Japan. J. Ichthyol.
40(1): 65–75, 1993

魚類学雑誌
40(1): 65–75, 1993

Electrophoretic Studies of Diploid, Triploid, and Tetraploid Forms of the Japanese Silver Crucian Carp, *Carassius auratus langsdorfii*

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(*Received October 29, 1992; in revised form February 8, 1993; accepted March 22, 1993*)

Abstract The diploid-polyploid complex in the Japanese silver crucian carp (ginbuna), *Carassius auratus langsdorfii*, includes a diploid bisexual form, a triploid gynogenetic form and a tetraploid gynogenetic form. However, the origin of the polyploids remains to be clarified. Flow cytometric and electrophoretic analyses of Japanese crucian carp demonstrated that all the individuals with a two-banded pattern for an AMY-2 gene were polyploid, and 90% of the polyploids gave such a two-banded pattern. One of the two bands was not found in diploid subspecies in Japan but was present in both Korean crucian carp and goldfish. The diploid subspecies, kinbuna (*C. auratus* subsp.), which is distributed along the Pacific coast of eastern Japan, generated a diagnostic band of phosphoglucomutase. This band was also found in the polyploid "ginbuna" from the same region, but was observed in neither triploids nor diploids from other regions. The region-specific distribution of patterns of muscle lactic dehydrogenases indicated that the crucian carp examined in the present study could be divided into three groups, which originated, respectively, from the Pacific coast of eastern Japan, the Sea of Japan coast of eastern Japan, and western Japan. These results suggest a possible polyphyletic origin of polyploid forms of "ginbuna" via multiple hybridizations.

Polyploid species are rare amongst vertebrates but have been reported in fish, amphibians and reptiles. In fish, a triploid form in natural populations is known in six genera of teleosts, namely, *Poeciliopsis*, *Poecilia*, *Carassius*, *Phoxinus*, *Rutilus*, and *Cobitis* (Vrijenhoek et al., 1989). A tetraploid form has also been observed in *Cobitis* and *Carassius*.

Ginbuna, the Japanese silver crucian carp, is widely distributed in Japan (Okada and Nakamura, 1948; Kobayasi, 1982; Onozato et al., 1983). There are three forms of ginbuna; a bisexual diploid form with 100 chromosomes; a unisexual (all-female) triploid form with 156 chromosomes; and a unisexual tetraploid (all-female) form with 206 chromosomes. Both unisexual forms reproduce by gynogenesis (Kobayasi et al., 1970, 1977; Kobayasi, 1971; Kobayasi and Ochi, 1972).

The classification and evolution of Japanese crucian carp remain to be clarified in spite of the wide distribution of these fish in the fresh waters of Japan and the efforts of Japanese ichthyologists. Nakamura (1984) recognised five subspecies: kinbuna (*C. auratus* subsp.); ginbuna (*C. a. langsdorfii*); nagabuna (*C. a. buergeri*); nigorobuna (*C. a. grandoculis*); and gengorobuna (*C. a. cuvieri*). The subspecies were distinguished by their coloration and some meristic characteristics, such as dorsal fin-ray and gill-raker numbers. Differences in these characteristics are not always clear, however, especially in the first three subspecies mentioned. Polyploidy found in "ginbuna" seems to render the classification more complicated, and triploid specimens with morphology intermediate between kinbuna and ginbuna have been found (Kobayasi, 1982). The diploid chromosome number of Japanese crucian carp ($2n=100$) is considered to be of tetraploid origin, rendering electrophoretic and chromosomal analyses more complicated and difficult.

