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Retarded Growth of Hexaploid Loaches

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Abstract: In the loach *Misgurnus anguillicaudatus*, hexaploid individuals were produced by chromosome manipulation to inhibit the second polar body release after the fertilization of diploid eggs from natural tetraploid female by sperm from tetraploid male. Eggs of mature hexaploid (6n) female were fertilized by sperm of diploid (2n), tetraploid (4n) and hexaploid (6n) males, respectively, to produce tetraploid (6n female × 2n male), pentaploid (6n × 4n) and hexaploid (6n × 6n) progeny. Each group was reared in a separate tank until the age of 1 year old and then long-term communal rearing experiments from the age of 1 year old to 3 years and 2 months old were carried out to compare performance of tetraploid, pentaploid and hexaploid loaches under a laboratory condition. At the end of experiments, significantly retarded growth was observed in hexaploid loaches and they exhibited about half body weight of counterpart tetraploid and pentaploid loaches. Hexaploid loaches also showed significantly lower body length/head length proportion than other polyploids.

Key words: Hexaploid; Polyploid; Loach; Growth

In the loach, *Misgurnus anguillicaudatus*, natural tetraploid individuals have been found in the specimens from fish dealers (Arai et al. 1991a). Despite of the unknown origin of these tetraploid loaches, tetraploid brood stock was produced by a cross using a tetraploid pair, because they are able to generate fertile diploid gametes (Arai et al. 1991ab; Matsubara et al. 1995; Zhang and Arai 1996). Using mature eggs and sperm of tetraploid loaches, hexaploid individuals were artificially induced by inhibiting the second polar body release with hydrostatic pressure shock, after the fertilization of eggs (with diploid egg nucleus plus diploid second polar body nucleus) with diploid sperm (Kijima et al. 1996). These hexaploid individuals were fertile, because females and males formed functional triploid eggs and sperm, respectively (Arai et al. 1999). Crosses between eggs of hexaploid females and sperm of hexaploid, tetraploid and diploid males gave rise to the production of hexaploid (6n female × 6n male), pentaploid (6n × 4n) and tetraploid (6n × 2n) progeny, respectively (Arai et al. 1999). Fertilization of eggs from diploid and tetraploid females by sperm of hexaploid male produced tetraploid (2n × 6n) and pentaploid (4n × 6n) progeny, respectively (Arai et al. 1999).

Performance of induced polyploidy has been evaluated in a large number of aquatic species mainly from the viewpoint of improvement of commercial traits for aquaculture by chromosome manipulation, but most of these studies were exclusively done for triploid fishes and shellfishes, because other higher polyploids such as tetraploid, pentaploid, and hexaploid are practically difficult to produce at present (Pandian and Kotteswran 1998; Arai 2001; Felip et al. 2001). Thus, performance of higher polyploid fishes is still unknown, except for

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several studies on induced and natural tetraploid individuals and their progeny (Chourrout et al. 1986; Chourrout and Nakayama 1987; Nam et al. 2001; Taniura et al. 2002; Horie et al. 2004). The loach, *Misgurnus anguillicaudatus* is considered a good material to evaluate performance of higher polyploids, because hexaploid, pentaploid and tetraploid individuals were already produced using gametes of hexaploid brood stock that previously induced from natural tetraploid individuals by chromosome manipulation techniques (Arai et al. 1999; Arai 2001).

In the present study, we report survival and growth of hexaploid (6n × 6n), pentaploid (6n × 4n) and tetraploid (6n × 2n) progeny of the hexaploid female loaches, reared in different tanks in the stage from 1 week to 1 year after fertilization, and then in a communal rearing condition in the age from 1 year old to 3 years and 2 months old. Sex ratio and morphometric characteristics of these progeny were also compared among them. However, traits of various progeny of hexaploid loaches were not compared with those of tetraploid (4n × 4n), triploid (4n × 2n, 2n × 4n) and diploid (2n × 2n) progeny from the crosses using diploid gametes of tetraploid loaches and haploid gametes of diploid ones, due to the shortage of surviving progeny and rearing space.

