2倍体および4倍体シマドジョウの赤血球径の比較

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Comparison of Erythrocytic Size between Diploid and Tetraploid in Spinous Loach, *Cobitis biwae*

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Spinous loach, *Cobitis biwae*, lives in slow-flowing and stagnant waters in Honshu, Shikoku and the eastern part of Kyushu. There are two local races in spinous loach different in body size. The small race of the Kantô district is diploid with 48 chromosomes and the large race of the Kansai district is tetraploid with 96 chromosomes. Both races were examined for the relationship between the somatic chromosome number and erythrocytic size. The small race from Aki River, Tokyo, had 48 chromosomes with small erythrocytes and the large race from Yoshii River, Okayama Pref., had 96 chromosomes with large erythrocytes. The ratios of surface areas of the tetraploid erythrocytes and of their nuclei to those of the diploid ones were 1.62 and 1.74, respectively. These results show that tetraploid spinous loach is exactly discriminated from diploid one by the determination of erythrocytic size.

MINAMORI[1–3] reported that physiological isolation resulting in hybrid sterility was found between the small and large races of spinous loach, *Cobitis biwae*. Recently, HITOTSUMACHI *et al.*[4] studied karyotypes of *Cobitidae* and counted 96 chromosomes in spinous loach. KOBAYASI[5] made further studies of karyotypes of some local races of spinous loach and reported that the small race was diploid with 48 chromosomes and the large race was tetraploid with 96 chromosomes. UENO and OJIMA[6] also noted the diploid-tetraploid complex in this species. It is well known that polyploid causes an increase in nuclear and cellular size. Erythrocytic sizes of polyploid fishes were reported on *Carassius*[7–9], *Poeciliopsis*[10], *Gasterosteus aculeatus*[11], *Pleuronectes platessa*[12] and *Tilapia aurea*[13].

In the preceding report[9] the authors showed that it is easy to distinguish the triploid ginbuna, *Carassius auratus langsdorfi*, from the diploid one by the determination of erythrocytic size. In this paper, the relationship between the erythrocytic size and polyploidy was examined for spinous loach.

Materials and Methods

The specimens used of spinous loach, *Cobitis biwae*, were two different local races: 12 specimens collected from Aki River, Tokyo (Lat. 35°43′N, Long. 139°14′E), in May, 1975, and 15 specimens from Yoshii River, Okayama Pref. (Lat. 35°10′N, LONG. 133°55′E), in June, 1976. The fish from Aki River were reared in the laboratory for about one year. The experiments were carried out in May, June and Aug., 1976. The techniques for chromosome study used were the same as KOBAYASI[13]. Some small pieces of the gonad cut off were placed in a disposable Petri dish and cultured at 30°C in a CO_2 incubator for 7 days. The culture media consisted of a mixture of 60 m*l* of TC 199 (Chiba Serum Institute Co., Ltd.), a very small quantity of 4 kinds (penicillin, streptomycin, kanamycin and mycostatin) of antibiotics, and 40 m*l* of calf serum. Chromosome preparations were made according to the standard flame-drying method. Chromosome numbers were counted at least 5 times on each preparation at metaphase stage under a 1,000-fold magnification.

Blood sample was obtained by cutting the caudal peduncle. Blood-smear preparations were made by the ordinary method and stained with Giemsa's solution[15]. The major and minor axes of 100 erythrocytes and of their nuclei on each smear preparation were measured by a micrometer under a 1,500-fold magnification, and then the ratio of major to minor axis and surface area (major axis × minor axis × $\pi/4$) were calculated.

Results

In the two races, females were usually larger

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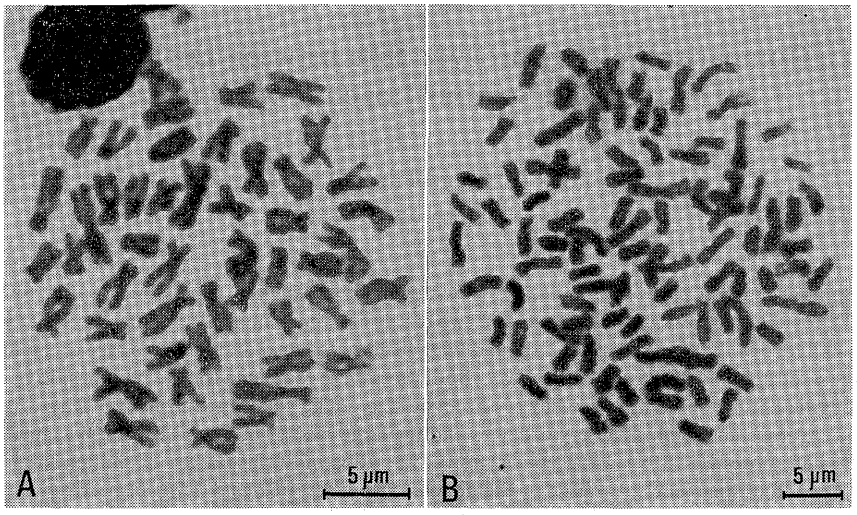