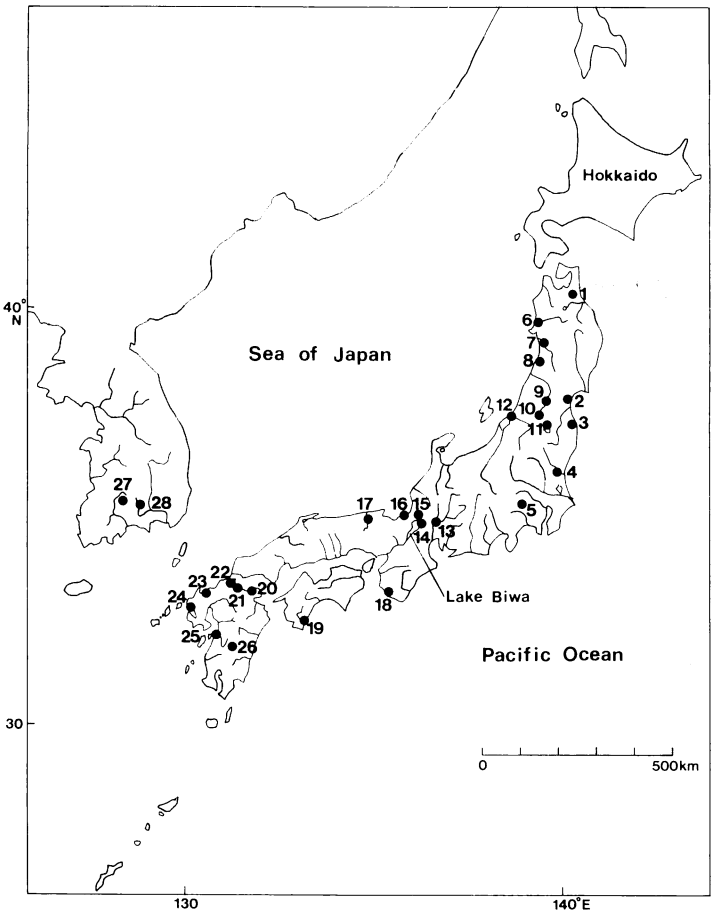

Fig. 1. Location of sites at which subspecies of *Carassius auratus* were collected. Numbers are explained in the text.

In order to solve these problems, it is necessary to examine both diploid and polyploid individuals of Japanese crucian carps. Electrophoretic data available to date are limited and direct comparisons between electromorphs and ploidy for each individual have not previously been reported (Amano et al., 1971; Taniguchi and Ishiwatari, 1972; Taniguchi and Sakata, 1977; Liu et al., 1980).

This report describes the largely consistent two-banded pattern of a specific amylase and the absence of one of the two bands in Japanese bisexual *C. auratus* subspecies, and notes that polyploid "ginbuna" differ regionally from a genetic perspective. These observations support the putative hybrid origin of polyploid "ginbuna" through multiple hybridizations with different combinations of genomes derived from ancestral forms.

Materials and Methods

Fish

Fish from wild populations of ginbuna and kinbuna were collected in 1989 at 26 sites in Japan and two in Korea (Fig. 1). Collection sites are enumerated below. The number of electrophoretically examined specimens from each site is given in parentheses.

1 = Uwano, Kamikita, Aomori (2); 2 = Gamo, Sendai, Miyagi (9); 3 = Obama, Souma, Fukushima (10); 4 = Kawawada, Mito, Ibaraki (11); 5 = Fuchigami, Akikawa, Tokyo (17); 6 = Fukkoshi, Noshiro, Akita (7): 7 = Hirasawa, Yuwa, Akita (21); 8 = Detomachi, Honjo, Akita (1); 9 = Miyajuku, Asahi, Yamagata (12); 10 = Nishi-ootsuka, Kawanishi,