Materials and Methods

Fish specimens

Production of the first generation of hexaploid loach (identification code, 3047) using gametes of tetraploids was reported by Kijima et al. (1996). Hexaploidy was induced by inhibiting the second polar body release with hydrostatic pressure treatment (700 kg/cm^2, 1 min duration, 5 min after fertilization). In experiment G, mature hexaploid female (identification code, 7039) was used for artificial fertilization to produce hexaploid female × diploid male (code, 2n-E) cross (6n × 2n), hexaploid female × tetraploid males (4n-C, D and E) cross (6n × 4n), and hexaploid female × hexaploid male (6n-A) cross (6n × 6n), in July 9, 1997 (Arai et al. 1999). In experiment H, eggs of the other female (7037) were fertilized with sperm of tetraploid (4n-C, D and E) and hexaploid (6n-A) males to produce pentaploid (6n × 4n) and hexaploid (6n × 6n) progeny, at the same day (Arai et al. 1999). Procedures of induced ovulation, artificial insemination and incubation of embryos and fry were already described in Arai et al. (1999) and Horie et al. (2004).

Rearing until one-year-old fish

Fertilized eggs of each cross were placed in a shallow plastic pan (length 300 mm, width 250 mm, height 30 mm) half filled with freshwater as in the previous study (Horie et al. 2004). Feeding fry at 1 week after fertilization was measured on selected 10 − 20 fishes using a Nikon Profile Projector Model 6C (Nihon Kogaku, Co. Ltd.). In experiment G, 15 progeny of female 7039 from each cross were placed in a container (length 620 mm, width 380 mm, height 180 mm, 0.006 fish/cm^2). In experiment H, 30 progeny of female 7037 from each cross were also placed in the container with same size and shape (0.013 fish/cm^2). Rearing containers had neither air-supplying pump nor re-circulating system. They were placed in the hatchery, which was used in the previous study (Horie et al. 2004). Fry after yolk absorption were fed with *Artemia* sp., *Daphnia* sp. and then artificial food (Tetrafin, Tetraberke GmbH, Germany). After the one year rearing, survivors were counted and total length was measured nearest to 0.01 mm after anesthesia with 0.1% phenoxyethanol.

Long-term communal rearing

At June 11, 1998, about 1-year-old 14 tetraploid (6n × 2n), 14 pentaploid (6n × 4n) and 13 hexaploid (6n × 6n) progeny of female 7039 were pooled and placed in a large plastic container (length 1300 mm, width 900 mm, height 220 mm), to conduct a communal rearing in experiment G (rearing density, 0.004 fish/cm^2). At the same date, 28 pentaploid (6n × 4n) and 29 hexaploid (6n × 6n) were mixed into the container with the same size and shape, to conduct a communal rearing in experiment H (0.006 fish/cm^2). These rearing containers were equipped with air-supplying pump. However, the

pump was removed in the winter season. Fishes of experiment G were moved to a polycarbonate cylinder tank (Upper diameter 1050 mm, bottom diameter 900 mm, height 750 mm) at March 10, 1999 and then reared until August 3, 2000. Loaches of experiment H were also transferred to the same size cylinder tank at the same day and then reared until August 3, 2000. In the period from March to November, appropriate quantity of artificial feed (Tetrafin, Tetraberke GmbH, Germany) was supplied.

Ploidy determination

Ploidy was determined by DNA content flow cytometry with PA (Ploidy Analyzer, Partec GmbH, Germany) after DAPI staining according to the previous study (Horie et al. 2004).

Comparison of performance

In October 26, 1999 (about 1 year and 4 months after the beginning of communal rearing of about 1-year-old fish), total length (TL), body length (BL) and body weight (BW) were measured in selected specimens, according to the procedure described in the previous paper (Horie et al. 2004).

