ranges of the wild-type rotifer and *Artemia* nauplii in seedling production (Fig. 1, red bars). Various larger mutant strains have been established by mutagenesis with heavy-ion-beam irradiation, and these strains may help reduce the mass mortality of domestic fish if used during the intermediate feeding stage between rotifers and *Artemia* nauplii.

The lorica lengths of all large mutant strains showed significant differences compared to the control group (Steel's multiple comparison tests, $p < 0.001$, $n = 20$). Boxplots indicate the lorica lengths of rotifers ($n = 20$). Strains were separated into classes according to the average lorica length.

References
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