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VARIABILITY OF SOME CELL PARAMETERS IN DI- AND TRIPLOID RAINBOW TROUT *ONCORHYNCHUS MYKISS* R.

Heat shock (26 °C during 20 min., started 27 min. after insemination) was used to induce triploidy in rainbow trout. Relative DNA content, relative cell volume, cell and nucleus length of erythrocytes, and diameters of liver-cell nuclei were used to estimate the ploidy level.

17 out of 24 studied adult fish could be classified as di- or triploids unequivocally, the erest showed intermediate values of some parameters or the results obtained by different methods were contradictory. Diameters of liver-cell nuclei do not correlate with the ploidy level. The relative DNA content was the most reliable parameter for distinguishing di- and triploids.

Several parameters must be analyzed simultaneously to estimate the ploidy level of rainbow trout with high reliability.

Introduction

The techniques of inducing polyploidy in fish have been developed rapidly and are widely used in modern aquaculture. To estimate the results of these experiments fast and large-scale express methods are needed. Chromosome counting is a complicated and slow method for ploidy determination, which cannot be used as an express method, although karyotyping remains the only definitive method for determining chromosome numbers precisely. As an increase of the chromosome number leads to an increase in the dimensions of somatic cells and their nuclei, the kidney and epithelial cells or chondrocytes (Purdom, 1969; Васецкий, 1967; Chourrout, 1982; Yamaha, Onozato, 1985; Wolters et al., 1982), but most often the erythrocytes and their nuclei have been measured to determine the ploidy level (Purdom, 1972; Valenti, 1975; Chourrout et al., 1986). There are various other methods of erythrocyte analysis, which have been used for this purpose — microdensitometry, flow cytometry (Gervai et al., 1980; Lincoln, 1981; Thorgaard et al., 1982; Allen, 1983; Solar et al., 1984) and cell volume measurement on the basis of the electronic density by means of Coulter-counter (Benfey, Sutterlin, 1984; Cassani, Caton, 1985; Wattendorf, 1986). The purpose of this study was to compare the variability of different parameters of diploid and triploid rainbow trout blood cells to estimate their suitability for ploidy determination.

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Material and methods

The experiment of triploidy induction was made in the Kotka trout farm (North Estonia). The Donaldson strain of rainbow trout was used. Eggs of two females were mixed, fertilized with milt of two males and activated with water during two minutes. 27 minutes after fertilization, the eggs were put into 100 l tank with 26 °C water for 20 minutes. The temperature of water used for sperm activating and incubation of eggs before and after shock fluctuated between 10.5—11.5 °C.

To reveal whether the triploidy was really achieved and to estimate the proportion of triploids metaphase chromosome slides were made from the kidney cells of 12 experimental group and 18 control group fish from another hatchery. The fish were injected with 0.3% colhicine solution (1 ml/kg). Pieces of kidney tissue were fixed in ethanol-acetic acid fixator (3 : 1), treated with hypotonic solution and stained by using the standard Giemsa techniques.

To study the variability of blood and liver-cell parameters samples were taken from 24 mature fish weighing 620—1420 g. The blood sample was taken from heart and pieces of liver tissue were fixed. Blood smears were made, fixed in ethanol-acetic acid fixator and stained with aceto orceine to measure the dimensions of erythrocytes and their nuclei. 30 erythrocytes were measured from every specimen with the aid of ocular micrometer. Blood cell nuclei were measured in a series of 50 juvenile fish to determine an approximate percentage of triploids. It appeared to be 33%. On the following day after taking blood samples, the DNA content and cell volume were determined by flow cytometer ATC 3000 (Bruker) connected with Coulter Counter. 30 000—40 000 cells from every fish were analyzed. Blood samples were prepared by the Allens (1983) method. RNAase was added to samples. The same samples were storaged in refrigerator at 2—7 °C and used for the second time after a year.

The pieces of liver were fixed in fixator of Susza and histological slices 7—9 μm thick were stained by Feulgen. Diameters of 30 cell nuclei from every fish were measured.

Results

Metaphase chromosomes. In the sample of experimental group, 3 fish occurred to be triploids (NF = 156) and 6 diploids (NF = 104). 3 fish had an intermediate karyotype (NF = 110 − 150). In control group 16 fish were diploids and 2 differed from the normal karyotype having fewer than 104 chromosome arms. Therefore we may conclude that not all the shocked embryos became triploids but at least 1/4 of the grown-up fish were real triploids.

