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Analyses of Histocompability and Isozyme Variations in Triploid Fish, Carassius auratus langsdorfii

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Abstract

Clonal diversity of triploid gynogenetic fish, *Carassius auratus langsdorfii,* was examined by tissue grafts and electrophoreses of several enzymes and muscle protein. The hypervariabilities of histocompatibility clones and electromorph clones were observed in wild-caught specimens. The ratio of DNA value of each specimen to control diploid fish varied from 1.38 to 2.01. Unidirectional histocompatibility and protein variations in histocompatibility clones were the characteristics of this gynogenetic fish. These facts seem to indicate the divergence of a clone.

Tissue-incompatibility was observed betweeen F_1 progeny of triploid ginbuna × Shubunkin. Some of offspring expressed the male-dependent transparent-scaled character. This indicates the fact that fusions of female and male pronuclei must have occured. In the other strain, random reductions of DNA values were observed. The divergence of a clone must have been caused by the gene addition or loss.

Introduction

In the unisexual vertebrates, following three reproductive types have been reported; 1) the parthenogenesis, which is observed in lizards as in the genes *Cnemidophorus* (MASLIN, 1967) 2) the gynogenesis, observed in amphibians as in the genus *Ambystoma* (MACGREGOR and UZZELL, 1964); and in fish as in the three genera of teleost, *Poecilia* (HUBBS, 1955, KALLMAN, 1962 b), *Poeciliopsis* (SCHULTZ, 1969) and *Carassius* (CHERFAS, 1972, KOBAYASHI,1971), 3) the hybridgenesis, observed in teleost, *Poeciliopsis monacha-luchida* (SCHULTZ, 1961). Among them, the genus *Carassius* was reported in two subspecies, silver crucian carp *Carassius auratus gibelio* (CHERFUS, 1972) and ginbuna *C. a. langsdorfii* (KOBAYASHI, 1971). Ginbuna have distributed most widely in Japan, and they include both bisexual diploid (2n=100) type and unisexual (all female) triploid (3n=156) and tetraploid (4n=206) types (KOBAYASHI, 1970, 1977). The unisexual type reproduces gynogenetically.

They omit the meiosis I resulting in a triploid egg, and during the first cleavage sperm nucleus remains condenced, without fuse with female pronucleus (KOBAYASHI, 1976, OJIMA, 1977). Then the progenies of these fish belong to the clone having the same genotype as a mother. The clonal reproduction is related to the diversity of histocompatibility clones in natural populations. Tissue transplantation experiment, which applied frequently for the investigation of a genetic similarity, can detect a slight difference of histocompatibility antigens between a donor and a host. If a donor and a host have an identical histocompatibility gene set, the grafts transplanted between them can be survive for a long time.

The origin of polyploid ginbuna is not clear because of the difficulty to decide its ploidy from morphological characteristics. Only one triploid specimen, caught in the lake Kasumigaura, had the intermediate morphological character between kinbuna (*C. a.* subsp.) and ginbuna. By the analysis of the electrophoretic pattern of muscle protein, it was presumed that this specimen would have two genomes of kinbuna and one genome of ginbuna (TANIGUCHI, 1975).

The genus *Carassius* had evolved from diploid species of cyprinid fish by total genomes duplication (OHNO *et al.*, 1967, OHNO, 1970). Several dehydrogenase, for example, 6-phospho-gluconate dehydrogenase and isocitrate dehydrogenase are controlled by duplicated loci in gold fish, *C. a. auratus* (BNEDER and OHNO, 1968, GUTIERREZ and OHNO, 1969). And electrophore-polymorphisms of muscle protein and hemoglobin which were separated in many components, were reported in Japanese crucian carp (TANIGUCHI and ISHIWATARI, 1972, TANIGUCHI and SAKATA, 1977, LIU *et al.*, 1978, 1980, AMANO *et al.*, 1971).

In this paper, using histocompatibility genes and several isozyme (containing muscle protein) genes as gene markers, the problems of the origin of triploid ginbuna and its clonal stability are analyzed.

Materials and Methods

I. Specimens

The experimental materials of ginbuna, *Carassius auratus langsdorfii,* were collected from the lake Kasumigaura and the lake Suwa. About 250 living specimens were brought into the laboratory, and bread in the water-tank till sacrified.

