Effects of Triploidy on Tissue Structure of Some Organs in Ayu

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The retina, optic tectum and trunk kidney of diploid and triploid ayu *Plecoglossus altivelis* were examined histologically. The differences between the two ploidies in sound and light sensitivity were also observed. No difference was found in the size of the organs between diploids and triploids. Histologically, the nucleus and cell in diploids were larger in number and smaller in size than those in triploids. Physiologically, triploids have a lower sensitivity to both sound and light than diploids.

The effects of polyploidy in fish have been studied in recent years, most of them being on the chromosome number¹⁻³⁾ and on the hematological characteristics.⁴⁻⁹⁾ By contrast, there are only a few studies on the effect of triploidy on the size of cells and nuclei of several tissues in stickleback *Gasterosteus aculeatus*¹⁰⁾ and on the physiological changes caused by triploidy in *G. aculeatus*¹¹⁾ and *Salmo salar*.¹²⁾ Swarup¹¹⁾ showed that both nucleus and cell sizes in the cartilage, blood and nerve cells of triploid stickleback are apparently larger than those of the corresponding cells of the diploid individuals, and increase in cell size does not affect the body size. The present study is undertaken for the purpose of showing the differences of tissue structure of some organs in ayu *Plecoglossus altivelis*, an amphidromous fish, between triploidy and diploidy, and of understanding the effect of polyploidic changes on their physiological characteristics and functions.

Materials and Methods

Triploid and diploid ayu *P. altivelis* used for histological observations were obtained from the Fukuoka Prefectural Freshwater Fisheries Experimental Station in April, 1987. Triploidy was induced by cold shock treatment following the methods of Taniguchi *et al.*¹³⁾ Ten triploid fish, 60.4-64.5 mm in fork length (FL); 1.65-2.35 g in body weight (BW) and 10 diploid fish,

68.1-75.4 mm FL; 3.12-3.89 g BW were fixed in Bouin's solution. The pieces of bodies were dehydrated, embedded in paraffin, sectioned serially at 5 μ m and stained chiefly with hematoxylin-eosin. Three hundred neuronal cells, 105 segments of secondary proximal tubule and 430 nuclei of secondary proximal tubule were examined in the length and width using micrometer under the light microscope. Based on the assumption that each cell and nucleus form was ellipsoids, their area and volume were determined using the following formulas:

surface area (S) = $1/4 \times ab\pi$ and

volume (V) = $4/3 \times \pi(a/2) \times (b/2)^2$

where: a = the length of a cell or nucleus

b = the width of a cell or nucleus.

Triploid and diploid ayu used for light and sound sensitivity observations were obtained from the Kochi Prefectural Freshwater Fisheries Experimental Stations in September, 1988. The observation of sound sensitivity was undertaken using 22 diploids, 19.94 cm in mean FL; 89.24 g in mean BW; and 22 triploids, 20.37 cm in mean FL, 98.38 g in mean BW. A long piece of wood was placed under two water tanks, each 100 l in capacity. Sound was made by hitting the wood between the water tanks by the hammer and the loudness was measured by the vibration level meter (Rion VM-14B). A pair of triploid and diploid fish of almost the same size were selected and placed in each water tank. After the fish became accustomed to the condition in the water

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tank, the wood was hit for 10 times at an interval of several seconds. On every hitting, the response of each individual was observed. Sign minus (−) was given to the fish which made no response to the loudness and scored by 0. Sign plus (+) and score 1 were given to the fish which was startled by the loudness by swimming in confusion in the water tank. Sign double plus (++) and score 2 were given to the fish which was much startled by the loudness by swimming and jumping in confusion in the water tank. Based on the total score of 10 observations of each pair fish, the sensitivity level was divided into four classes: total score 0, fish making no response; total score 1–5, making weak response; total score 6–15, making fair response and total score 16–20, making strong response. Four levels of loudness were used: below 60 dB, between 60–70 dB, between 70–80 dB and above 80 dB.

