



Comparison of genome size of diploid and tetraploid of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Tetraploidization of rainbow trout (*Oncorhynchus mykiss*) was induced by hyperthermia and designed in three treatments (28°C for 10, 12 and 14 min), 7 h after fertilization. Immature fishes were anaesthetised using standard method in tank (Table 1). Blood samples were collected by caudal venipuncture using 2 mL syringes fitted with 0.5 x 35 mm (25 gauge) needles (Sima, Iran) pre-dosed with heparin (Caspian Tamin, Iran). Blood samples subjected to various conditions, were analysed by FC. Methodology was adapted from established protocols for human blood FC analysis, but modified as required due to the nucleated nature of fish erythrocytes. Blood sample evaluations by FC were performed in surface-deactivated polypropylene tubes to minimize cell-tube adhesion and associated quantitation error. Five millilitre 12 x 75 mm polypropylene Falcon tubes (Maxwell, Italy) were filled with a 4% solution of bovine serum albumin (BSA; Sigma, USA) in PBS and stored overnight at 4°C (Lecommandeur *et al.*, 1994). Prior to use, the tubes were emptied and centrifuged at 2000 rpm for 5 min. For each treatment, we also measured the size of erythrocytes and genome size. Genome size was positively correlated with erythrocyte nucleus size and chromosome number when using PI as the fluorescent dye. This work provides new knowledge on *Oncorhynchus mykiss* genetics/genomics, important for future research in basic cellular/molecular mechanisms and for the development of molecular techniques in this species. However, further investigation is required to obtain a high percent tetraploid Rainbow trout population.

Keywords: Aquaculture, Flow cytometry, Genome size, Ploidy determination

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Introduction

Polyploidy at fish is characterized by modification of normal diploid chromosome set ($2n$) to triploid ($3n$), tetraploid ($4n$) etc. Polyploid cells are having in plus one or more sets of chromosomes ($3n$, $4n$), so they will have a bigger nucleus. Standards and investigated cells were stained with a mixture of propidium iodide, citric acid, and Nonidet P40 in the presence of RNase, and fluorescence of at least 50,000 nuclei was analyzed by flow cytometry. Average cell size was determined by flow cytometry, using fresh cell suspension in relation to latex beads of known diameter. The size of nuclei was examined on the basis of digital micrographs. The aim of this study was to evaluate genome size and ploidy of the Rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Tetraploidization of rainbow trout (*Oncorhynchus mykiss*) was induced by hyperthermia and designed in three treatments (28°C for 10, 12 and 14 min), 7 h after fertilization. Immature fishes were anaesthetised using standard method in tank (Table 1). Blood samples were collected by caudal venipuncture using 2 mL syringes fitted with 0.5 x 35 mm (25 gauge) needles (Sima, Iran) pre-dosed with heparin (Caspian Tamin, Iran). Blood samples subjected to various conditions, were analysed by FC. Methodology was adapted from established protocols for human blood FC analysis, but modified as required due to the nucleated nature of fish

erythrocytes. Blood sample evaluations by FC were performed in surface-deactivated polypropylene tubes to minimize cell-tube adhesion and associated quantitation error. Five millilitre 12 x 75 mm polypropylene Falcon tubes (Maxwell, Italy) were filled with a 4% solution of bovine serum albumin (BSA; Sigma, USA) in PBS and stored overnight at 4°C (Lecommandeur *et al.*, 1994). Prior to use, the tubes were emptied and centrifuged at 2000 rpm for 5 min. The remaining BSA solution was removed with a Pasteur pipette. Blood samples were generally diluted to 0.1×10^6 cells mL⁻¹, the optimal cell concentration in flow cytometry practice according to Juchno *et al.*, (2010), in PBS prior to propidium Iodide (PI) dye staining. This was typically achieved by adding 4 μL of whole blood to 4 mL of diluent.

Results and discussion

Flow cytometry is a well-recognized technique for nuclear DNA content analysis. It has gained an increasing use in DNA measurements in fish tissues for its speed, accuracy, and reproducibility. A flow cytometric analysis performed on a large number of fluorescent-stained fish nucleated erythrocytes has proven to be an ideal tool for DNA content and ploidy determinations (Figs. 1 and 2).

The statistical testing to verify differences between the treatments was carried out using a one-way analysis of variance (ANOVA).

