

Tetraploidy Determination in Rainbow Trout (*Oncorhynchus mykiss*) Based on Erythrocytes Dimensions

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Abstract

Tetraploidy induction at fish is characterized by modification of normal diploid chromosome set (2n) into tetraploid set (4n). Experiments were carried out on biological material from rainbow trout (*Oncorhynchus mykiss*) during the natural breeding season. Polyploidy was induced by exposing the eggs to heat shock. Blood smear was used as a technical method, to determine diploid and tetraploid status. Staining of blood smear was performed by Pappenheim method. The erythrocytes area and perimeter measurements done comparatively on tetraploid and diploid individuals may represent an indicator to determine the ploidy level of individuals. Erythrocytes area for tetraploid individuals is 2.18 times higher than at diploid individuals, and perimeter 1.45 times higher than in diploid individuals.

Keywords: erythrocytes, heat shock, rainbow trout, tetraploidy

1. Introduction

Tetraploidy induction [1-4] at fish is characterized by modification of normal diploid chromosome set (2n) into tetraploid set (4n) [1]. Tetraploid cells are having four sets of chromosomes, in comparison with diploids were two sets are present, this is leading further to bigger nucleus. Rapport of the cytoplasm and nucleus is constant, so if nucleus volume is increasing proportionally will increase also the cell cytoplasm volume. Theoretically, tetraploid cell size will be greater than in diploid cells. Size increase of the polyploid cells is valid for all tissues and cells.

Polyploidy can be identified by karyotype analysis, flow cytometry, cell size measurement with Coulter Counter Channellizer, silver staining of nuclear organization regions (Ag-NOR), izozimes analysis using markers. Karyotype analysis is the most accurate method, but the

technique is slow and laborious [5]. Flow cytometry is a fast method but the equipments are very expensive. Coulter Counter Channelyzer analysis is a rapid method and the equipment is cheaper. Cell size measurement based on blood smear is an enough precise method [4]. Ag-NOR analysis [3] is cheaper and easier to perform than karyotype analysis, and can be applied without sacrificing the young fish. The principle of this method is based on establishing the numbers of NORs (1 NOR per haploid genome) [6].

2. Materials and methods

To study the biological material from rainbow trout (*Oncorhynchus mykiss*), blood smear was used as a technical method, to determine diploid and tetraploid status. Staining of blood smear was performed by Pappenheim method, using two coloring solution May-Grunwald and Giemsa. Smear examination has been made using a microscope with immersion objective, and to

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calculate perimeter and area of erythrocytes was used Quick photo program.

Tetraploidy induction was performed on biological material from rainbow trout (*Oncorhynchus mikiss*) obtained during natural breeding season. Eggs were collected from two females, in the age of three and respectively four years, by abdominal pressure (cca.4000 eggs).

Semen was collected from 4 males in the age of two years and after that eggs were fertilized by wet method. After fertilization to remove the excess of sperm, eggs were rinsed with water.

Heat shock was induced after 6 hours after fecundation at 28°C for 15 minutes and then the fertilized eggs were incubated. Water temperature for incubation was 7.5-8°C, the same temperature of water was used for activation and rinsing. Eggs were incubated for 45 days, summing a temperature of 340°C. For control group were

used normal fertilized eggs, incubated in same condition as per experimental group.

3. Results and discussion

To determine the tetraploidy induction rate in rainbow trout we started from the idea that the heat shock applied after 6 hours after fertilization will block cell division when diploid set of chromosomes is doubled ($2n+2n=4n$). From the experimental group 15 tetraploid trouts, in the age of two and a half years, were chosen randomly and we have collected blood samples for smear preparation. Smear was performed also for the individuals from the control group.

In Figure 1 are presented erythrocytes from tetraploid rainbow trout and in Figure 2 erythrocytes from diploid rainbow trout.

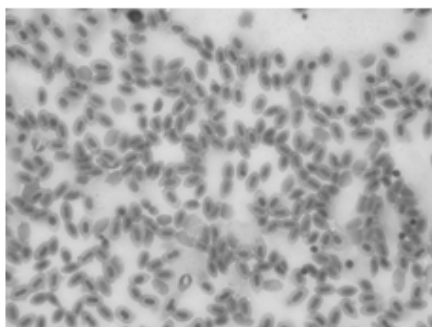


Figure 1. Erythrocytes from tetraploid rainbow trout

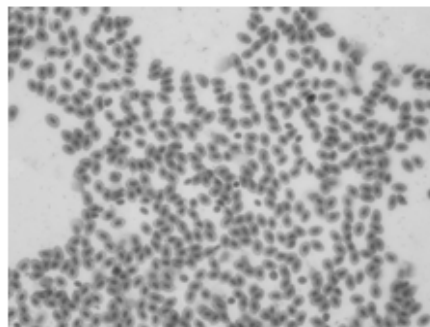


Figure 2. Erythrocytes from diploid rainbow trout

Erythrocytes were examined on blood smears, on minimum 10 fields. To determine the exact dimensions for erythrocytes we measured both, cell area and cell perimeter. Results from these measurements done on tetraploid trout are presented in Table 1. From those data we observe

that the erythrocytes area has an average of 61.48 μm^2 with values ranging between 51.4 to 88.6 μm^2 . Erythrocytes perimeter average is 30.12 μm , with minimum and maximum variation between 27.2 - 33 μm .