The light sensitivity was examined on 21 diploid fish and 21 triploid fish by placing a pair of the triploid and diploid in the 100 l water tank under the low intensity of light. When both ones took almost the same position against the light, the room was suddenly lighted. The first startled one at the lightening was given plus (+) record and the fish which made response later was given minus (−) record.

The difference in mean values of each features between triploid and diploid fish was compared statistically by Student's *t*-test.¹⁴⁾

Results

Retina

Structures of the retina in triploid ayu were the same as those in the diploid, formed out of eight distinct component layers: pigment epi-

thelium layer, rod and cone layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer. The thickness (%) of each layer of the retina in both triploids and diploids is shown in Table 1. Statistically significant differences in the thickness could be found in the epithelial layer, outer nuclear layer ($P<0.001$) and inner plexiform layer ($P<0.05$) between triploids and diploids. The former two layers, the diploids showed larger values than the triploids. By contrast, the inner plexiform layer of the triploids was thicker than that of the diploids. There was a difference in tissue structure between triploids and diploids. In triploids, the outer nuclear layer consisted of, in general, two strata of nuclei (Fig. 1A), while in diploids, the layer consisted of three strata of nuclei (Fig. 1B).

Optic Tectum

The optic tectum is a main optic center in the mesencephalon of fish. The nucleus size of the granular layer of this tectum was compared between triploids and diploids. The thickness of the granular layer in diploids was $70.21\ \mu\text{m}$ on the average, almost the same as that in triploids. The value was $67.36\ \mu\text{m}$ on the average in triploids and not significantly different at the level of 5% ($P>0.05$). The nucleus sizes of 300 neuronal cells of the granular layer in both triploids and diploids were compared by measuring their length and width. The nuclei of the neuronal cells of triploids were larger than those of diploids (Table 2, Figs. 2A and B). The mean length of nuclei in triploids was $4.88\ \mu\text{m}$, and significantly larger than that of diploids ($3.51\ \mu\text{m}$, $P<0.001$). The mean width of nuclei was $4.41\ \mu\text{m}$ in triploids, also significantly different from that in diploids

Table 1. Thickness of each component layer of retina in triploid and diploid ayu (Mean $\pm$ SD)

	Triploid	Diploid
Body weight (g)	2.03 $\pm$ 0.27	3.51 $\pm$ 0.31
Fork length (mm)	62.90 $\pm$ 1.50	70.80 $\pm$ 2.90
Thickness of retina (μm)	227.19 $\pm$ 19.15	242.87 $\pm$ 9.81
Thickness of each layer of retina (%)		
1. Epithelial layer	25.00 $\pm$ 1.16* ¹	28.72 $\pm$ 1.31
2. Rod and cone layer	13.16 $\pm$ 0.64	12.64 $\pm$ 1.29
3. Outer nuclear layer	6.99 $\pm$ 0.40* ¹	7.98 $\pm$ 0.54
4. Outer plexiform layer	7.69 $\pm$ 0.70	7.94 $\pm$ 0.86
5. Inner nuclear layer	11.18 $\pm$ 1.28	10.55 $\pm$ 1.10
6. Inner plexiform layer	20.69 $\pm$ 1.78* ²	18.71 $\pm$ 1.37
7. Ganglion cell layer	2.61 $\pm$ 0.27	2.44 $\pm$ 0.27
8. Nerve fiber layer	12.69 $\pm$ 1.55	11.46 $\pm$ 1.39

*¹ Significant at $P<0.001$; *² Significant at $P<0.05$.

Fig. 1. The outer nuclear layer of retina in triploid (A): 6.23 cm FL and diploid (B): 6.81 cm FL ayu. ONL: outer nuclear layer; RCL: rod and cone layer. $\times 500$.

($3.25 \mu\text{m}$, $P < 0.001$). The difference in nucleus length and width between triploids and diploids resulted in the difference in area and volume of neuronal cell nuclei, being larger in triploids than in diploids ($P < 0.001$).