DNA content. On the basis of the relative DNA content of erythrocyte nuclei, two groups of fish could be distinguished (Fig. 1). The fish of the first group had on an average 1.9 (1.5—2.1) times more DNA in their blood cells than had sperms of diploid rainbow trout. In the second group this parameter was 2.8 (2.6—3.0). The results of the repeated analyses of the DNA content in the samples storaged over a year gave an entirely different picture. There was no correlation between the results of the first and second analyses. It allows us to conclude that blood samples could not be storaged for such a period.

The blood of two triploid specimens (No. 16, 18) also contained diploid cells (0.2 and 0.5% of blood cells analysed).

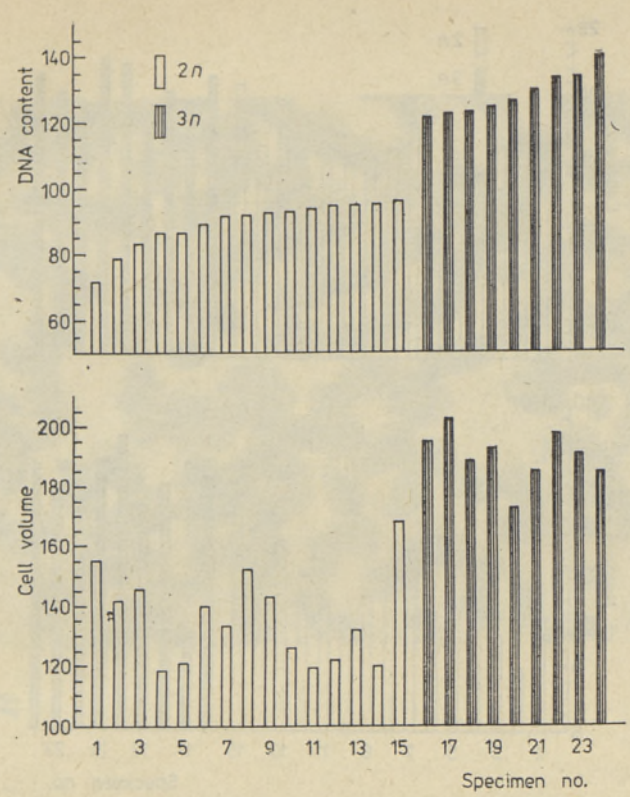

Fig. 1. DNA content and cell volume (characterized by mean channel number of cytometer) of di- and triploid rainbow trouts.

Volume of erythrocytes. On the basis of the volume of erythrocytes most fish could also be classified as belonging to two groups (Fig. 1). The first group (14 specimens) consisted of diploids. Their erythrocytes fell into channels 118—156 of Coulter Counter. The fish of the second group (8 specimens) were triploids and fell into channels 184—202. The ratio of the average volumes of erythrocytes of these groups is 1.45. There were two fish with intermediate size of erythrocytes. One of them (No. 15) was classified as diploid, and the other (No. 20) as triploid on the basis of DNA content. Four fish (two triploids — No. 18 and 23, and two diploids — No. 6 and 13) had multipeak histograms of the distribution of cell volumes. From the slides it appeared that their blood contained much more lymphocytes and neutrophile granulocytes than that of other fish. The peak corresponding to blood cells with the biggest volume was taken as erythrocytes.

Length of erythrocytes and their nuclei. By the length of erythrocytes the fish could also be divided into two groups (Fig. 2). The first group (14 fish) had erythrocytes on an average 14.71 (13.06—16.12) μm long, the second group (10 specimens) 20.58 (19.17—22.47) μm long, which means that the erythrocytes of triploids are on an average 1.4 times longer. However, one fish (No. 15), classified as triploid by the length of erythrocytes was classified as diploid by the DNA content. There were two fish (No. 7 and 13) which had almost intermediate cell-length values.

