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Performance of the Progeny of Natural Tetraploid Loaches in Long-Term Communal Rearing Experiments under a Laboratory Condition

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Abstract: In the loach *Misgurnus anguillicaudatus*, triploid (3n) and tetraploid (4n) individuals were produced by crosses using diploid (2n) gametes of natural tetraploids, which had been found in the specimens obtained from fish dealers. Long-term communal rearing experiments from the age of 1 month old to 3 years 1 month old were carried out among diploid (2n female × 2n male), triploid (4n × 2n) and tetraploid (4n × 4n) to compare their performance under a laboratory condition. At the end of experiments, significantly retarded growth was observed in tetraploids and they showed significantly lower body length/head length proportion than diploid and triploid individuals. Triploid females exhibited less developed gonad than tetraploid and diploid individuals, when compared gonad somatic index (GSI). Triploid and tetraploid males showed lower GSI than diploid males, but tetraploid males gave better gonadal development than triploid males.

Key words: Polyploid, Tetraploid, Triploid, Loach

Triploid fish has been produced to improve growth, survival rate, meat quality and external appearance, because these characteristics are generally deteriorated in the period of maturation. Performance of induced triploid has been evaluated in a relatively large number of fish species (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001). In general, premature triploids exhibit growth equivalent or sometimes inferior to the counterpart diploids, but induced triploids at the age of sexual maturation often outperform diploid in growth, survival, food conversion and other characteristics (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001). In the loach, *Misgurnus anguillicaudatus*, induced triploids showed undeveloped gonads in both sexes and exhibited better growth of females at the end of one year experiment using 0+ fish (Suzuki et al. 1985). Similar results were obtained in a closely related species, *M. mizolepis* (Kim et al. 1994).

Artificial induction of tetraploid fish by inhibition of the first cleavage after fertilization is technically very difficult and a few examples of long-surviving and/or reproducible tetraploids have been reported (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001). Recently, successful production of tetraploid *M. mizolepis* was reported and survival and growth similar to the counterpart diploids were observed (Nam et al. 2001). In the loach, *M. anguillicaudatus*, however, induced tetraploid individuals have not been produced yet, but this species includes polyploid biotypes in nature (Ojima and Takai 1979; Arai et al. 1991a; Zhang and Arai 1999; Morishima et al. 2002). Diploid individuals (2n = 50) are most common in Japanese populations, although triploids (3n = 75) and tetraploids (4n = 100) have been found in the specimens obtained from some wild populations and in those from fish dealers, respectively (Arai et al. 1991a; Zhang and Arai 1999;

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Morishima et al. 2002). Despite the origin of natural tetraploids being unknown, tetraploid breeding line has been established by an experimental cross using a tetraploid pair (Arai et al. 1991b, 1993; Matsubara et al. 1995; Zhang and Arai 1996) and then utilized not only to produce triploid family by cross fertilization between normal diploid and tetraploid loaches, but also to induce hexaploid family by chromosome set manipulation to inhibit the second polar body release after intra-tetraploid crosses (Matsubara et al. 1995; Zhang and Arai 1996; Arai et al. 1999). Size of eggs, ploidy of gametes, sex ratio and reproductive potential of these tetraploid, triploid and hexaploid loaches have been already reported in a series of studies (Matsubara et al. 1995; Zhang and Arai 1996; Arai et al. 1999; Arai and Mukaino 1997, 1998; Zhang et al. 1998). Survival and growth comparison among diploid, triploid and tetraploid loaches have been preliminarily conducted at the age of 6 months old and 2 years 3 months old under the communal rearing conditions, but no clear differences were found yet (Taniura et al. 2002).

In the present study, we conducted a long-term communal rearing of diploid (diploid female × diploid male), triploid (tetraploid × diploid) and tetraploid (tetraploid × tetraploid) until the age of 3 years 1 month old, in order to compare their survival and growth performance under a laboratory condition. Comparison of morphometric characteristics among different polyploid loaches was also carried out to disclose their biological differences.