In August 3, 2000 (about 2 years and 2 months after the beginning of communal rearing of 1-year-old fish), counting of survivors, measurements of TL, BL, and BW, and determination of sex were done. At the same day, head length (HL), body depth (BD) and caudal peduncle length (CL) were also measured in the same specimens according to the procedure described in Horie et al. (2004), to compare BL/HL, BL/BD and BL/CL ratios among different ploidies. After these measurements, survivors were unfortunately lost by unexpected accident. Thus, further examination on gonadal development and nutritional conditions was not conducted.

Statistics was made according to the previous paper (Horie et al. 2004).

Results

Survival and growth of tetraploid (6n × 2n), pentaploid (6n × 4n) and hexaploid (6n × 6n) loaches of experiment G and H in the stage from 1 week to 1 year post fertilization are shown in Table 1. In both experiments, no significant difference was observed in survival rates of one-year-old progeny between different polyploid groups.

In total length of one-week-old progeny, no difference was observed between pentaploid and hexaploid loaches in experiment H. However, in experiment G, tetraploid loaches were significantly larger than pentaploid loaches, but no difference was observed between tetraploid and hexaploid loaches and between pentaploid and hexaploid loaches. One-year-old pentaploid progeny gave significantly larger total length than hexaploid progeny in both experiments. In experiment G, no difference was observed in total length between tetraploid and hexaploid loaches.

Survival rates in the period of communal rearing from June, 1998 to August, 2000 are shown in Table 2. Ploidy of each individual in a

Table 1. Survival and growth of tetraploid (4n), pentaploid (5n) and hexaploid progeny of hexaploid (6n) females of the loach in the stage from 1 week to 1 year post-fertilization (pf.)

Exp.	Female	Male	Cross	Ploidy	Survival		Total length (n) *1	
					Initial (1 week pf.) n	Final (1 year pf.) n (%)	Initial (1 week pf.) *Mean ± S.D.*	Final (1 year pf.) *Mean ± S.D.*
G	7039	2n-E	6n × 2n	4n	15	14 (93.3)^{a*2}	6.21 ± 0.15 (32)^{a*3}	36.26 ± 2.27 (14)^{a}
		4n-C,D,E	6n × 4n	5n	15	14 (93.3)^{a}	6.07 ± 0.21 (35)^{b}	39.30 ± 2.56 (14)^{b}
		6n-A	6n × 6n	6n	15	13 (86.7)^{a}	6.09 ± 0.19 (36)^{ab}	36.74 ± 2.56 (13)^{a}
H	7037	4n-C,D,E	6n × 4n	5n	30	28 (93.3)^{a}	5.91 ± 0.18 (32)^{a}	29.85 ± 2.37 (28)^{a}
		6n-A	6n × 6n	6n	30	29 (96.7)^{a}	5.90 ± 0.14 (33)^{a}	25.06 ± 3.94 (29)^{b}

*1 Number of fish measured, *2 different superscript letters among different crosses in the same experiment indicate significant difference ($P<0.05$, by χ^2-test), *3 different superscript letters among different crosses in the same experiment indicate significant difference ($P<0.05$, by Scheffe test).

same tank was flow-cytometrically determined. In both experiment G and H, no significant difference was observed in survival rates between different ploidies.

Normal sex ratio (female : male = 1 : 1) was detected in hexaploid and pentaploid progeny in both experiments. However, only female progeny appeared in tetraploid (6n × 2n) group of experiment G.

TL measured at June 1998, TL, BL and BW at October 1999 and those at August 2000, in experiment G are shown in Table 3. At the beginning of experiment G, TL of pentaploid loaches were significantly larger than tetraploid and hexaploid loaches. However, sizes of 2-year-old hexaploid loaches, measured by TL, BL and BW were significantly smaller than those of tetraploid and pentaploid loaches. Retarded growth of hexaploid loaches became conspicuous in about 3-year-old progeny. TL, BL and BW of hexaploid loaches were significantly lower than those of pentaploid and tetraploid loaches. Hexaploids exhibited about half body weight, compared with those of tetraploids and pentaploids. No difference was observed in growth between pentaploid and tetraploid loaches. In hexaploid, females were significantly smaller than males. No such difference was observed between females and males of pentaploids.