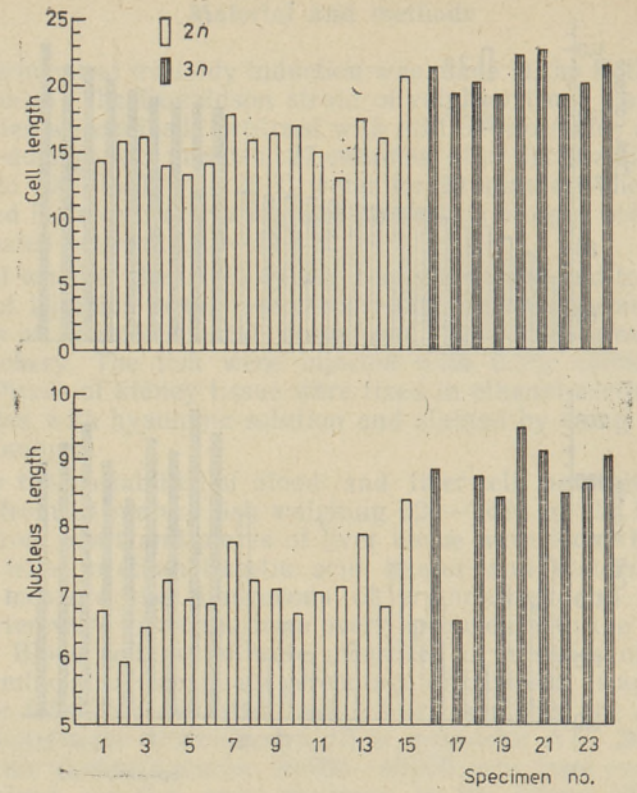

Fig. 2. Average erythrocyte cell length and nucleus length of di- and triploid rainbow trouts.

The average length of erythrocyte nuclei of the probably diploid group (15 specimens) was 6.79 (5.93—7.19) μm and that of the probably triploid group (9 specimens) was 8.69 (8.39—9.46) μm. As in the case of the length of blood cells, the diploid fish No. 15 was classified as triploid by this parameter and the specimens No. 7 and 13 had intermediate values of the nucleus length. In addition, the fish No. 17, classified as diploid by the nucleus length, was classified as triploid on the basis of the DNA content. The ratio of the average lengths of nuclei of tri- and diploid group fish cells was 1.3.

The nuclei of erythrocytes of the triploid group fish were of more elongated shape (Fig. 3). In a special series of the measurements of blood cells of 50 juvenile fish the ratio of the length and width of the erythrocyte nuclei of diploids was estimated 1.71 ± 0.03 and of triploids 2.0 ± 0.04. All the fish had a small number of dividing erythrocytes in their blood (Fig. 4). There was no correlation between different blood-cell parameters inside a diploid and triploid group. The coefficients of the variation of the blood parameters of diploid and triploid fish did not differ significantly.

Liver cell nuclei. The diameters of the liver cell nuclei of 16 specimens were measured. They varied in the range 6.17—7.55 (on an average 6.94) μm and no clustering of fish on the basis of this parameter was observed (Fig. 5). They did not correlate with dimensions of erythrocytes.