Materials and Methods

Fish specimens

Diploid females ($n = 2$; individual identification code 7028 and 7030) were collected from the rearing stock, originally obtained in the population of Hirokami village, Niigata prefecture in the fall of 1995 and the spring of 1996. Diploid males ($n = 3$; individual identification code A, B and C) were collected in Iwamizawa city, Hokkaido. Tetraploid loaches (female $n = 3$; 7022, 7023 and 7024, male $n = 2$; A and B) were selected from the second generation of the tetraploid breeding line originated in the cross #024 made in 1990 (Arai et al. 1991b, 1993).

Eggs and sperm

Human chorionic gonadtropin (HCG) was administered to induce ovulation according to the procedure described by Suzuki and Yamaguchi (1975). Injected females were placed in a tank. Water temperature was adjusted to 25℃ for 10-12 h until the ovulation. Mature eggs were taken by squeezing abdomen gently after confirming the ovulation. Mature milt was also taken by using capillary tube and diluted to 100 times with physiological saline for fertilization.

Fertilization and incubation of fry

As shown in Table 1, eggs collected from diploid females were fertilized with sperm of diploid males (2n × 2n) in June 18, 1997. Eggs from tetraploid females were fertilized with

Table 1. Experimental design of communal rearing experiments of diploid, triploid and tetraploid loaches

Exp.	Date of fertilization	Date beginning experiment	Femele #	Male #	Cross	Ploidy	No. of fish
E	June 18, 1997	July 30, 1997	7030	2n-A	2n × 2n	2n	46
	June 10, 1997		7022	2n-C, D	4n × 2n	3n	48
	June 10, 1997		7022	4n-A	4n × 4n	4n	38
						Sum	132
F	June 18, 1997	July 30, 1997	7028, 7030	2n-A, B	2n × 2n	2n	70
	June 10, 1997		7022, 7030	2n-C, D	4n × 2n	3n	37
	June 10, 1997		7022, 7024	4n-A, B	4n × 4n	4n	27
						Sum	134

sperm of diploid males (4n × 2n) and tetraploid males (4n × 4n) in June 10, 1997. 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. The rearing pan was placed in the laboratory at room temperature. Each pan had neither air-supplying pump nor re-circulating system. Dead eggs were removed daily and water was changed everyday. Fry after yolk absorption were fed with *Artemia* larvae. After equalizing the rearing density to 40 or 50 fish per pan, each experimental cross was reared in the pan placed in the hatchery until the end of July 1997. The hatchery has no wall, but roof to protect fish from the sun. After the one month rearing, survivors were counted and total length was measured nearest to 0.01 mm after anesthesia with 0.1% phenoxyethanol for 1 min duration.

Long-term communal rearing

About 1-month-old diploid (2n × 2n), triploid (4n × 2n) and tetraploid (4n × 4n) loaches were mixed to conduct a long-term communal rearing experiment. In experiment E, 46 diploids produced from eggs of female 7030, 48 triploids and 38 tetraploids produced from eggs of female 7022 were mixed at July 30, 1997 and then reared in a plastic container (length 1300 mm, width 900 mm, height 220 mm, initial density 0.013 fish/cm^2) placed in the hatchery. The rearing container was equipped with air-supplying pump. *Artemia* and *Daphnia* sp. were fed until the mid November, 1997. No foods were supplied in winter season from November to February. Air supply pump was also removed in the winter season. In experiment F, 70 diploids developed from eggs of females 7028 and 7030, 37 triploids from females 7022 and 7023, and 27 tetraploids from females 7022 and 7024 were also mixed at July 30, 1997 and then placed in a different container with the same dimension to experiment E. The initial density was same to that in experiment E. Fishes of experiment E were moved to a polycarbonate cylinder tank (upper diameter 1050 mm, bottom diameter 900 mm, height 750 mm) at March 10, 1999. Those of experiment F were also transferred to the same size cylinder tank at the same day. These tanks were equipped with air supply pump and placed in the same hatchery. In the period between March 1998 and November 1998, that between March 1999 and November 1999 and that between March 2000 and August 2000, appropriate quantity of artificial feed (Tetrafin, Tetraberke GmbH, Germany) was supplied.