In addition to these wild-caught specimens, some of the F_1 and F_2 progenies of triploid ginbuna (caught in the lake Suwa and Ueda fisheries experimental station) × shubunkin (goldfish ; homozygous transparent-scaled chracter, *TT*) were also used in this experiments.

II. Estimation of Ploidy

The ploidy of experimental materials were estimated from the DNA content of erythrocyte nucleus by microspectrophotometly. Erythrocyte nuclei were stained by

Feulgen reagent according to the method of RASCH *et al.* (1970). After dehydration through alcohol series, the samples were mounted with Canada balsam. DNA value of individual nuclei was determind at 560 nm by one-wavelength scanning method with OLYMPUS MMSP. In each set of blood smears, average DNA content of ten erythrocyte nuclei of each specimen was compared with that of control fish (goldfish or kinbuna).

III. **Tissue Graft**

To investigate the histocompatibility relationships among polyploid ginbuna, the scale transplantations were performed. The scale transplantations were carried out by the method of HILDEMANN (1957). Sample fish had been maintained in the aquariums over a week at 23-25°C prior to operations. The donor and host fish were anesthetized with 0.08 % MS-222. Lateralline scales were removed alternately by a fine forceps and the scales from the donor, previously removed, were inserted into the empty spaces of the host. The host fish generally received two allografts from each donor and a control autograft simultaneously. The conditions of allo- and autograft had been observed every day for 30 days after operations. The results of scale grafts were based on a intensity of inflammation and remaining time of melanophores : 1) accute rejection (−) ; Severe inflammation appeared within three days after grafting, and after 6-10 days all melanophores disappeared. The graft absorbed by the host at last. 2) chronoc rejection (±) ; The rejection bsaed on weak histo-incompatibility was characterized by slight inflammation and melanophores remained healty on the graft throughout the experimental period, but the graft had been absorbed gradually. 3) acception (+) ; The graft showed no inflammation and survived more than 30 days.

IV **Electrophoresis**

1. *Sample Preparation*

The variations of several enzymes and muscle protein of the specimens used in transplantation experiment were analysed electrophoretically. After decapitation, kidney,liver and muscle were removed immediately. The isozyme variations of malate dehydrogenase and 6-phosphogluconate dehydrogenase in kidney extract and alcohol dehydrogenase, lactate dehydrogenase, isocitrate dehydrogenase, aspartate aminotranspherase, creatine kinase and esterase in liver extract were examined. Kidney was stored in a refrigerator and employed in electrophoresis within the same day. Liver and muscle were stored at −20°C for 2-21 days till analysis. A small quantity of each tissue was homogenized with three volumes of buffer solution (0.01M Tris-HCl, pH 7.0) and centrifuged at 15,000 rpm for 30 minutes at 0°C. The supernatant fraction was used for the electrophoretic analyses.

2. *Electrophoresis system*

Each enzyme was separated on horizontal starch gels (14×26×0.6 cm). About 40 μl of sample solution was added in the slot (0.5×1.2×0.1 cm) made in the gel. Muscle

protein was separated on slab acrylamide gels (11×18×0.3 cm). Sample solution was absorbed into filter paper (Toyo No. 2, 0.2×0.8 cm) and inserted in a slit cut in the gel.

3. *Staining Solution*

Starch gels were sliced horizontally in identical two parts and each slab was stained histochemically for a specific enzyme. The staining solutions for enzymes were accorded to the method of PHILIPP *et al.* (1979) and SHAW and PRASAD (1970).

Results

I. DNA Value in Polyploid Ginbuna

The DNA value of 15 specimens collected from the L. Suwa (designated ST) and 16 specimens from the L. Kasumigaura (designated KT) were in triploid range, and one

Table 1. (a)-(b). DNA values of erythrocytes from F_1 and F_2 progeny of four strains (Triploid ginbuna × Shubunkin), and the ratio of DNA value of each sample to control fish.

(a).