In experiment H, such a retarded growth of hexaploid was also detected when compared with pentaploid progeny during the experiment from June 1998 to August 2000 (Table 4). In the

Table 2. Survival rate and sex ratio of tetraploid (4n), pentaploid (5n) and hexaploid (6n) progeny of hexaploid females of the loach in the communal rearing from June 11, 1998 (about 1-year-old fish) to August 3,2000 (about 3-year-2-month-old-fish)

Exp.	Cross	Ploidy	Survival		Sex ratio
			Initial (June 11, 1998)	Final (August 3, 2000)	Female : Male
			n	*n* (%)	*n* *n*
G	6n × 2n	4n	14	13 (92.9)[a]*	13 : 0**
	6n × 4n	5n	14	9 (64.3)[a]	5 : 4
	6n × 6n	6n	13	11 (84.6)[a]	7 : 4
H	6n × 4n	5n	28	26 (89.7)[a]	9 : 17
	6n × 6n	6n	29	20 (71.4)[a]	14 : 6

* Same superscript letters mean no significant difference among different ploidies in the same experiment ($P<0.05$).
** Deviation from the normal female1 : male1 sex ratio ($P<0.05$, χ^2-test).

Table 3. Comparison of growth among tetraploid (4n), pentaploid (5n) and hexaploid (6n) loaches in communal rearing experiment G

Date of measurement		Sex	Tetraploid (6n × 2n)	Pentaploid (6n × 4n)		Hexaploid (6n × 6n)	
			Mean ± *S.D.*(*n*)	*Mean* ± *S.D.*(*n*)		*Mean* ± *S.D.*(*n*)	
June, 1998 (Initial)	TL[*1] (mm)	All	36.26 ± 2.27(14)[a*4]	39.30 ± 1.77 (14)[b]		36.74 ± 2.56 (13)[a]	
Oct., 1999	TL (mm)	All	73.54 ± 4.34(10)[a]	72.65 ± 5.83 (11)[a]		58.87 ± 5.94 (10)[b]	
	BL[*2] (mm)	All	63.36 ± 4.07(10)[a]	60.63 ± 5.54 (11)[a]		48.70 ± 7.79 (10)[b]	
	BW[*3] (g)	All	1.49 ± 0.19(10)[a]	1.54 ± 0.41 (11)[a]		0.84 ± 0.33 (10)[b]	
Aug., 2000 (Final)	TL (mm)	All	79.92 ± 4.16(13)[a]	81.33 ± 4.43 (9)[a]		64.46 ± 6.66 (11)[b]	
		Female	79.92 ± 4.16(13)[a]	83.12 ± 4.39 (5)[a]	n.s.[*5]	60.66 ± 5.65 (7)[b]	*[*6]
		Male	–	79.08 ± 3.96 (4)[a]		70.32 ± 4.46 (4)[b]	
	BL (mm)	All	67.52 ± 3.13(13)[a]	67.75 ± 3.96 (9)[a]		53.54 ± 5.69 (11)[b]	
		Female	67.52 ± 3.13(13)[a]	68.86 ± 3.92 (5)[a]	n.s.	49.92 ± 4.25 (7)[b]	**[*7]
		Male	–	66.35 ± 4.07 (4)[a]		58.95 ± 3.67 (4)[b]	
	BW (g)	All	2.02 ± 0.30(13)[a]	2.16 ± 0.34 (9)[a]		1.09 ± 0.29 (11)[b]	
		Female	2.02 ± 0.30(13)[a]	2.27 ± 0.33 (5)[a]	n.s.	0.93 ± 0.20 (7)[b]	*
		Male	–	2.01 ± 0.28 (4)[a]		1.36 ± 0.24 (4)[b]	

[*1] Total length, [*2] body length, [*3] body weight, [*4] different superscript letters among different ploidies in a line at the same date mean significant difference ($P<0.05$, Scheffe test), [*5] not significant between female and male, [*6] significant difference between female and male ($P<0.05$, Scheffe test), [*7] significant difference between female and male ($P<0.01$, Scheffe test).

course of experiment, hexaploid loaches gave significantly smaller sizes than pentaploids. No significant growth difference was detected between the two sexes.