Ploidy determination

Ploidy was determined by DNA content flow cytometry of erythrocytic cells or gill epithelia with FACS (Becton Dickinson, USA) after PI (Propidium iodide) staining or with PA Ploidy Analyzer (Partec, Germany) after DAPI (4'-6-diamidino-2-phenylindole) staining according to Zhang and Arai (1996) and Yamaha et al. (2001).

Comparison of survival and growth

In October 26, 1999 (about 2 years 3 months old), total length (TL, nearest to 0.01 mm), body length (BL, nearest to 0.01 mm) and body weight (BW, nearest to 0.01 g) were measured in randomly selected specimens. In August 3, 2000 (about 3 years 1 month old), all fishes in each experiment were taken to count survivors as well as to examine growth, sex ratio and morphology. Survival rates of each ploidy group were examined after the confirmation of ploidy in all the specimens of the same experiment. Growth was evaluated by measuring TL, BL and BW.

Sex and maturation

Sex was determined by observing the morphology of pectoral fins and body shape (Suzuki 1983). In experiment E, gonad somatic index (GSI) was calculated from measurements of gonad weight (GW, nearest to 0.01 g) and body weight (BW) of five females and five males taken from each ploidy group according to the formula: GW/BW × 1000.

Morphometric comparison

Total length (TL), body length (BL), head

length (HL, the distance from the anteriormost point of the farthest margin of the pactoral fin, nearest to 0.01 mm), body depth (BD, the greatest dimension, exclusive of fins, and fleshly or scaly structures which pertain to the fin base, nearest to 0.01 mm) and caudal peduncle length (CL, the distance from the base of the last anal ray to the posterior end of the hypural bone, nearest to 0.01 mm) were measured at the end of communal rearing experiment for morphometric comparison. BL/HL, BL/BD and BL/CL ratios were calculated and compared among different ploidies.

Statistics

Survival rates and sex ratios were statistically examined by χ^2 test. Results of growth and morphometrics were tested by Fisher's PLSD or Scheffe by using software package StatView J-4.5 (Abacus Concepts).

Results

Survival rates of polyploid loaches under a long-term communal rearing are shown in Table 2. In experiment E, no significant difference was observed in survival rates at the age of 3 years 1 month old among diploid, triploid and tetraploid loaches, determined by flow cytometry ($\chi^2 = 1.739$, df = 2, $P > 0.05$). In experiment F, significantly low survival rate of diploid loaches was detected ($\chi^2 = 14.665$, df = 2, $P < 0.01$), when compared with triploid and tetraploid groups. In both experiments, diploid, triploid and tetraploid exhibited the normal 1 : 1 sex ratio.

Table 2. Survival rate and sex ratio of diploid, triploid and tetraploid loaches

Exp.	Ploidy	Survival		Sex ratio
		July 30, 1997 (beginning) *n*	August 3, 2000 (end) *n* (%)	Female : Male
Exp. E	2n	46	25(54.3)[a]*	10:15[a]**
	3n	48	21(43.8)[a]	14:7[a]
	4n	38	12(31.6)[a]	6:6[a]
Exp. F	2n	70	14(20.0)[a]	6:8[a]
	3n	37	27(73.0)[b]	9:18[a]
	4n	27	20(74.1)[b]	14:6[a]

* Different superscript letters between different ploidies in the same experiment indicate significant difference. ($P<0.05$, χ^2-test).
** Same superscript letters mean no significant difference from the normal female 1 : male 1 sex ratio ($P>0.05$, χ^2-test).