Strain	Specimen designition	DNA value per cell mean ± S.D.	Ratio
F_1 (10S33 × Shubunkin)	a	22.35 ± 1.61	2.04
	b	17.37 ± 1.16	1.58
	c	22.46 ± 1.38	2.05
F_2 (Clone I × Shubunkin)	a	15.91 ± 1.18	1.45
	b	16.05 ± 1.41	1.46
F_1 (U29 × Shubunkin)	a	16.60 ± 0.57	1.51
	b	16.27 ± 1.96	1.48
F_1 (10S22 × Shubunkin)	a	14.47 ± 1.33	1.32
Control fish (goldfish)		10.98 ± 0.66	1

(b).

Strain	Specimen designition	DNA value per call mean ± S.D.	Ratio
F_1 (10S22 × Shubunkin)	b	18.97 ± 1.37	1.31
	c	20.28 ± 1.55	1.40
	d	15.37 ± 1.36	1.06
	e	15.40 ± 0.85	1.06
	f	18.45 ± 1.18	1.28
	g	16.00 ± 0.90	1.11
Control fish (goldfish)		14.47 ± 0.72	1

specimen from the L. Suwa (designated STe) was tetraploid range.

DNA values of F_1 and F_2 progenies of four strains obtained form the cross mating experiments are given in Table 1 (a)−(b). In F_1 progeny of U29 × Shubunkin and F_2 progeny of Clone I × Shubunkin, apparent difference in DNA value was not observed. On the other hand, in F_1 progeny of 10S33 × Shubunkin, some of which showed male dependent transparent-scaled character, DNA values of "a" and "c" were elevated to the tetraploid state whereas DNA value of "b" remained the triploid state. The ratios of DNA values of F_1 progeny of 10S22 × Shubunkin varied from 1.06 to 1.40, and the DNA values of "d" and "e" were apparently reduced to the diploid state. Sex of five F_1 offspring of 10S22 × Shubunkin were male without female according to dissection.

II. Histocompatibility among Polyploid Ginbuna

Among 15 triploid and one tetraploid ginbuna collected from the L. Suwa, 69 donor-host combinations were examined. As shown in Table 2, in 16 specimens, allografts were survived in eight donor-host combinations and two histocompatibility clones were found; Clone IS (ST4 and ST5) and Clone IIS (ST12 and ST14). When the transplants were made from ST11 to ST12 and ST14, one allograft was survived but the other was rejected. This type of random reaction of allograft may be due to a weak histo-incompatibility between a donor and a host (KALLMAN, 1960). Reverse transplants from ST12 and ST14 to ST11 were succeeded. Allografts from ST2 to ST7 were survived, but clonal relationship between them is not clear because reverse transplantations were not performed. In the other donor-host combinations, allografts were rejected. From these results it was concluded that eight specimens of ST1, ST2, ST3, ST6, ST10, ST15, ST16 and STe17 had different histocompatibility gene sets from Clone IS and two specimens of ST11 and ST13 had different histocompatibility gene sets from Clone IIS.

In this experiment, 16 polyploid ginbuna from the L. Suwa can be distinguished into minimum 8 and maximum 14 histocompatibility clones. Tetraploid fish (STe17) did not received no allografts from six triploid fish.

In 16 triploid ginbuna collected from the L. Kasumigaura, two histocompatibility clones were found (Table 3); Clone IK (KT25 and KT26) and Clone IIK (KT50 and KT53). Unidirectionl histocompatibility was observed in the donor-host combination of KT15 and KT54. Allografts from KT15 to KT54 were rejected chronically, but reverse transplants were succeeded. The hyper-variability of histocompatibility clones were also observed in triploid ginbuna from the L. Kasumigaura. Among 10 triploid fish of KT44-KT53, allografts except made between KT50 and KT53 were all rejected in 75 donor-host combinations. From these results it became apparent that 16 specimens from the L. Kasumigaura can be distinguished into minimum 8 and maximum 14 histocompatibility clones.

The intra-strain grafts were made to determine whether the offspring surely possess the same genotype. Six fish of F_2 progeny of Clone I and 13 fish of F_1 progeny

Table 2. Histocompatibility relationships among polyploid ginbuna collected from the lake Suwa.