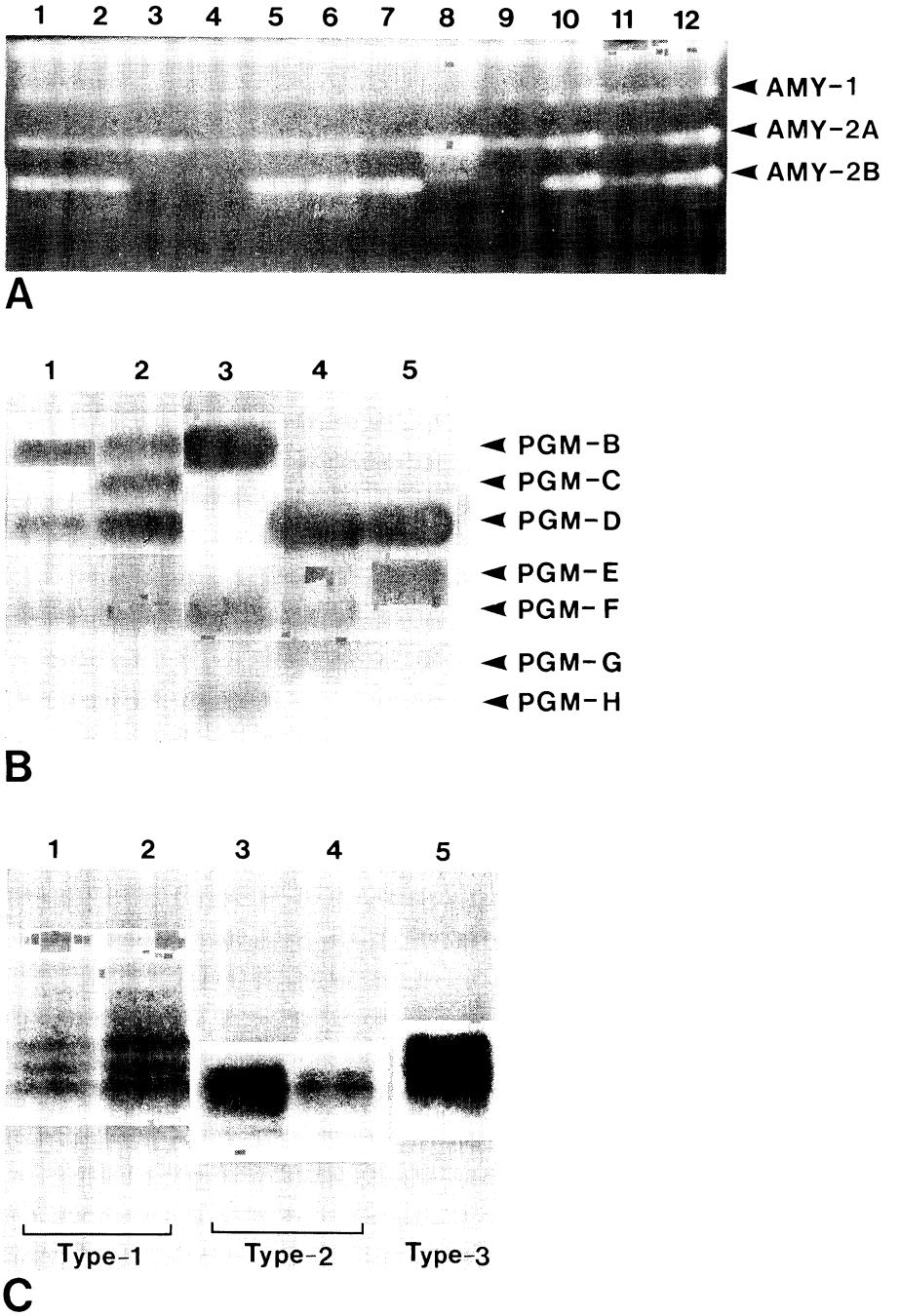

Fig. 2. Electrophoretic patterns of enzymes from subspecies of *Carassius auratus.* A) Amylases: lane 1, Yukuhashi-3 (3n); lane 2, Yukuhashi-4 (3n); lane 3, Yukuhashi-5 (2n); lane 4, Taragi-1 (2n); lane 5, Sasebo-1 (3n); lane 6, Sasebo-2 (3n); lane 7, Sasebo-3 (3n); lane 8, Uto-1 (2n); lane 9, Uto-2 (2n); lane 10, Shima-1 (3n); lane 11, Shima-2 (3n); lane 12, Shima-3 (3n). B) Phosphoglucomutases: lane 1, Sendai-5 (3n); lane 2, Sendai-4 (4n); lane 3, Sendai-3 (2n); lane 4, Tanabe-3 (2n); lane 5, Yanaizu-7 (2n). C) Muscle lactate dehydrogenases: lane 1, Asahi-5 (2n); lane 2, Asahi-6 (2n); lane 3, Mihama-3 (2n); lane 4, Mihama-4 (2n); lane 5, Mito-3 (2n). Direction of electrophoresis was from top to bottom in each case.