TL measured at the age of one month old (beginning of experiment), TL, BL and BW at the age of 2 years 3 months old, and those at 3 years 1 month old (end of experiment) in the E group are shown in Table 3. At the initial of communal rearing, there was no significant difference in TL among different ploidies in experiment E. However, it was evident that diploid loaches grew faster than triploids and growth of triploids was faster than tetraploids, when parameters were measured at the age of 2 years and 3 months. At the end of the communal rearing, no significant difference was seen in TL, BL and BW between 3 years 1month old diploid and triploid loaches, but tetraploid loaches gave significantly retarded growth than diploid and triploid progeny. No difference was detected in growth of diploid and tetraploid loaches between the sexes, but TL and BW of triploid males were significantly larger than those of triploid females (Table 3).

Such a retarded growth of tetraploids and no different growth between diploid and triploid loaches at the age of 2 years 3 months and 3 years 1 month were also recorded in the experiment F (Table 4). In this experiment, TL, BL and BW of diploid loaches were smaller than those of triploid and tetraploid loaches in the beginning of experiment. However, in the age of 2 years 3 months, TL, BL and BW of diploid loaches were not only similar to those of triploids, but also larger than those of tetraploids. Through the growth comparison in the experiment F, no difference was detected between the two sexes (Table 4).

In the experiment E, GSI was compared among different ploidies at the end of experiment (Table 3). Triploid females gave significantly lower GSI than diploid and tetraploid females. No difference was observed in GSI between diploid and tetraploid females. Triploid males gave significantly lower GSI than diploid and tetraploid males. Tetraploid males showed significantly lower GSI when compared with diploid males.

Table 3. Comparison of total length, body length, body weight and gonad-somatic index among diploid, triploid and tetraploid loaches in experiment E

Date of measurements			Diploid (2n × 2n)		Triploid (4n × 2n)		Tetraploid (4n × 4n)	
July, 1997 (Beginning)	TL*1		21.63 ± 2.65 (46)a*6		20.45 ± 2.38 (46)a		21.98 ± 4.07 (38)a	
Oct., 1999	TL		60.42 ± 3.80 (36)a		55.66 ± 4.82 (21)b		51.33 ± 4.14 (28)c	
	BL*2		51.40 ± 3.51 (36)a		47.29 ± 4.20 (21)b		45.29 ± 4.14 (28)c	
	BW*3		0.80 ± 0.21 (36)a		0.55 ± 0.15 (21)b		0.47 ± 0.15 (28)b	
Aug., 2000 (End)	TL		62.52 ± 5.27 (25)a		61.83 ± 3.52 (21)a		55.64 ± 3.77 (12)b	
		F*4	64.42 ± 6.13 (10)a	n.s.*7	60.85 ± 3.32 (14)a	*8	54.90 ± 3.09 (6)b	n.s.
		M*5	61.03 ± 4.11 (15)ab		63.79 ± 3.25 (7)a		56.38 ± 4.52 (6)b	
	BL		52.86 ± 4.26 (25)a		52.74 ± 3.17 (21)a		46.47 ± 3.04 (12)b	
		F	54.30 ± 4.33 (10)a	n.s.	51.89 ± 3.07 (14)a	n.s.	45.87 ± 2.04 (6)b	n.s.
		M	51.73 ± 3.99 (15)ab		54.44 ± 2.83 (7)a		47.07 ± 3.91 (6)b	
	BW		1.04 ± 0.29 (25)a		0.93 ± 0.17 (21)a		0.65 ± 0.17 (12)b	
		F	1.12 ± 0.32 (10)a	n.s.	0.88 ± 0.14 (14)b	*	0.62 ± 0.37 (6)b	n.s.
		M	0.98 ± 0.25 (15)a		1.04 ± 0.18 (7)a		0.67 ± 0.23 (6)b	
	GSI	F	3.50 ± 0.79 (5)a		1.67 ± 0.74 (5)b		3.88 ± 1.01 (5)a	
		M	0.79 ± 0.18 (5)a		0.34 ± 0.09 (5)b		0.58 ± 0.15 (5)c	

*1 Total length, Mean ± SD mm (*n*), *2 Body length, Mean ± SD mm (*n*), *3 Body weight, Mean ± SD g (*n*), *4 Female, *5 Male, *6 Different superscript letters between different ploidies within the same age group indicate significant differences ($P<0.05$, Scheffe test), *7 Not significant difference between females and males, *8 Significant difference between females and males ($P<0.05$, Scheffe test or Fisher's PLSD).