Host Donor	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST10	ST11	ST12	ST13	ST14	ST15	ST16	STe17
ST1	+	−	−	−		−			−					−	−	−
ST2	−	+	−		−		+	−						−	−	−
ST3	−	−	+		−			−						−	−	−
ST4	−	−		+	+	−			−							
ST5		−	−	+	+	−								−	−	−
ST6	−	−	−	−	−	+			−							
ST7							+	−								
ST8								+	−							
ST10								−	+							
ST11										+	+,−	−	+,−			
ST12										+	+	−	+			
ST13										−	−	+	−			
ST14										+	+	−	+			
ST15								−	−					+	−	−
ST16								−	−					−	+	−
STe17														−	−	+

Table 3. Histocompatibility relationships among polyploid ginbuna collected from the lake Kasumigaura.

Host Donor	KT25	KT26	KT44	KT45	KT46	KT47	KT48	KT49	KT50	KT51	KT52	KT53	KT15	KT54	KT55	KT56
KT25	+	+														
KT26	+	+														
KT44			+			−	−	−	−	−	−					
KT45			−	+	−	−	−	−	−	−	−					
KT46				−	+	−	−	−	−	−	−					
KT47				−		+		−	−	−	−					
KT48	−	−	−	−	−	−	+	−	−	−	−					
KT49			−	−	−	−	−	+	−	−	−	−				
KT50			−	−	−	−	−	−	+	−	−	+				
KT51			−	−		−	−	−	−	+	−	−				
KT52			−	−	−	−	−		−	−	+	−				
KT53			−	−	−	−	−	−	+	−	−	+				
KT15													+	±	+	−
KT54													+	+		
KT55															+	−
KT56															−	+

Table 4. Fate of intra-strain tissue grafts.Numerals of the table indicate the number of donor-host combinations.

Strain	Fate of graft
F_2 (Clone I × Shubunkin)	8+
F_1 (U29 × Shubunkin)	14+
F_1 (10S33 × Shubunkin)	2+, 3±, 1−
F_1 (10S22 × Shubunkin)	10+

of U29 in addition to the specimens analysed their DNA values were also used in transplantation experiments. F_1 progenies of 10S22 and 10S33, and F_2 progeny of Clone I were two-year old fish. F_1 progeny of U29 were three-year old fish.

The results of intra-strain grafts are given in Table 4. In three progenies of Clone I, U29 and 10S22, all intrastrain grafts were accepted. In these strains, clonal inheritances concerning histocompatibility genes were ascertained. But the results of grafts transplanted among F_1 progeny of 10S33 was not uniform. In each donor-host combination, autografts were survived but unidirectional histocompatibility was observed in two donor-host combinations of "a"-"b" and "a"-"c". The allografts from "a" to "b" showed acute-type rejections and when the transplants were made from "c" to "b", from "b" to "c" and "a" to "c", the allografts showed chronictype rejections. These results suggest the possibility that a new histocompatibility-distinct individual may produce from a existent clone.

III. Protein Variations in Polyploid Ginbuna

§ 1. *Electrophoretic Phenotypes of Enzymes and Muscle Protein in Wild-caught Specimens*

Genetic variability in several proteins besides histocompatibility antigens was surveyed. Among the polyploid ginbuna used in transplantation experiments, 14 specimens from the L. Suwa and 15 specimens from the L. Kasumigaura were employed in electrophoresis. The samples for electrophoresis from the specimens which belonged to the identical histocompatibility clones, or which were related in histocompatibility were separated on the same gel to compare the electrophoretic patterns directly.

Electrophoretic phenotypes of enzymes and muscle protein are summarised in Table 5 and Figure 1. Each protein variation is explained in detail as follows;

1. *Alcohol dehydrogenase*

By electrophoresis, three isozyme bands were detected. Homozygote had only "a" band (phenotype B) and heterozygote had three bands of "a", "b" and "c". A "b" band was always accompanied with "c" band. As ADH is dimer enzyme, it is considered that "b" band may be heterodimer isozyme including "a" and "b" subunit. Homozygous "c" band was not observed in 19 specimens. Both KT25 and KT26, which belonged to Clone

Table 5. Electrophoretic phenotypes for the proteins examined in this study.