Fig. 1. Chromosomes in somatic cells of diploid and tetraploid spinous loach. A, diploid form with 48 chromosomes; B, tetraploid form with 96 chromosomes.

Table 1. Comparison of erythrocytic size between diploid and tetraploid in spinous loach. One hundred cells were measured on each specimen.

	Diploid (12 fish)*1	Tetraploid (15 fish)*1	Ratio*2
Erythrocyte			
Major axis (μm)	11.50±0.73 (10.54~12.63)	15.50±0.41 (14.84~16.03)	1.35
Minor axis (μm)	7.74±0.41 (6.84~8.60)	9.27±0.33 (8.81~9.93)	1.20
Major a./Minor a.	1.49±0.16 (1.23~1.85)	1.67±0.08 (1.53~1.78)	1.12
Area (μm^2)	69.7±3.6 (63.1~75.0)	112.8±4.4 (105.6~120.5)	1.62
Nucleus of erythrocyte			
Major axis (μm)	5.13±0.37 (4.50~5.75)	7.43±0.28 (6.97~7.84)	1.45
Minor axis (μm)	3.00±0.14 (2.81~3.21)	3.60±0.21 (3.27~4.00)	1.20
Major a./Minor a.	1.72±0.14 (1.51~1.99)	2.07±0.15 (1.82~2.38)	1.20
Area (μm^2)	12.1 ±1.1 (10.5~13.8)	21.0±1.5 (18.6~24.2)	1.74

*1 mean±standard deviation (range).
*2 represents each ratio of tetraploid to diploid.

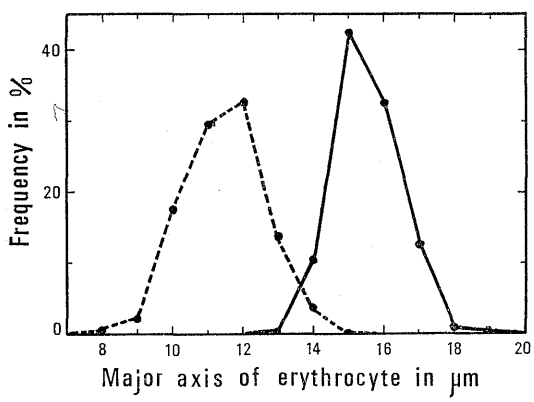

Fig. 2. Comparison of Price-Jones curves between diploid and tetraploid in spinous loach. Broken line indicates diploid fish (average of 12 specimens) and solid line the tetraploid fish (average of 15 specimens).

than males in the body size. The mean of total length of specimens of Aki River race was 49.0± 4.2 mm in 7 males and 59.4±2.0 mm in 5 females. In Yoshii River race, 10 males were 79.2±3.9 mm in length and 5 females 100.2±13.2 mm. The chromosome number of specimens of Aki River race was 48 and that of Yoshii River race 96 (Fig. 1). The small race of Aki River was diploid and the large race of Yoshii River tetraploid.

Table 1 shows mean values of the erythrocytic and nuclear sizes. In the diploid fish, the mean of erythrocytic size was 11.50×7.74 μm, and that of nuclear size 5.13×3.00μm, whereas the corresponding sizes to the tetraploid fish were respectively 15.50×9.27 μm and 7.43×3.60 μm. Price-Jones curves are shown in Fig. 2. The distribution (and frequency in percent) of the major axis of 1,200 erythrocytes in 12 diploid fish was 8 μm (0.2%),