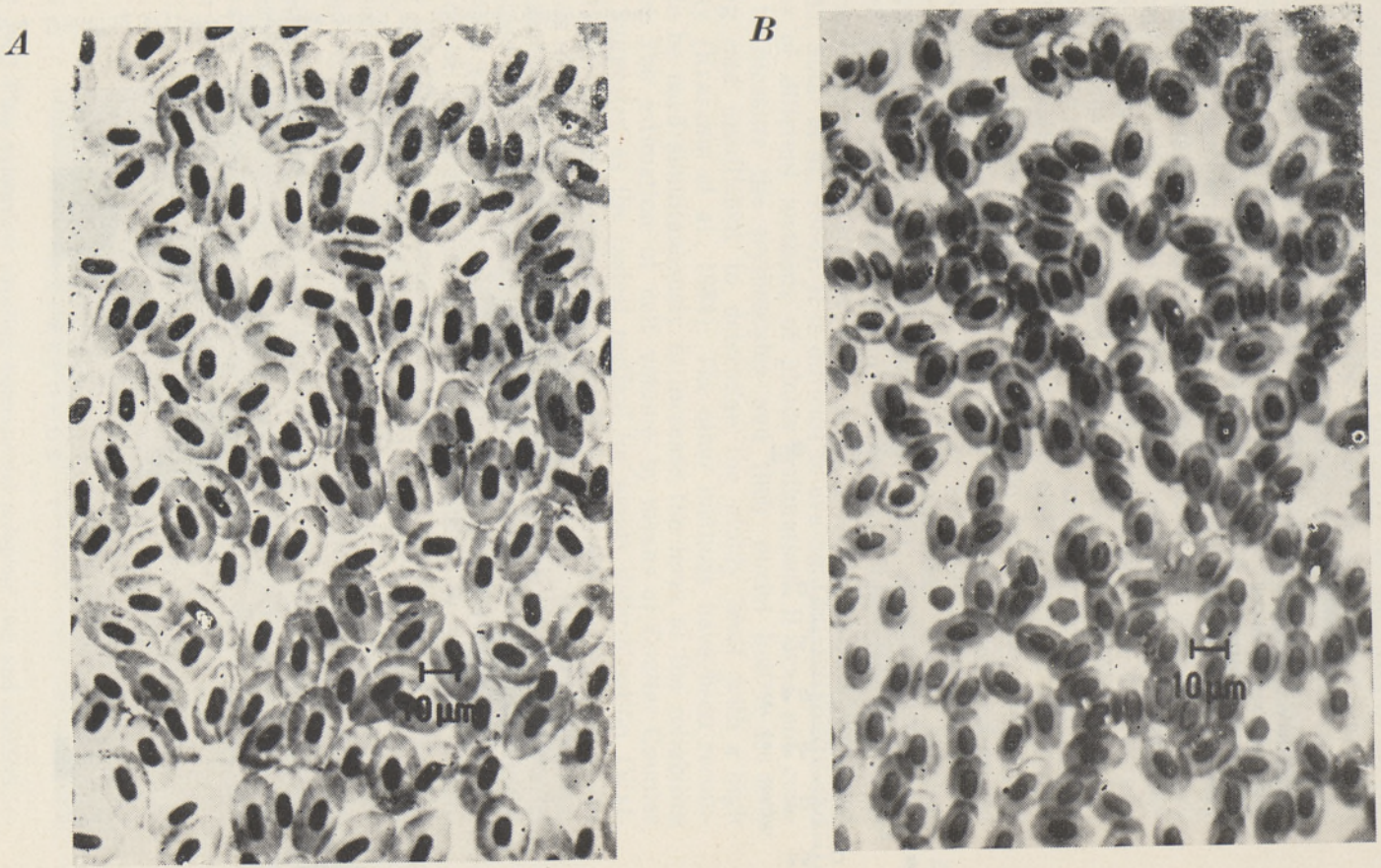

Fig. 3. Erythrocytes of di- (*A*) and triploid (*B*) rainbow trout.

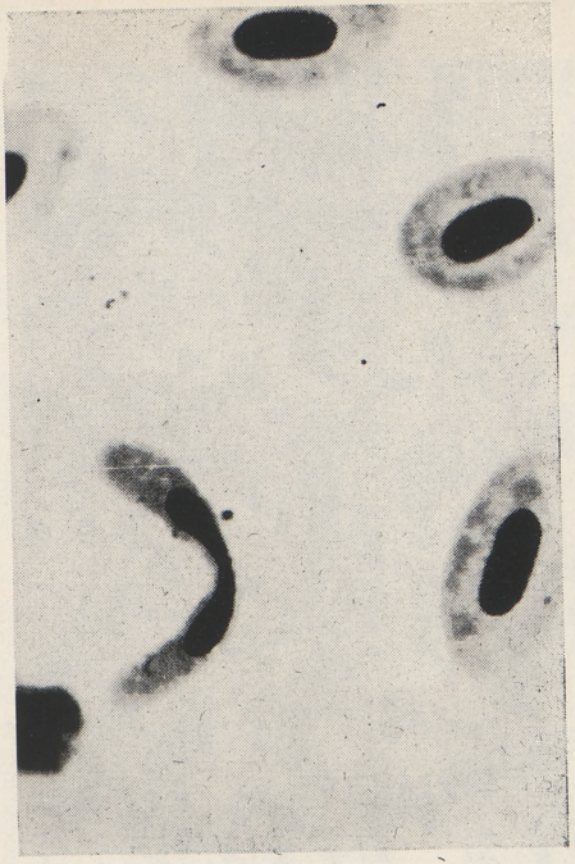

Fig. 4. Dividing erythrocyte in the blood of triploid rainbow trout.

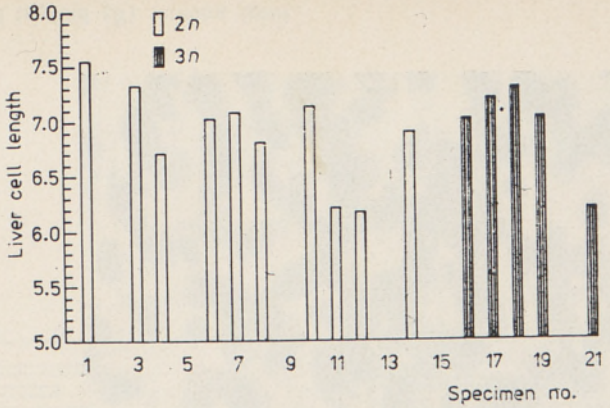

Fig. 5. Average diameter of liver cell nuclei of di- and triploid rainbow trouts.