At the end of communal rearing experiments, BL/HL, BL/CL and BL/BD were compared among tetraploid, pentaploid and hexaploid loaches in the experiment G and H (Table 5). In experiment G, mean BL/HL value of hexaploid females was significantly lower than those of pentaploid and tetraploid loaches. In experiment H, both female and male hexaploid loaches showed significantly lower BL/HL values. These results denote that the proportion of the head to body length is significantly large in hexaploid loaches. No difference was seen in BL/CL and BL/BD among three different ploidies, except for BL/BD of female hexaploids in experiment H.

Table 4. Comparison of growth between pentaploid (5n) and hexaploid (6n) loaches in communal rearing experiment H

Date of measurement		Sex	Pentaploid (6n × 4n)		Hexaploid (6n × 6n)	
			Mean ± S.D. (n)		*Mean ± S.D. (n)*	
June, 1998 (Initial)	TL[*1] (mm)	All	29.85 ± 2.37 (28)[a*4]		25.06 ± 3.94 (29)[b]	
Oct., 1999	TL (mm)	All	59.59 ± 6.36 (25)[a]		46.83 ± 3.59 (14)[b]	
	BL[*2] (mm)	All	49.93 ± 5.35 (25)[a]		39.19 ± 2.95 (14)[b]	
	BW[*3] (g)	All	0.85 ± 0.25 (25)[a]		0.40 ± 0.13 (14)[b]	
Aug., 2000 (Final)	TL (mm)	All	69.95 ± 6.03 (26)[a]		55.43 ± 3.79 (20)[b]	
		Female	69.71 ± 7.58 (9)[a]	n.s.[*5]	55.70 ± 3.21 (14)[b]	n.s.
		Male	70.07 ± 4.96 (17)[a]		54.98 ± 4.79 (6)[b]	
	BL (mm)	All	58.53 ± 5.14 (26)[a]		46.43 ± 3.20 (20)[b]	
		Female	58.40 ± 6.48 (9)[a]	n.s.	46.58 ± 2.53 (14)[b]	n.s.
		Male	58.59 ± 4.20 (17)[a]		46.08 ± 4.29 (6)[b]	
	BW (g)	All	1.57 ± 0.38 (26)[a]		0.78 ± 0.18 (20)[b]	
		Female	1.54 ± 0.46 (9)[a]	n.s.	0.77 ± 0.14 (14)[b]	n.s.
		Male	1.58 ± 0.32 (17)[a]		0.81 ± 0.25 (6)[b]	

[*1] Total length, [*2] body length, [*3] body weight, [*4] different superscript letters among different ploidies in a line at the same date mean significant difference ($P<0.05$, Scheffe test), [*5] not significant between female and male.

Table 5. Comparison of BL/HL, BL/CL and BL/BD among tetraploid (4n), pentaploid (5n) and hexaploid (6n) loaches at the end of communal rearing experiment G and H