Table 1. Area and perimeter measurements results of erythrocytes from tetraploid rainbow trout

Ref. no.	Number of measurements	Area (μm^2)	Standard deviation(s)	Perimeter (μm)	Standard deviation (s)
1	10	56.3	9.7	28.9	2.5
2	10	53.1	7.5	28.8	2.6
3	10	51.4	8.5	27.5	1.7
4	10	58.9	12.3	30.2	4.4
5	10	56.8	8.2	28.1	1.8
6	10	57.37	8.89	29.0	1.7
7	10	76.4	6.0	33.3	1.0
8	10	67.8	6.2	31.2	1.2
9	10	66.5	7.5	30.5	1.7
10	10	67.8	7.9	32.2	2.7
11	10	51.9	10.4	28.1	2.3
12	10	51.8	5.5	28.1	2.1
13	10	57.4	8.1	28.9	2.0
14	10	58.3	9.9	29.8	2.4
15	10	88.6	10.9	36.8	2.2
16	10	79.3	12.3	34.7	3.1
17	10	61.8	4.2	30.2	1.3
18	10	50.0	7.3	29.1	2.0
19	10	64.85	5.4	29.8	2.2
20	10	63.25	20.28	30.8	4,1
21	10	51.7	10.3	27.2	2.0
22	10	59.3	13.4	29.5	3.2
Average		61.48	9.7	30.12	2.3

Raw data in Table 1 were processed statistically and are presented in Table 2. From the Table 2 data can be observed that the area has an average and error of the mean value of $61.48 \pm 2.11 \mu\text{m}^2$, standard deviation has a value of 9.7, and coefficient of variation is 15.77, which means that

our measurements have medium variability. In case of erythrocytes perimeter, media and medium error of the mean was $30.12 \pm 0.5 \mu\text{m}$, standard deviation had a value of 2.3 and the coefficient of variation has a value of 7.63 which represents a small variation of this character.

Table 2. The statistical interpretation on area and perimeter on tetraploid rainbow trout erythrocytes

Ref. No.	Specification	$\bar{x} \pm s_x$	Standard deviation (s)	Variance(s^2)	Coefficient of variation (C.V.)
1	Aria	61.48 ± 2.11	9.7	97.5	15.77
2	Perimeter	30.12 ± 0.5	2.3	5.57	7.63

The results for erythrocytes measurements for area and perimeter done on diploid rainbow trout from control lot are shown in Table 3. In Table 3 can be

observed that the average for erythrocytes area measurements is $28.1 \mu\text{m}^2$ and erythrocyte mean perimeter is $20.68 \mu\text{m}$.

Table 3. Results of area and perimeter of erythrocytes from diploid rainbow trout

Ref. no.	Number of measurements	Aria (μm^2)	Standard deviation(s)	Perimeter (μm)	Standard deviation (s)
1	10	25.3	3.4	20	1.3
2	10	23.4	4.4	19.1	1.6
3	10	30.4	2.9	21.5	1.2
4	10	31.7	9.08	21.3	1.3
5	10	30.6	3.8	21.8	1.5
6	10	27.5	4.2	20.4	1.4
Media		28.15	3.30	20.68	1.03

From Table 4 can be observed that the erythrocyte area has an average and medium error of $28.15 \pm 1.35 \mu\text{m}^2$, standard deviation has a value of 3.3 and coefficient of variability 11.72, which indicates a low variability of this character. Media

and medium error of the mean for erythrocytes perimeter from diploid individuals has a value of $20.68 \pm 0.42 \mu\text{m}$, standard deviation is 1.03, and coefficient of variation is small 4.98.

Tabel 4. The statistical interpretation on area and perimeter on diploid rainbow trout erythrocytes

Ref. No.	Specification	$\bar{x} \pm s_x$	Standard deviation(s)	Variance(s^2)	Coefficient of variation (C.V.)
1	Area	28.15 ± 1.35	3.30	10.95	11.72
2	Perimeter	20.68 ± 0.42	1.03	1.03	4.98

From the above presented results can be seen that the difference between the erythrocytes area from tetraploid individuals and diploid individuals is $33.33 \mu\text{m}^2$, which shows that erythrocytes from tetraploid individuals are having a higher area by 2.18 than diploid individuals.

The difference observed on erythrocytes perimeter from tetraploid and diploid individuals is $9.44 \mu\text{m}$, which shows that erythrocytes from tetraploid are having a higher perimeter by 1.45 than diploid individuals.

4. Conclusions

The erythrocytes area measurements done comparatively on tetraploid and diploid individuals may represent an indicator to determine the ploidy level of individuals.

The erythrocytes perimeter measurements done comparatively on tetraploid and diploid individuals may represent an indicator to determine the ploidy level of individuals.

Erythrocytes area for tetraploid individuals, measures in μm^2 , is 2.18 times higher than at diploid individuals.

Erythrocytes perimeter measured on tetraploid individuals, measures in μm , is 1.45 times higher than in diploid individuals.

Coefficient of variation is lower to medium for erythrocytes area, which indicates that the method has acceptable precision and accuracy for determining ploidy level in rainbow trout.

Coefficient of variation is small for erythrocytes perimeter, which indicates that the method has acceptable precision and accuracy for determining ploidy level in rainbow trout.

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