Discussion

The proportion of fish which could be classified on the basis of all the parameters of blood cells unequivocally as diploids or triploids was 75%. However, there were two cases of intermediate cell volume, two cases of intermediate erythrocyte nucleus diameter and three cases of intermediate erythrocyte diameter (altogether 7 fish). Two fish (No. 15 and 17) were included into different groups on the basis of different parameters. So we can conclude that one parameter is not sufficient for determining the ploidy level. Combined methods should be recommended. All the parameters used here (except liver cell measurements) can be used in ploidy determinations. But, the DNA content measurements showed the most distinct and reliable differences between di- and triploids. The fish which have intermediate values of the indices of ploidy or give contradictory results when using different methods must be missed in practical purpose screenings. However, they need more detailed karyological investigation. We found some specimens with intermediate karyotype too, but it is difficult to say how these karyotypes have arisen. Diploid — polyploid mosaicisms have been described among induced polyploids of salmonids. (Allen, Stanley, 1978, 1979; Lemoine, Smith, 1980; Черненко, 1985). In our analyzes the per cent of diploid cells in the blood of triploids was negligible. This is in good accordance with literature data about rainbow trout (Thorgaard et al., 1982; Solar et al., 1984). It is difficult to evaluate the reliability and usefulness of different methods of ploidy estimation. It was not complicated to measure the erythrocytes and their nuclei but it is time consuming and insufficient to determine the ploidy level with a high probability (Krasznai et al., 1984). The other methods have been recommended to be used simultaneously to increase the level of the reliability of results. The estimation of cell volume by means of Coulter Counter has been suggested as the fastest method for ploidy determination (Wattendorf, 1986). However, as some trouts had multipeak histograms of the distribution of cell volume, the slides had to be studied, too, to distinguish, which peak corresponded to erythrocytes. The multipeak distribution of salmon blood cells has been explained with abnormal blood or contamination of blood sample with proteins. Such specimens have been recommended to be discarded (Benfey, Sutterlin, 1984). To measure simultaneously the DNA content and cell volume we have used blood cells treated by the method of Allen (Allen, 1983). It could be one of the causes of high intraindividual variability of erythrocytes. Usually the cells are not stained before volume determinations on the basis of electronic density.

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MÕNEDE RAKUPARAMEETRITE VARIEERUVUS DI- JA TRIPLOIDSETEL VIKERFORELLIDEL *ONCORHYNCHUS MYKISS* R.

Vikerforellide triploidsuse indutseerimiseks kasutati kuumašokki (27 min. pärast seemendamist 26 °C 20 min. vältel). 24 katsekala ploidsuse analüüsil määrati erütrotsüütide pikkused, suhteline maht ja tuumade pikkused ning DNA suhteline hulk erütrotsüütides, samuti maksarakkude tuumade diameetrid.

Kõigi nimetatud parameetrite (v. a. maksarakkude tuumade diameetrid) alusel klassifitseeriti di- või triploidideks 17 vikerforelli. Ülejäänud kuuel jäi mõne uuritud parameetri väärtus di- ja triploidi vahepeale või andis ploidsuse määramine erinevate parameetrite järgi erinevaid tulemusi.

Parim parameeter di- ja triploidide eristamiseks oli DNA suhteline hulk erütrotsüütides. Maksarakkude tuumade diameetrite väärtused ei olnud ploidsusega korrelatsioonis.

Usaldatava tulemuse saamiseks tuleb vikerforellide ploidsuse määramisel analüüsida mitut parameetrit.

Яак ТАМБЕТС, Тийт ПААВЕР, Ану ПАЛЬМ, Арно ПИХЛАК, Рихо ГРОСС

ВАРИАЦИЯ НЕКОТОРЫХ КЛЕТОЧНЫХ ПАРАМЕТРОВ У ДИ- И ТРИПЛОИДНЫХ РАДУЖНЫХ ФОРЕЛЕЙ *ONCORHYNCHUS MYKISS* R.

Для индукции триплоидий у радужной форели был применен тепловой шок (26 °С в течение 20 мин через 27 мин после осеменения). Для определения плоидности было измерено относительное количество ДНК в ядрах эритроцитов, относительный объем, длина эритроцита и его ядра, диаметр ядер клеток печени.

17 рыб из 24 исследованных могли быть однозначно классифицированы как ди- и триплоиды; у остальных рыб часть параметров была промежуточной или же результаты, полученные разными методами, были противоречивыми.

Диаметр ядер печеночных клеток не коррелировал с плоидностью. Наиболее достоверным признаком разграничения ди- и триплоидов является содержание ДНК.

Для достоверного определения плоидности у радужной форели необходимо у каждой рыбы проанализировать несколько параметров.