Exp.		Sex	Tetraploid (6n × 2n)	Pentaploid (6n × 4n)		Hexaploid (6n × 6n)	
			Mean ± S.D. (n)	*Mean ± S.D. (n)*		*Mean ± S.D. (n)*	
G	BL[*1]/HL[*2]	All	5.25 ± 0.15 (13)[a*5]	5.30 ± 0.19 (9)[a]		4.97 ± 0.38 (11)[b]	
		Female	5.25 ± 0.15 (13)[a]	5.17 ± 0.14 (5)[a]	* [*6]	4.88 ± 0.19 (7)[b]	n.s.
		Male	–	5.46 ± 0.02 (4)[a]		5.17 ± 0.56 (4)[a]	
	BL/CL[*3]	All	6.05 ± 0.43 (13)[a]	6.35 ± 0.46 (9)[a]		6.18 ± 0.38 (11)[a]	
		Female	6.05 ± 0.43 (13)[a]	6.41 ± 0.38 (5)[a]	n.s.[*7]	6.18 ± 0.41 (7)[a]	n.s.
		Male	–	6.29 ± 0.56 (4)[a]		6.30 ± 0.20 (4)[a]	
	BL/BD[*4]	All	8.63 ± 0.26 (13)[a]	8.28 ± 0.28 (9)[a]		8.40 ± 0.48 (11)[a]	
		Female	8.63 ± 0.26 (13)[a]	8.42 ± 0.31 (5)[a]	n.s.	8.22 ± 0.54 (7)[a]	n.s.
		Male	–	8.11 ± 0.11 (4)[a]		8.64 ± 0.36 (4)[b]	
H	BL/HL	All	–	5.09 ± 0.20 (26)[a]		4.75 ± 0.24 (20)[b]	
		Female	–	5.01 ± 0.18 (9)[a]	n.s.	4.75 ± 0.22 (14)[b]	n.s.
		Male	–	5.13 ± 0.19 (17)[a]		4.75 ± 0.31 (6)[b]	
	BL/CL	All	–	6.34 ± 0.23 (26)[a]		6.45 ± 0.66 (20)[a]	
		Female	–	6.41 ± 0.22 (9)[a]	n.s.	6.59 ± 0.66 (14)[a]	n.s.
		Male	–	6.30 ± 0.22 (17)[a]		6.14 ± 0.62 (6)[a]	
	BL/BD	All	–	7.78 ± 1.54 (26)[a]		7.76 ± 0.32 (20)[a]	
		Female	–	7.80 ± 0.42 (9)[a]	n.s.	7.90 ± 0.24 (14)[b]	*
		Male	–	7.74 ± 0.37 (17)[a]		7.43 ± 0.27 (6)[a]	

[*1] Body length, [*2] head length, [*3] caudal peduncle length, [*4] body depth, [*5] different superscript letters among different ploidies in a line at the same date mean significant difference ($P<0.05$, Scheffe test), [*6] significant difference between female and male ($P<0.05$, Scheffe test), [*7] not significant between female and male.

Discussion

Survival rates of all polyploids examined were generally good (87－97%) in the period from 1-week-old to 1-year-old loaches. No difference was observed in survival rates among tetraploid (6n×2n), pentaploid (6n×4n) and hexaploid (6n×6n) loaches, each of which was reared in a separate tank. At the end of communal rearing experiment for 2 years and 10 months after pooling 1-year-old loaches with different ploidies, no difference was observed in survival rates among three different ploidies.

Hexaploid (6n×6n) and pentaploid (6n×4n) loaches gave normal sex ratio (female 1: male 1), but no male occurred in tetraploid (6n×2n) loaches. In the *Misgurnus* loach, morphologically distinct sex chromosome has not been detected. However, the *Misgurnus* loach is estimated to have male heterogametic sex determination system (XX female: XY male), because artificially induced gynogenetic diploids were all-female (Suzuki et al. 1985). Normal sex ratio was also observed in the progeny of tetraploid pairs and those of hexaploid pairs and all the gynogenetic tetraploids produced from eggs of tetraploid loaches were female (Arai et al. 1999). These results strongly suggested that the male should be determined by functional Y chromosome, even in tetraploid and hexaploid loaches. Thus, in the case of tetraploid from 6n×2n cross, the male was predicted to appear due to the involvement of functional Y chromosome. However, the progeny of the 6n×2n cross were all female. High survival rate (92.9%) of this cross (6n×2n) did not justify selective death of males. Therefore, this result may be explained by the accidental involvement of sex reversed XX male on the fertilization. Sex reversal from genetic females to physiological males by ambient water temperature probably during sex differentiation period has been proved experimentally in the loach species (Nomura et al. 1998).