Table 1. Ploidy and electromorphs of each specimen

	Locality	Individual no. (relative DNA content)	Ploidy	AMY-2	PGM	LDH
1.	Kamikita	1 (3.0), 2 (2.8)	3n	AB	BDF	2
2.	Sendai	1 (2.9)	3n	AB	BDF	—[1]
		2 (2.6)	3n	AB	BDF	2
		3 (2.0)	2n	A	BFH	3
		4 (3.8)[2]	4n	AB	BCDEFH	3
		5 (3.0)	3n	AB	BDF	2
3.	Souma	1 (2.9)	3n	AB	D	2
		2 (3.0)	3n	AB	CDF	2
		3 (1.8)	2n	A	CF	—
		4 (2.7)	3n	AB	BDFH	—
		5 (2.7)	3n	AB	DFG	3
		6 (2.7)	3n	AB	BDFH	2
4.	Mito	1 (3.0)	3n	AB	DF	2
		2 (2.7)	3n	A[3]	BDF	3
		3 (1.9), 7 (1.9)	2n	A	BF	3
		4 (2.7)	3n	AB	BDEG	—
		5 (2.7)	3n	AB	BDF	—
		6 (2.9)	3n	A[3]	BDEG	—
		8 (2.8)	3n	A[3]	BDF	3
5.	Akikawa	1 (1.9), 2 (1.9), 3 (1.9), 5 (1.9), 10 (1.9), 11 (1.9), 12 (1.9), 13 (1.8), 14 (1.9), 15 (1.9), 16 (1.8), 17 (1.8)	2n	A	BF	3
		4 (2.8)	3n	AB	DEFG	2
		6 (1.9), 7 (1.9)	2n	A	BF	—
		8 (1.9)	2n	A	BFG	3
		9 (2.8)	3n	AB	BDF	2
6.	Noshiro	1 (3.0)	3n	AB	DF	1
		2 (2.0)	2n	A	DEF	1
		3 (2.9)	3n	AB	ADEFG	—
7.	Yuwa	1 (2.9)	3n	AB	DF	1
		2 (1.9), 4 (1.8), 6 (1.9), 7 (1.9), 8 (1.9), 11 (1.8), 13 (1.8), 14 (1.9), 15 (2.0)	2n	A	DE	1
		3 (1.8), 10 (1.8)	2n	A	DEF	1
		5 (1.8)	2n	A	D	1
		9 (2.7), 12 (2.8)	3n	AB	ABE	—
8.	Honjo	1 (3.0)	3n	A[3]	DF	—
9.	Asahi	1 (1.8)	2n	A	E	1
		2 (2.8)	3n	AB	DEF	—
		3 (2.0)	2n	A	DF	1
		4 (1.9), 5 (1.9), 9 (1.9)	2n	A	DEFG	1
		6 (1.8)	2n	A	EFG	1
		7 (1.8)	2n	A	DEF	1
		8 (1.9)	2n	A	DEG	1
10.	Kawanishi	1 (2.0)	2n	A	DE	1
		2 (1.9)	2n	A	DEF	1
		3 (2.7)	3n	A[3]	BD	—
11.	Inawashiro	1 (2.9)	3n	AB	DE	—
		2 (1.8)	2n	A	DF	2
		3 (2.8), 4 (2.8)	3n	AB	DEFH	—
12.	Nishikawa	1 (2.0)	2n	A	DFG	1
		2 (2.9)	3n	AB	DFG	—
		3 (2.9)	3n	AB	DEFG	—

Table 1. Continued

	Locality	Individual no. (relative DNA content)	Ploidy	AMY-2	PGM	LDH
13.	Yanaizu	1 (1.8)	2n	A	DF	2
		2 (1.5)	hypodiploid	A	DF	2
		3 (1.8), 4 (2.0), 6 (2.0)	2n	A	DFG	2
		5 (1.9)	2n	A	DEFG	2
		7 (1.9)	2n	A	DEF	2
14.	Maibara	1 (1.8)	2n	A	DEF	2
		2 (1.8)	2n	A	DF	2
16.	Mihama	1 (1.9), 2 (1.8), 3 (1.9), 4 (1.9)	2n	A	DE	2
		5 (2.0), 7 (1.9)	2n	A	DFG	2
		6 (1.9)	2n	A	DEG	2
17.	Toyooka	1 (2.9)	3n	AB	DEF	2
		2 (1.9)	2n	A	DEFG	2
		3 (2.8)	3n	AB	DEFG	2
		4 (2.8)	3n	AB	DEF	2
18.	Tanaka	1 (2.9)	3n	AB	DE	2
		2 (1.8)	2n	A	DEF	2
		3 (1.9)	2n	A	DEFG	2
20.	Madama	1 (2.9)	3n	AB	DEFG	2
21.	Shiida	1 (2.9)	3n	AB	DFGH	2
22.	Yukuhashi	1 (1.9)	2n	A	DEF	2
		2 (1.9), 5 (2.0)	2n	A	DF	2
		3 (2.8), 4 (2.8)	3n	AB	DEFG	2
23.	Shima	1 (2.8), 2 (2.8), 3 (2.9), 4 (2.9)	3n	AB	DEFG	2
24.	Sasebo	1 (2.8), 2 (2.9)	3n	AB	DFG	2
		3 (2.8)	3n	AB	DEFG	2
25.	Uto	1 (1.9)	2n	A	DEFH	2
		2 (1.9)	2n	A	DE	2
26.	Taragi	1 (1.9)	2n	A	DEFG	2

[1]Others; [2]chimeric tetraploid; [3]five of 47 polyploid individuals (10.6%) lacked the AB bands but possessed the A phenotype of AMY-2.

Yamagata (7); 11=Osada, Inawashiro, Fukushima (8); 12=Zenkoji, Nishikawa, Niigata (3); 13=Saba, Yanaizu, Gifu (13); 14=Irie, Maibara, Shiga (8); 15=Ooto, Kinomoto, Shiga (6); 16=Sakajiri, Mihama, Fukui (11); 17=Kehi, Toyooka, Hyogo (4); 18=Inaricho, Tanabe, Wakayama (3); 19=Gudo, Nakamura, Kochi (1); 20=Usuno, Madama, Ooita (1); 21=Minato, Shiida, Fukuoka (1); 22=Imai, Yukuhashi, Fukuoka (5); 23=Kofuji, Shima, Fukuoka (4); 24=Sakioka, Sasebo, Nagasaki (3); 25=Nagahama, Uto, Kumamoto (2); 26=Taragi, Taragi, Kumamoto (1); 27=Shinpyong, Chollabuk-do, Korea (4); 28=Sudong, Kyonsangnam-do, Korea.