Kidney

The area of the secondary proximal tubule of the trunk kidney of triploid and diploid ayu and the number and size of nuclei in it were compared. Areas of 15 secondary proximal tubule segments in 3 triploids and 4 diploids were measured. Mean area of proximal tubule was $697.29 \mu\text{m}^2$ in triploids and $758.95 \mu\text{m}^2$ in diploids. The area (%) of the tubule in triploids and diploids was compared. However, no statistically significant difference was found between them ($P > 0.05$) (Table 3). The number of nuclei within the tubule segment was counted. In triploids, the mean number of nuclei was 7.31, ranging from

4 to 12, and that in diploids was 10.08, ranging from 7 to 18, which showed a definite difference. Fig. 3 shows the relation between the area of the secondary proximal tubule cell (%) and the number of its nuclei in triploid and diploid ayu. The triploid has a smaller number of nuclei than the diploid in comparison using the same area of the tubule. Each nucleus of triploids was significantly larger than that of diploids ($P < 0.001$) (Table 3).

Sound Sensitivity

The response of diploids and triploids to the four levels of loudness is shown in Table 4. It shows that though the statistical examination for difference between the two ploidies was not done, diploids seem to be more sensitive to sound than triploids at every level.

Light Sensitivity

Table 2. Mean size, area and volume for neuronal cell nucleus of granular layer of optic tectum in triploid and diploid ayu (Mean $\pm$ SD)

	Triploid	Diploid
Body weight (g)	1.86 $\pm$ 0.19	3.53 $\pm$ 0.39
Fork length (mm)	61.17 $\pm$ 1.00	70.70 $\pm$ 4.08
Neuronal cell nucleus:		
Length (μ m)	4.88 $\pm$ 0.08*	3.51 $\pm$ 0.16
Width (μ m)	4.41 $\pm$ 0.10*	3.25 $\pm$ 0.14
Area (μ m ²)	16.93 $\pm$ 0.65*	9.01 $\pm$ 0.69
Volume (μ m ³)	50.47 $\pm$ 3.10*	19.84 $\pm$ 2.38

* Significant at $P < 0.001$ **Fig. 2.** The neuronal cell in the granular layer of optic tectum in triploid (A) and diploid (B) ayu. $\times 500$.

A simple test was carried out to examine the difference in light sensitivity between diploids and triploids (Table 5). Of 21 observations, in 14 cases diploids made faster response to light than triploids. In 1 case the triploids made faster response than the diploids. These suggest that diploids have higher sensitivity than triploids to light.

Discussion

The results obtained in this study on the tissue structure of the retina, granular layer of the optic tectum and the secondary proximal tubule of the trunk kidney in triploid ayu is generally in accordance with those of triploid stickleback *Gasterosteus aculeatus*.¹⁰⁾ The outer nuclear

Table 3. Area of secondary proximal tubule of trunk kidney (%) and its nucleus size and area in triploid and diploid ayu (Mean $\pm$ SD)

	Triploid	Diploid
Body weight (g)	2.03 $\pm$ 0.27	3.56 $\pm$ 0.31
Fork length (mm)	62.90 $\pm$ 1.50	71.00 $\pm$ 2.60
Area of secondary proximal tubule (%)	85.66 $\pm$ 0.45* ²	86.35 $\pm$ 2.74
Nucleus of secondary proximal tubule:		
Length (μ m)	5.24 $\pm$ 0.45* ¹	4.25 $\pm$ 0.33
Width (μ m)	4.19 $\pm$ 0.30* ¹	3.44 $\pm$ 0.25
Area (μ m ²)	17.34 $\pm$ 2.63* ¹	11.55 $\pm$ 1.66

*¹ Significant at $P < 0.001$; *² Not significant at $P > 0.05$. **Fig. 3.** Scatter diagram showing the relation between area of secondary proximal tubule and its nucleus number in triploid and diploid ayu.**Table 4.** Response of triploid and diploid ayu to four levels of sound loudness