Table 4. Comparison of total length, body length and body weight among diploid, triploid and tetraploid loaches in experiment F

Date of measurements			Diploid (2n × 2n)		Triploid (4n × 2n)		Tetraploid (4n × 4n)	
July, 1997 (Beginning)	TL*1		20.08 ± 5.34 (70)a*6		24.69 ± 2.69 (37)b		25.71 ± 2.84 (27)b	
Oct.,1999	TL		59.94 ± 8.55 (17)a		59.81 ± 4.36 (28)a		54.11 ± 3.89 (22)b	
	BL*2		50.60 ± 7.02 (17)a		50.69 ± 3.70 (28)a		45.11 ± 3.36 (22)b	
	BW*3		0.83 ± 0.36 (17)a		0.72 ± 0.21 (28)a		0.56 ± 0.19 (22)b	
Aug., 2000 (End)	TL		67.09 ± 6.65 (14)a		66.64 ± 4.44 (27)a		58.78 ± 4.10 (20)b	
		F*4	64.40 ± 7.21 (6)ab	n.s.*7	67.02 ± 6.24 (9)a	n.s.	58.92 ± 4.57 (14)b	n.s.
		M*5	69.11 ± 5.84 (8)a		66.45 ± 3.42 (18)a		58.46 ± 3.08 (6)b	
	BL		56.95 ± 5.75 (14)a		56.91 ± 4.08 (27)a		49.64 ± 3.16 (20)b	
		F	54.76 ± 6.42 (6)ab	n.s.	57.63 ± 5.64 (9)a	n.s.	49.60 ± 3.61 (14)b	n.s.
		M	58.59 ± 4.97 (8)a		56.55 ± 3.18 (18)a		49.73 ± 2.05 (6)b	
	BW		1.25 ± 0.35 (14)a		1.08 ± 0.24 (27)a		0.77 ± 0.15 (20)b	
		F	1.10 ± 0.31 (6)a	n.s.	1.11 ± 0.30 (9)a	n.s.	0.77 ± 0.16 (14)b	n.s.
		M	1.37 ± 0.36 (8)a		1.07 ± 0.20 (18)b		0.77 ± 0.14 (6)b	

*1 Total length, Mean ± SD mm (*n*), *2 Body length, Mean ± SD mm (*n*), *3 Body weight, Mean ± SD g (*n*), *4 Female, *5 Male, *6 Different superscipt letters between different ploidies within the same age group indicate significant differences ($P<0.05$, Scheffe test), *7 Not significant difference between females and males.

At the end of communal rearing experiments, BL/HL, BL/CL and BL/BD were compared among diploid, triploid and tetraploid loaches in the experiment E and F (Table 5). In both experiments, mean BL/HL value of tetraploid loaches was significantly lower than those of diploid and triploid loaches. This means that the proportion of the head to body length is significantly large. No difference was seen in BL/CL and BL/BD among diploid, triploid and tetraploid loaches.