Specimen	Phenotype									
	ADH	LDH	MDH	IDH	6-PGDH	AAT	CK	PGM	ES	MP
Clone IS										
ST-4	—	—	A	C	I	A	A	A	C	D
ST-5	—	—	A	C	I	A	A	A	C	D
Clone IIS										
ST-12	—	A	A	B	H	B	D	D	H	A
ST-14	A	A	A	B	A	B	D	G	H	A
ST-11	A	A	A	B	H	B	D	D	H	A
ST-2	—	—	A	C	A	A	B	E	J	G
ST-7	A	—	A	C	A	A	B	E	J	A
ST-1	—	—	A	C	C	A	B	H	E	A
ST-3	—	—	A	A	F	A	A	I	H	A
ST-6	B	—	A	C	F	B	—	—	I	E
ST-13	A	—	A	C	C	A	—	—	F	B
ST-15	A	—	A	C	A	A	C	F	E	A
ST-16	A	—	A	C	A	A	C	J	H	A
STe-17	A	—	A	C	B	B	A	K	H	D
Clone IK										
KT-25	A	—	A	E	D	A	—	—	F	C
KT-26	A	—	A	E	D	A	—	—	F	C
Clone IIK										
KT-50	A	—	A	D	B	A	D	C	H	C
KT-53	A	—	A	D	A	A	D	C	C	F
KT-15	—	—	A	E	E	A	D	B	C	F
KT-54	—	—	A	E	A	A	D	B	C	F
KT-55	—	—	A	E	A	A	D	B	C	F
KT-44	A	A	A	E	A	A	E	L	F	C
KT-45	A	B	A	E	A	A	F	M	F	A
KT-46	—	C	A	F	A	A	E	N	D	F
KT-47	—	B	A	E	A	A	E	O	A	C
KT-49	A	—	A	E	—	A	—	—	B	E
KT-51	A	—	A	E	G	A	—	—	G	E
KT-52	B	—	A	F	A	A	—	—	G	F
KT-56	—	—	A	E	H	A	B	F	E	B