Level of sound loudness	Ploidy	No. of fish	Response			No response
			Strong	Fair	Little	
<60 dB	3N	6	—	—	1	5
	2N	6	—	1	2	3
60–70 dB	3N	16	—	—	6	10
	2N	16	—	4	12	—
70–80 dB	3N	8	—	2	5	1
	2N	8	2	5	1	—
>80 dB	3N	14	—	11	3	—
	2N	14	3	9	2	—

Table 5. Response of triploid and diploid ayu to light

	Triploid		Diploid		1)	2)	3)	4)
	Body weight (g)	Fork length (cm)	Body weight (g)	Fork length (cm)				
1.	91.80	21.63	85.77	19.77	*			
2.	116.04	20.82	93.11	19.71	*			
3.	144.26	23.39	98.55	21.31	*			
4.	110.52	20.88	92.24	20.45			*	
5.	77.50	19.21	85.05	19.69	*			
6.	116.25	22.86	90.38	20.47	*			
7.	114.20	21.30	95.97	20.25				*
8.	86.61	19.87	108.93	20.51	*			
9.	121.25	22.00	107.61	21.89				*
10.	98.43	19.42	75.15	19.02	*			
11.	99.20	21.16	93.84	19.88	*			
12.	109.68	22.03	76.19	18.28	*			
13.	124.44	21.86	98.63	20.08				*
14.	99.34	21.18	91.11	19.25	*			
15.	144.42	22.12	85.82	21.18				*
16.	118.74	21.85	89.95	19.64	*			
17.	110.80	21.75	109.09	21.03	*			
18.	137.45	23.41	88.49	19.86	*			
19.	115.51	21.28	93.95	20.19		*		
20.	128.17	20.54	88.65	20.33				*
21.	116.32	22.20	90.95	19.78	*			

1). First response was made by diploid fish.

2). First response was made by triploid fish.

3). Both ploidies made response at the same time.

4). Both ploidies made response several seconds after lightening.

layer of the retina consisted of three strata of nuclei in diploids and two strata in triploids in these two species. This layer in diploids was thicker than in triploids. It is generally accepted that nuclear size increases in proportion with chromosome number. The number of nuclei of visual cells (cones and rods) in diploids is, therefore, larger than that in triploids, which means that the density of these cells is higher in diploids than in triploids. This fact suggests that diploids possess higher acuity of vision than triploids. Swarup¹⁰⁾ also reported that the number of cells of the pronephric ducts of *G. aculeatus* was 26 in the diploid and 18 in the triploid, *i. e.* nearly in the ratio of 3:2. The same ratio was also obtained in the secondary proximal tubule of the trunk kidney in *P. altivelis*, most tube consisted of 9 nuclei in diploids and 6 nuclei in triploids. Concerning the nervous system, in which cell divisions cease in the early developmental stage, triploid ayu showed a reduction in the cell number as in other organs. Swarup¹⁰⁾ suggested that the cell division in the polyploids is undergone at a slower rate at least up to the end of the cleavage period, so that a polyploid gastrula consists of a

smaller number but larger in the size of cells than in diploids. The same mechanism might operate also in above-mentioned 3 organs of *P. altivelis*.

The present study suggests that diploid ayu are more sensitive to both light and sound than triploid ayu. This suggestion is also supported by the fact that triploids of the rainbow trout tend to be "shy" and in mixed populations are at a disadvantage in the competition for food with diploids.¹⁵⁾ We also observed that when feeding while diploid ayu swam up toward the water surface to take foods, triploid ayu tended to wait near the bottom to take sinking foods. The shyness of triploids might be related with low sensitivity to environmental stimulations.

The low sensitivity to sound in triploids seems profitable for fish culture, because it makes them feel easy in the reared environment where is full of noise. Since triploid ayu also have lower sensitivity to light than diploids, the former must be reared in isolation as in rainbow trout.¹⁶⁾

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