Gengorobuna, *C. a. cuvieri*, were taken from stocks at the Tokyo University of Fisheries. Goldfish (wakin) were purchased from a fish dealer.

Flow cytometry

For the preparation of erythrocyte samples, the caudal fin was cut from the fish with a disposable surgical blade and the tail dipped into a solution of 10 mM EDTA, 1% (w/v) gelatin and 5 mM veronal buffer (pH 7.4). After two washes with the same buffer, a small aliquot of a suspension of erythrocytes was put into 1 m*l* of propidium iodide staining solution, which was composed of 50 μg/m*l* propidium iodide, 0.1% sodium citrate, and 0.2% (v/v) Nonidet P-40. After incubation for 30 min at 4°C, the cells were monitored for fluorescence with an EPICS CS flow cytometer (Coulter Electronics, Hialeah, FL). 10,000 cells were counted per sample.

Erythrocytes of *Oryzias latipes*, from the stock at The Tokyo Metropolitan Institute of Medical Sci-

ence, were used as controls. Histograms showed discrete peaks for *O. latipes*, and diploid, triploid, and tetraploid cells in the case of the crucian carp. The relative DNA content was determined by calculating the ratio of mean fluorescence of crucian carp cells to that of cells of *O. latipes*.

Table 2. Electromorphs of specimens whose ploidy was not determined

Locality	Individual no.	AMY	PGM	LDH
2. Sendai	6, 7	AB	BDF	2
	8	A	BF	3
	9	AB	BDF	—[1]
3. Souma	7, 8	AB	DF	2
	9	A	BCF	3
	10	AB	BDEFG	3
4. Mito	9	AB	CDEF	2
	10	A	BDF	3
	11	AB	BE	—
6. Noshiro	4, 5, 7	AB	DF	1
	6	AB	DE	—
7. Yuwa	16, 18, 19	A	DEF	1
	17, 20, 21	A	DE	1
8. Asahi	9, 10	A	CDEF	1
	11	AB	DEF	—
	12	A	DE	1
9. Kawanishi	4	AB	BEF	3
	5, 7	A	DEF	1
	6	AB	BDEF	3
10. Inawashiro	5	A	DF	2
	6	A	DF	—
	7	AB	DEF	—
	8	A	BCDEF	—
13. Yanaizu	8, 9	A	DF	2
	10, 11, 12, 13	A	DEF	2
14. Maibara	3, 4	A	DE	2
	5	AB	DE	2
	6	AB	DEF	2
	7	A	DEF	2
	8	A	BDEF	2
15. Kinomoto	1	A	DFG	2
	2	A	DE	2
	3	AB	DG	2
	4, 5, 6	A	DEF	2
16. Mihama	8, 11	A	DE	2
	9	A	DEF	2
	10	B[2]	BDG	2
19. Nakamura	1	AB	DEG	2
27. Imshil	1	B	DF	2
	2	B	DE	2
	3, 4	B	DEFH	2
28. Sudong	1, 2	B	DEF	2

[1] Others; [2] a single individual from Japan with the B phenotype of AMY-2.

Electrophoresis and staining

The liver, intestines, and skeletal muscle of each individual were dissected out and homogenized with 10 mM Tris-HCl (pH 7.0). Each homogenate was centrifuged at 15,000 × *g* for 10 min and the resultant supernatant used for electrophoretic analysis. Superoxide dismutase (SOD) from liver, lactic dehydrogenases (LDH) from muscle, and amylases (AMY) from intestines were analysed on native, discontinuous, vertical polyacrylamide slab gels in the buffer system of Davis (1964). Each gel sheet of 140 × 100 × 1 mm^3 was composed of a 12% acrylamide plus 0.125% bisacrylamide separation gel and a 3% acrylamide plus 0.8% bisacrylamide stacking gel. Phosphoglucomutases (PGMs) from liver were separated by electrophoresis on horizontal agarose gels. Each gel sheet of 140 × 180 × 1 mm^3 was composed of 0.9% agarose and 2% polyvinylpyrrolidone (K-90; Nacarai Tesque, Kyoto, Japan). The buffer system for analysis of PGMs consisted of 135 mM tris-hydroxymethylaminomethane (Tris) and 43 mM citric acid (pH 7.0) for the electrode buffer, and 9 mM Tris and 3 mM citric acid (pH 7.0) for the gel.