The most important observation in the present study was the retarded growth of hexaploid (6n×6n) loaches at the communal rearing experiment with pentaploid (6n×4n) and tetraploid (6n×2n) fishes produced from the same female. In both experiments G and H, total and body length of hexaploids were much smaller than those of pentaploid and tetraploid loaches. Body weight of hexaploid loaches was about half (50－53%) of counterpart pentaploid and/or tetraploid loaches. Similar retarded growth has been observed in the communal rearing experiment using diploid (2n×2n), triploid (2n×4n) and tetraploid (4n×4n) loaches in the previous paper (Horie et al. 2004). Body weight of tetraploid loaches was about 62－63% of normal diploid loaches and the rates to counterparts were generally better than those in hexaploid loaches observed in the present study. Although we could not compare the growth of hexaploid and other higher polyploids with normal diploid and spontaneous tetraploid fishes, these results suggest the decreasing tendency of growth in proportion to the elevation of ploidy status.

Possible harmful effect of higher polyploidy itself might be involved in this phenomenon, as observed in polyploidy in plant kingdom. Certain polyploid plants exhibit gigantism in proportion to the increase of ploidy, but the sizes reduce paradoxically in much higher polyploidy plants (Blakeslee 1941). The other possibility of the retarded growth of higher polyploid loaches may be inbreeding depression. Since hexaploid progeny were induced by means of chromosome manipulation, from the third generation tetraploid loaches that originated from the single pair of natural tetraploid loaches found in the specimens collected from fish market (Arai et al. 1999), homozygosity should be increased to express harmful effects of recessive deleterious genes. This might explain lower growth of tetraploid loaches in the previous study (Horie et al. 2004). On the contrary, however, such retarded growth may be intrinsic characteristics of tetraploid loaches, because natural tetraploid loaches are considered as an exotic fish originally living in Yang-tze River, China (Li et al. 1983) and hexaploid loaches were produced from gametes of such tetraploid loaches by chromosome manipulation (Arai et al. 1999).

Significant morphological difference was

detected in BL/HL value. Hexaploid (6n × 6n) loaches had significantly lower values (4.75 – 4.97) of BL/HL than those of pentaploid (6n × 4n, 5.09 – 5.30) and tetraploid (6n × 2n, 5.25) loaches. This means hexaploid loaches have relatively larger head, i.e. the proportion of the head length to the body length is significantly large. BL/HL values (4.75 – 4.97) of hexaploid loaches observed in the present study were also much lower than those of tetraploid (4n × 4n, 5.09 – 5.11), triploid (4n × 2n, 5.29 – 5.48) and diploid (2n × 2n, 5.48 – 5.65) reported in the previous study (Horie et al. 2004). BL/HL values of pentaploid (6n × 4n, 5.09 – 5.30) were similar to those of tetraploid (4n × 4n, 5.09 – 5.11). BL/HL value of tetraploid (6n × 2n, 5.25) was similar to those of triploid (4n × 2n, 5.29 – 5.48), but all these values were lower than those of diploid (2n × 2n, 5.48 – 5.65) loaches. These results suggest the general tendency that HL/BL values should decrease in proportion to the elevation of ploidy status. However, other morphometric values were similar among loaches with different ploidies.

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六倍体ドジョウの成長低下

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ドジョウ *Misgurnus anguillicaudatus* 自然四倍体（4n）の産する二倍体（2n）配偶子の受精と染色体操作により作成した六倍体（6n）雌の卵と二倍体，四倍体，および六倍体雄の精子を受精し，各々，四倍体（6n雌×2n雄），五倍体（6n×4n）および六倍体（6n×6n）子孫を作出した。そして，これらを1年間倍数体毎に個別の水槽で，そして，その後2年2カ月間同一の水槽で混合飼育し，特性の比較を行った。その結果，3年2カ月齢の六倍体の成長は五倍体，四倍体に著しく劣り，体重で約半分程度であった。また，体長/頭長比が有意に低いことが判明した。