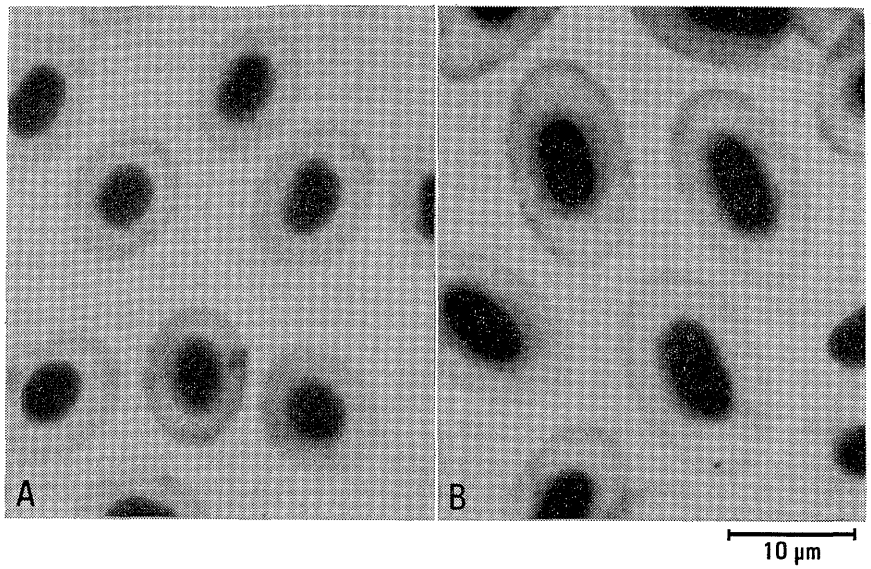

Fig. 2. Microphotographs of diploid (A) and tetraploid (B) erythrocytes of spinous loach.

9 μm (2.1%), 10 μm (17.8%), 11 μm (29.5%), 12 μm (32.8%), 13 μm (13.7%), 14 μm (3.8%) and 15 μm (0.2%), and that of 1,500 erythrocytes of 15 tetraploid fish was 13 μm (0.5%), 14 μm (10.6%) 15 μm (42.5%), 16 μm (32.5%), 17 μm (12.7%), 18 μm (1.1%) and 19 μm (0.1%).

Erythrocytes and their nuclei of tetraploid were larger in both axes than those of diploid (Table 1 and Fig. 3). The means of the surface areas of erythrocytes and their nuclei were respectively 69.7 ± 3.6 μm^2 and 12.1 ± 1.1 μm^2 in the diploid fish, and those of tetraploid fish were 112.8 ± 4.4 μm^2 and 21.0 ± 1.5 μm^2. Therefore, the ratio of the area of the tetraploid to diploid was 1.62 for erythrocyte and 1.74 for nucleus.

Discussion

MINAMORI[1-3] reported that there are two local races in spinous loach, one of which is larger in size than the other, and that hybrids of small race × large race were nearly sterile: abnormal spermatogenesis, sterility in females and inviability in second generation. HITOTSUMACHI *et al.*[4] compared karyotypes in several species of *Cobitidae* with each other. They assumed that spinous loach which had 96 chromosomes was a tetraploid species since another species examined had 50 chromosomes. KOBAYASI[5] made further studies of karyotypes in some local races of spinous loach and reported that small race is a diploid and the large race a tetraploid. Generally, the polyploid cell and nucleus are larger in size than the diploid ones. Therefore, the determination of erythrocytic size by the blood-smear preparation is often used to discriminate the polyploid from the diploid. The relationship between the polyploidy and cellular size, especially erythrocytic size in fish, was reported on *Carassius*[7-9], *Poeciliopsis*[10], and artificial triploid specimines of *Gasterosteus aculeatus*[11], *Pleuronectes platessa*[12], and *Tilapia aurea*[13] The ratios of area of erythrocytes and their nuclei of triploid to diploid in *Carassius* and *Poeciliopsis* increase by 1.30–1.42[7,9,10]. The ratios in spinous loach increased by about 1.62 and 1.74, respectively (Table 1). It is of interest that the increase in erythrocytic and nuclear sizes was almost proportional to the increase in chromosome set. Although the triploid erythrocyte of *Carassius auratus langsdorfi* is more elongated in the direction of major axis than that of diploid one[9], the tetraploid erythrocyte of spinous loach is enlarged in both directions of major and minor axes than that of diploid one (Table 1 and Fig. 3). Thus, tetraploid spinous loach is easily discriminated from the diploid by erythrocytic size.

In the plants, polyploid produces a gigantism resulting from the increase in nuclear and cellular sizes. But in the animals, polyploidy is not suffered from the gigantism except for some invertebrates since many polyploid animals compensate for the increase in cellular size with decreasing cellular number[11,16,17]. A possiblity exists that tetraploid spinous loach with large erythrocytic and body size is suffered from the gigantism which is rare in the vertebrates.

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