Potentials of 400 V were applied for two hours at 4°C for acrylamide gel electrophoresis For agarose gel electrophoresis a potential of 300 V was applied for 1.5 hours. Staining procedures were those described by Shaw and Prasad (1970) and Sakaizumi et al. (1992).

Results

Flow cytometric analyses of erythrocytes from 119 wild-caught specimens demonstrated that they included 71 diploids, 46 triploids, one tetraploid, and one hypodiploid. The tetraploid showed chimerism with two small, broad peaks at 2n and 5n, in addition to a 4n peak that included 75% of all cells. All six goldfish were determined to be diploid.

Electrophoretic patterns were examined for four different proteins. The phenotypic patterns of these enzymes showed polymorphism. Most of the specimens gave a single band for SOD. The C phenotype with a single, rapidly migrating band was observed in the case of *C. a. cuvieri* exclusively. Three individuals from Noshiro and two from Yuwa had a three-banded phenotype AB. Other specimens had the B phenotype.

The AMY band that migrated most slowly toward

the anode, AMY-1, was monomorphic. The rapidly migrating AMY-2 occurred as two bands (Fig. 2A). All of the Japanese diploid specimens, including *C. a. cuvieri* possessed only the A band (A phenotype). By contrast, all of the Korean specimens and the goldfish possessed only the B band (B phenotype). Of 47 Japanese polyploids, 42 specimens (89.4%) had a pattern with both the A and B bands (AB phenotype). Specimens with the AB phenotype were always polyploid. Five triploid specimens with the A phenotype were all from eastern Japan. A single individual from Mihama possessed the B phenotype, but the DNA content of the specimen was not examined (Table 2).

PGM gave multiple banding patterns. For convenience, the bands were designated in alphabetical order, starting from that with the slowest mobility (Fig. 2B). The diploid specimens from Akikawa, Sendai and Mito possessed the B band. This band was also detected frequently in the analyses of polyploids from the Pacific coast of eastern Japan (15 out of 20 specimens) and three triploids from the Sea of Japan coast of eastern Japan, but it was not observed in specimens from western Japan or Korea. The B band was not detected in *C. a. cuvieri.*

The polymorphic patterns of muscle LDH could be classified into at least three types, as shown in Figure 2C. All specimens from western Japan were type 2, irrespective of ploidy. By contrast, type 1 was predominant in fish from the Sea of Japan coast of eastern Japan, irrespective of ploidy. Type 3 was observed in the majority of diploids and in some triploids from the Pacific coast of eastern Japan. The polymorphic patterns were very complicated and have not yet been interpreted.

On the basis of the geographic distribution of electrophoretic patterns of PGM and LDH, the results were summarized as shown in Table 3. Fish from the Pacific coast of eastern Japan, which had a high frequency of the B band of PGM, were composed of diploid kinbuna and polyploid "ginbuna." Along the coast of the Sea of Japan, diploid and triploid ginbuna, which had the type 1 LDH pattern, were distributed. Diploid and triploid ginbuna, that had the type 2 LDH pattern and PGM patterns lacking the B band were distributed in western Japan.

Discussion

The gynogenetic form of ginbuna is known to exist

Table 3. Geographic variation of ploidy and electrophoretic characteristics

Locality	Ploidy	Number of specimen	AMY			PGM		LDH			Others
			A	AB	B	B(+)	B(−)	1	2	3	
Pacific coast of eastern Japan (locality no. 1–5)	2n	19	19	0	0	18	1	0	0	16	3
	3n	18	3	15	0	13	5	0	10	3	5
	4n	1	0	1	0	1	0	0	0	1	0
	ND[1]	11	3	8	0	8	3	0	5	4	2
	Total	49	25	24	0	40	9	0	15	24	10
Sea of Japan coast of eastern Japan (locality no. 6–12)	2n	25	25	0	0	0	25	24	1	0	0
	3n	13	2	11	0	3	10	2	0	0	11
	ND	22	14	8	0	3	19	14	1	2	5
	Total	60	41	19	0	6	54	40	2	2	16
Western Japan (locality no. 13–26)	2n	24	24	0	0	0	24	0	24	0	0
	3n	15	0	15	0	0	15	0	15	0	0
	ND	23	18	4	1	2	21	0	23	0	0
	Total	62	42	19	1	2	60	0	62	0	0
Korea (locality no. 27, 28)	ND	6	0	0	6	0	6	0	6	0	0
	Total	6	0	0	6	0	6	0	6	0	0

[1] Not determined.