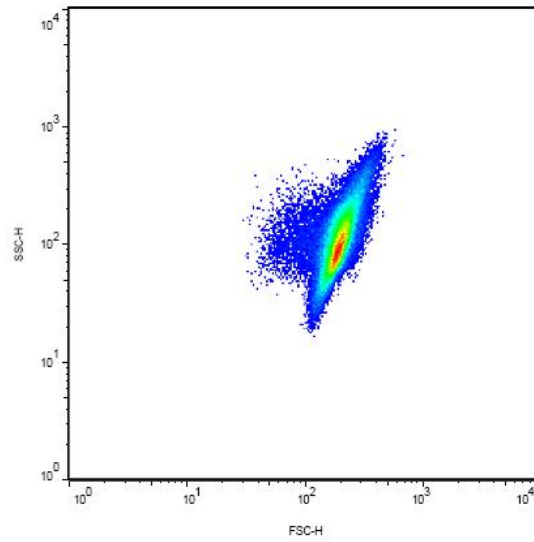


Figure 1: Flow cytometric dot plots.

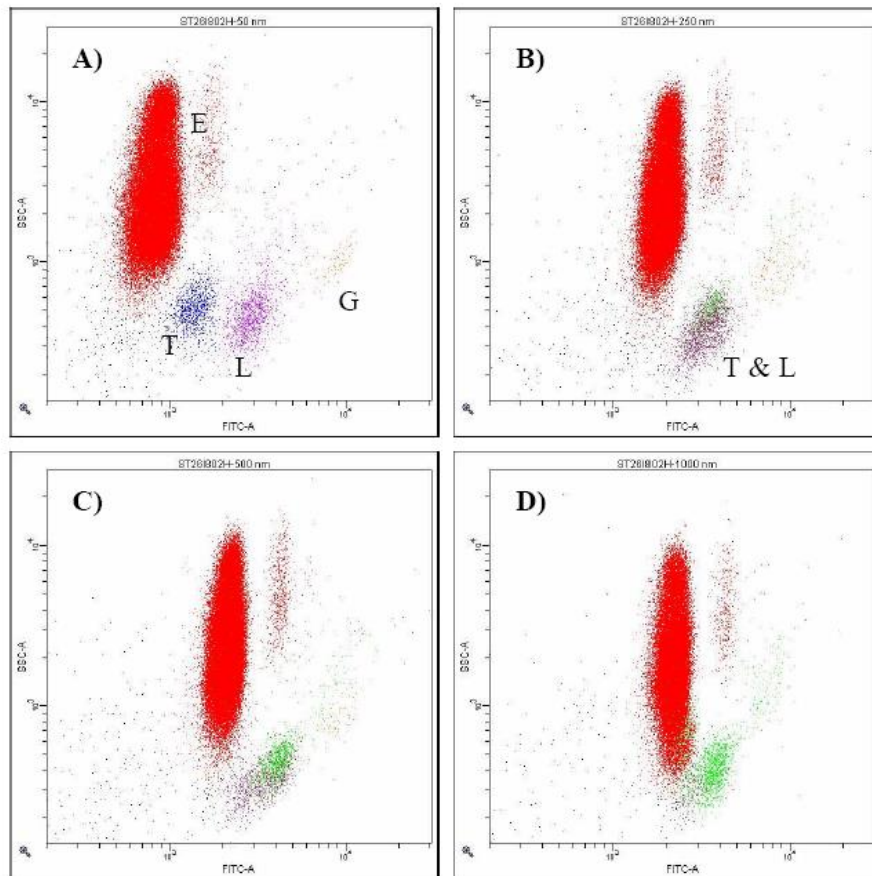


Figure 2: Flow cytometric side scatter (SSC) vs. 3,3'-dihexyloxcarbocyanine iodide (DiOC6(3)) fluorescence (FITC-A) dot plots of rainbow trout blood cells treated with 50 to 1000 nM dye, indicating erythrocytes (e), thrombocytes (t), lymphocytes (l), granulocytes (g) and Sytox® Red-positive events (non-viable cells) indicated in green.

For each treatment, we also measured the size of erythrocytes and genome size. Genome size was positively correlated with erythrocyte nucleus size and chromosome number when using PI as the fluorescent dye. The method used in the present study was based on fresh unfixed material obtained from fish directly collected from water. This is important as precision and reproducibility of the measurements may depend on chromatin condensation. Processing specimens with any fixative solutions before flow cytometric analysis might interfere with the DNA structure what could result in a shift of PI fluorescence intensity.

This work provides new knowledge on *Oncorhynchus mykiss* genetics/genomics, important for future research in basic cellular/molecular mechanisms and for the development of molecular techniques in this species. However, further investigation is required to obtain a high percent tetraploid Rainbow trout population.

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