Table 5. Comparison of body length/head length, body length/caudal peduncle length and body length/body depth among diploid, triploid and tetraploid loaches

Experiment			Diploid (2n × 2n)		Triploid (4n × 2n)		Tetraploid (4n × 4n)	
Exp. E	BL/HL*1		5.48 ± 0.27 (5)^{a}*6		5.29 ± 0.13 (21)^{ab}		5.11 ± 0.23 (12)^{b}	
		F*4	5.46 ± 0.15 (10)^{a}	]	5.21 ± 0.27 (14)^{b}	]	5.10 ± 0.23 (6)^{b}	]
		M*5	5.49 ± 0.33 (15)^{a}	] n.s.*7	5.43 ± 0.30 (7)^{a}	] n.s.	5.11 ± 0.25 (6)^{b}	] n.s.
	BL/CL*2		6.31 ± 0.49 (25)^{a}		6.59 ± 1.20 (21)^{a}		6.68 ± 0.64 (12)^{a}	
		F	6.27 ± 0.45 (10)^{a}	]	6.47 ± 0.70 (14)^{a}	]	6.69 ± 0.83 (6)^{a}	]
		M	6.34 ± 0.50 (15)^{a}	] n.s.	6.77 ± 1.76 (7)^{a}	] n.s.	6.67 ± 0.44 (6)^{a}	] n.s.
	BL/BD*3		8.44 ± 0.50 (25)^{a}		8.67 ± 0.31 (21)^{a}		8.75 ± 0.85 (12)^{a}	
		F	8.54 ± 0.51 (10)^{a}	]	8.76 ± 0.28 (14)^{a}	]	8.82 ± 0.78 (6)^{a}	]
		M	8.35 ± 0.48 (15)^{a}	] n.s.	8.51 ± 0.31 (7)^{a}	] n.s.	8.67 ± 0.90 (6)^{a}	] n.s.
Exp. F	BL/HL		5.65 ± 0.31 (14)^{a}		5.48 ± 0.21 (27)^{a}		5.09 ± 0.35 (20)^{b}	
		F	5.62 ± 0.34 (6)^{a}	]	5.38 ± 0.20 (9)^{a}	]	4.96 ± 0.25 (14)^{b}	]
		M	5.68 ± 0.27 (8)^{a}	] n.s.	5.53 ± 0.19 (18)^{ab}	] n.s.	5.40 ± 0.32 (6)^{b}	] *
	BL/CL		6.20 ± 0.42 (14)^{a}		6.44 ± 0.56 (27)^{a}		6.66 ± 0.62 (20)^{a}	
		F	6.33 ± 0.36 (6)^{a}	]	6.60 ± 0.39 (9)^{a}	]	6.70 ± 0.65 (14)^{a}	]
		M	6.10 ± 0.38 (8)^{a}	] n.s.	6.36 ± 0.58 (18)^{a}	] n.s.	6.56 ± 0.43 (6)^{a}	] n.s.
	BL/BD		7.88 ± 0.38 (14)^{a}		8.22 ± 0.58 (27)^{a}		8.00 ± 0.49 (20)^{a}	
		F	8.09 ± 0.32 (6)^{ab}	]	8.55 ± 0.45 (9)^{a}	] *	8.00 ± 0.50 (14)^{b}	]
		M	7.72 ± 0.33 (8)^{a}	] n.s.	8.05 ± 0.54 (18)^{a}	] *8	7.96 ± 0.44 (6)^{a}	] n.s.

*1 Body length/Head length, Mean ± SD (*n*), *2 Body length/Caudal peduncle length, Mean ± SD (*n*), *3 Body length/Body depth, Mean ± SD (*n*), *4 Female, *5 Male, *6 Different superscript letters between different ploidies within the same experiment indicate significant differences ($P<0.05$, Scheffe test), *7 Not significant difference between females and males, *8 Significant difference between females and males ($P<0.05$, Scheffe test).

Discussion

In the experiment E, no difference was observed in survival rates among three different ploidies after the long-term communal rearing for 3 years 1 month. In contrast, significantly reduced survival was found in diploid loaches of the experiment F. In general, normal diploids show poorer survival after spawning season (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001), but they have not spawned yet. In the previous work (Taniura et al. 2002), no such difference was found in survival at about 2 years 3months among diploid, triploid (diploid female × tetraploid male) and tetraploid loaches. At present, the reason for poor survival of diploid loaches in one of the two experiments has not been identified.