alongside the usual bisexual form of this subspecies. The two forms are morphologically indistinguishable, suggesting that gynogenesis in ginbuna is not of hybrid origin, although such an origin is typical of other gynogenetic vertebrates. Initial evidence, however, in favor of a hybrid origin of the gynogenetic form of ginbuna is provided by the peculiar distribution of chromosomes in the first meiotic metaphase (Cherfas, 1966, 1981; Yamashita and Nagahama, 1989). This observation may reflect genetic nonuniformity of the genomes that constitute the triploid set of chromosomes. Further evidence comes from the C-banding patterns of chromosomes in six subspecies of Japanese and Chinese crucian carp (Ueda and Ojima, 1978; Ojima et al., 1979; Ojima and Takai, 1979). Ginbuna with triploid chromosomes from Lake Biwa carry a pair of markers characterized by conspicuous C-bands on the short arms of the second largest submetacentrics. In kinbuna (*C. auratus* subsp.) and Chinese crucian carp (*C. a. auratus*), two such markers occur in the female, whereas there is only one in the male. These results suggest possible heterogeneity of the genomes that constitute the triploid set of chromosomes. Yet more evidence comes from interspecific hybridization experiments. Triploid hybrids were found among the offspring from backcrosses of F_1 females (common carp×*C. a. cuvieri*) with males of the common carp or *C. a. cuvieri* (Ojima et al., 1975; Zhang et al., 1992b). Kobayasi (1985) succeeded in obtaining triploid females among the F_2 progeny of a cross between a female diploid ginbuna and a male goldfish (*C. a. auratus*). Furthermore, he obtained a fertile tetraploid female, which reproduced gynogenetically, from a cross between an F_1 female (*C. a. cuvieri* female×*C. a. gibelio* male) and an F_1 male of the reciprocal cross. Production of allotriploid medaka by interspecific hybridization between *Oryzias latipes* and *O. curvinotus* was achieved by Sakaizumi et al. (1992) and Kurita et al.(1992).

In the present study, specimens were classified into diploids, triploids or tetraploids by flow cytometry and subsequently subjected to individual electrophoretic analyses. Examination of various protein and enzyme polymorphisms resulted in diagnostic polymorphisms in association with ploidy or geographic regions, being recognized for three enzyme systems.

The two-banded pattern (AB) of AMY-2 could be generated by hybrids between subspecies with bands AMY-2-A and AMY-2-B, respectively. This pattern was found exclusively in polyploid (3n and 4n) individuals. The B band was not found in any Japanese diploids but was present in Korean crucian carp and goldfish, the latter being considered to be derived from a Chinese subspecies, *C. a. auratus* (Ojima and Takai, 1979). All gynogenetic offspring of triploid ginbuna with the AB phenotype had the same phenotype as their mothers (data not shown). These results suggest that the polyploid form of "ginbuna" has a hybrid origin. If such is the case, one candidate as a parent seems to be a Continental subspecies of *C. auratus*. It should be noted that triploids with the A phenotype were detected in eastern Japan. Zhang et al. (1992a) reported that one of the PGM bands observed in the mother was lost in her gynogenetic offspring at fairly high frequency. It is probable that triploids with the A phenotype lost the B band owing to crossing-over or by deletion after establishment of the triploid form in eastern Japan. An exceptional individual from Mihama, which had the B phenotype and was therefore assumed to be triploid, seemed to have lost the A band after polyploidization.

The B band of PGM was observed in most of the specimens from the Pacific coast of eastern Japan, regardless of their ploidy, and in some polyploids from the Sea of Japan coast. Since all the diploids from Akikawa, which had the B band, could be identified as kinbuna on the basis of gill-raker numbers (31–34), this band can be regarded as a diagnostic characteristic of kinbuna. Thus, it is highly probable that the B band generated by polyploids in eastern Japan is derived from kinbuna, which has a diploid chromosome number. By contrast, neither triploids nor diploids from western Japan possessed this band, suggesting a different origin of polyploids in eastern and western Japan.

While all the western specimens gave the type 2 pattern of muscle LDH regardless of ploidy, eastern fish exhibited polymorphism. In particular, most fish from the Sea of Japan coast of eastern Japan gave the type 1 pattern, which was not found in other regions. These results indicated that the population on the Sea of Japan coast of eastern Japan is distinct from those in other regions. The type 3 pattern can be regarded as another diagnostic marker for kinbuna.

Considering all these results, the specimens examined in this study can be classified into eight groups (Table 4). Gengorobuna (*C. a. cuvieri*), with a diploid chromosome number, are distinct from all other groups, both morphologically and electrophoretically. Korean crucian carp, although their

ploidy was not determined, are electrophoretically different from Japanese crucian carp. Kinbuna (*C. auratus* subsp.) can be considered a true subspecies, with the diagnostic characteristics shown in Table 4. "Ginbuna," however, is composed of at least five different forms from a genetic perspective: polyploid-Pacific; diploid- and polyploid-Sea of Japan; and diploid- and triploid-western Japan. Since it is probable that the polyploids are of hybrid origin, those from different regions appearing to have arisen polyphyletically through multiple hybridizations, it is considered inappropriate to include all five groups in the single taxon, *C. a. langsdorfii.* It should be emphasized that "ginbuna," which are distributed alongside kinbuna, may have a genome(s) derived from the latter.