Growth observed in the previous (Taniura et al. 2002) and the present study was inferior to that observed in wild and near natural aquaculture conditions. Sizes of natural 0-yea-old loaches were reported to be about 50 – 60 mm in total length and 2 – 5 g in body weight (Suzuki 1983). Ito and Suzuki (1977) reported that loach larvae released to the pond grew to the size with 30 mm total length and 0.15 g body weight in one month after releasing. Kim et al. (1994) raised mud loaches to 3.0 – 3.2 g body weight within two months. Low growth in our experiments suggests that laboratory conditions are different from appropriate natural conditions, probably due to the small aquarium size and the high density of materials. Thus, rearing conditions should be examined again in near future.

However, significantly retarded growth of tetraploid loaches was evident at the end of communal rearing in both experiments. As natural tetraploids have not been found in wild populations of Japan after the flow cytometry examination (Zhang and Arai 1999; Morishima et al. 2002), they are considered to be an exotic fish. The loach *M. anguillicaudatus* with tetraploid chromosome number of 100 was reported in the specimens obtained from Yang-tze River in China (Li et al. 1983). In that case, tetraploid loaches are likely to show different characteristics from the common

diploid loaches living in wild populations in Japan. Such a possibility may be supported by the significant difference in BL/HL value between tetraploid and other ploidies. This morphological characteristic specific to tetraploid might suggest genetically remote relationship between common Japanese diploids and naturally occurred tetraploids.

As the other possible explanation, inbreeding depression might be involved in the retarded growth of tetraploids, because tetraploid progeny used in the present study was the second generation of the original tetraploid family produced in 1990 (Arai 2001; Arai et al. 1991b, 1993) and the elevation of homozygosity cannot be ruled out. Thus, the retarded growth and low BL/HL proportion might be explained by inbreeding and the subsequent expression of recessive genes.

In rainbow trout *Oncorhynchus mykiss*, induced triploids were reported to show better growth than diploids under separate rearing condition and at high feeding rates (Kobayashi and Fushiki 1997). However, competition for food between the two ploidies became evident under communal rearing condition at low percentage (30–50%) of the appropriate feeding amount and this gave rise to the retarded growth of triploids (Kobayashi and Fushiki 1997). Since the feeding behaviour of triploid ayu *Plecoglossus altivelis* was reported to be less active than diploids, triploid fish should be beaten by diploids in the competition for food under communal rearing (Aligh et al. 1990). As a cause of inactive feeding of triploid, an involvement of reduced cellular density in the brain volume was suggested (Lou and Purdom 1984). Although tetraploid loaches showed slower growth in the present work, no difference was observed in growth between diploid and triploid loaches. In this study, reduction of growth due to a defeat in feeding competition in triploids might be compensated by energy reallocation from reproduction to somatic growth in triploids due to the expression of sterility or near-sterility. Improvement of growth in triploid fishes due to sterile reproduction has been often reported (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001). The other possibility may be the harmful effect of high polyploidy itself. Certain polyploid plants often exhibit gigantism in proportion to the elevation of ploidy, but the paradoxical reduction in plant size has been observed in higher polyploids such as pentaploid, hexaploid and others (Blakeslee, 1941). This phenomenon is still unknown and unclear in animals (Pandian and Kotteswaran 1998; Arai 2001; Felip et al. 2001).

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長期混合飼育実験における自然四倍体ドジョウ子孫の特性

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ドジョウ *Misgurnus anguillicaudatus* の自然四倍体（4n＝100）と通常二倍体（2n＝50）の配偶子を用いた人工受精により，二倍体，三倍体，四倍体種苗を作出し，それらの特性比較を行うため，1ヵ月令から3年1ヵ月令まで，同一の水槽において長期の混合飼育実験を行った。その結果，四倍体の成長は，二倍体，三倍体に有意に劣ること，これらの体長／頭長比が低いことが判明した。雄の生殖腺指数は三者で差がないが，三倍体雌の卵巣は二倍体，四倍体に比べて未発達であった。