It is possible that the triploid forms from western Japan and the Sea of Japan coast of eastern Japan have two different genomes which may be derived from diploid ginbuna with AMY-2-A and an unknown subspecies with AMY-2-B. The triploid form from the Pacific coast of eastern Japan seems to have two distinct genomes that may be derived from kinbuna and an unknown subspecies. Examination of the C-bands of the eastern triploids may provide information useful to such a discussion because kinbuna also have metacentrics with large C-bands.

It should be noted that tetraploids have never been found in western Japan (Kobayasi, 1982). This is further evidence in favor of the multiple origin of the Japanese polyploid "ginbuna." It is probable that tetraploids arose from the rare incorporation of the sperm of diploid species into a triploid egg, since the tetraploid from Sendai had electrophoretic characteristics similar to those of triploids from the same location. Unidirectional acceptance of scales transplanted from triploids to tetraploids (Onozato et al., 1983) gives some supports to this suggestion.

An analysis of restriction endonucleases of mitochondrial DNA has demonstrated that kinbuna and eastern "ginbuna" have similar haplotypes, which are distinct from that of western ginbuna (Okazaki et al., unpublished data). This supports the hypothesis regarding the origin of polyploids and is relevant to the classification problem mentioned above. Moreover, it is well known that diploid, triploid, and tetraploid forms are distributed in Hokkaido (Kobayasi, 1982; Onozato et al., 1983). Fish from this area are particularly important for elucidation of the origin of polyploids in Japan and for studies of the relationship between Japanese "ginbuna" and the Russian silver crucian carp, *C. a. gibelio.* Direct comparisons between ploidy, electrophoretic characteristics of the nuclear genome, and analysis of mtDNA, which allow identification of the maternal form, in the crucian carp from Japan and adjacent Continental areas will provide a more complete picture of the relationship between the different forms of the "ginbuna complex" and its origin, and will enable a revision of the nomenclature of that complex.

Table 4. Diagnostic characteristics and distribution of Japanese and Korean forms of *Carassius auratus*

Form	Distribution	Ploidy	AMY	PGM	LDH	SOD
Polyploid "ginbuna" —Pacific—	Pacific coast of eastern Japan	3n 4n	AB	B(+)[1]	type 2, type 3 or others	B
Diploid ginbuna —Sea of Japan—	Sea of Japan coast of eastern Japan	2n	A	B(−)[2]	type 1	B
Triploid ginbuna —Sea of Japan—	Sea of Japan coast of eastern Japan	3n	AB	B(−)	type 1	B
Diploid ginbuna —Western—	Western Japan	2n	A	B(−)	type 2	B
Triploid ginbuna —Western—	Western Japan	3n	AB	B(−)	type 2	B
Kinbuna	Pacific coast of eastern Japan	2n	A	B(+)	type 3	B
Korean crucian carp	Korea	—[3]	B	B(−)	type 2	B
Gengorobuna	Lake Biwa	2n	A	B(−)	—	C

[1] Band-B positive; [2] band-B negative; [3] not examined.

Acknowledgments

We are grateful to Dr. Sang-Rin Jeon, Sang Myung Women's University, Seoul, for his kind help in the collection of the specimens. The assistance in the collection of the specimens provided by Dr. Satoshi Hamaguchi, Niigata University, Niigata, and Dr. Choji Taya, The Tokyo Metropolitan Institute of Medical Science, Tokyo, is also greatly appreciated.

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電気泳動パターンからみた倍数性ギンブナの特性

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日本各地で採集したギンブナについて，その倍数性とアイソザイムパターンを調べ，キンブナ，ゲンゴロウブナ，韓国産フナと比較した．AMY-2 のバンドパターンに関して 2 倍体はギンブナ，キンブナ，ゲンゴロウブナとも A 型であったのに対し，AB 型の個体はすべて 3 倍体で，3 倍体の約 90%（西日本ではすべての個体）が AB 型を示した．一方，韓国産フナは B 型であった．キンブナに特徴的な PGM の B バンドが，東日本の太平洋側産の 3 倍体の 75%と日本海側の 3 倍体の一部で観察された．また，東日本の日本海側には倍数性を問わずこの地域に特異的な LDH のパターンが認められた．これらの結果は，倍数性ギンブナが雑種起源であり，しかも多系統的であるとする仮説を支持するものと考えられる．

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