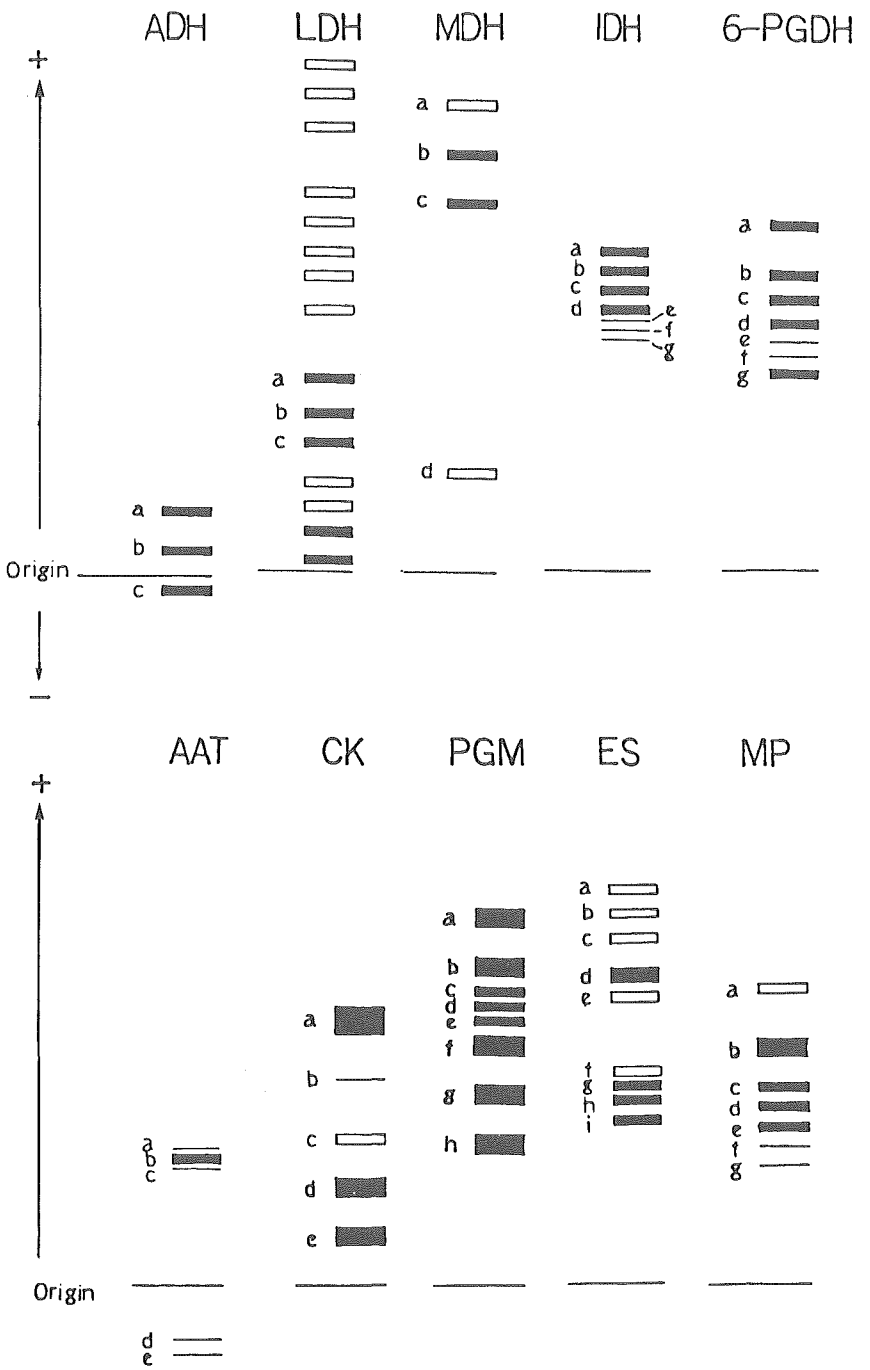

Figure 1. Electrophoretic pattern observed in each protein. Diagrams show the relative mobility of each band. Except LDH, the most anodally migrating band is designated as "a", the next in anodal mobility as "b", etc.

IK, showed phenotype A.

2. *Lactate dehydrogenase*

More than 10 bands were separated on the gel. KLOSE *et al.* (1969) has reported that the number of LDH locus is at least five in goldfish. Various hybrid enzymes are produced since LDH is tetramer enzyme. By the staining intensity of three bands of "a", "b" and "c", seven specimens were classified into three electrophoretic phenotypes. In Clone IIS, both ST2 and ST4 showed phenotype A which was characterized by the most deeply stained "c" band.

3. *Malate dehydrogenase*

All specimens had four isozyme bands. The most slowest band (designated "d") seems to be mitochondoria-dependent MDH. In anodal three bands, "c" band was always stained more intense than other two bands.

4. *Isocitrate dehydrogenase*

GUTIERREZ and OHNO (1979) suggested the duplication of IDH locus in goldfish. They have reported that three electrophoretic phenotypes were observed in the population of goldfish from the L. Erie. Heterozygote had five isozyme hands, and two kinds of homozygotes possessed anodal three bands or cathodal three bands among five bands of heterozygote, respectively.

In this study, liver extract was used for electrophoresis. Six phenotypes were confirmed in 29 specimens. Phenotype A included five bands of "a", "b", "c", "d" and "f". Phenotype D lacked "c" band from phenotype A. Phenotype B possessed three bands of "c", "d" and "f". C and E indicate two phenotypes having two bands of "c"-"d" and "d"-"f", respectively. In phenotype F, two bands were contained : "e" band had intermediate mobility between "d" and "f" bands, and "g" band less mobility than "f" band. Intra-histocompatibility clonal variations in this enzyme was not observed.

5. *6-Phosphogluconate dehydrogenase*

From the result of electrophoretic analysis of this dimer enzyme, BENDER and OHNO (1968) have proposed the hypothesis that autosomal two loci for 6-PGDH must exist in goldfish, and two kinds of allele, A and B had been probably taken into a genome by a tetraploidy event occurred during the evolutionary development of cyprinid fish. They have found five estimated genotypes in kidney extracts in 107 goldfish : that is AB_2/AB_2, AB_2/AB_3, AB_3/AB_3, AB_1/AB_3 and AB_1/AB_2.

In this study, two double-heterozygotes, AB_2/AB_3 (phenotype A) and AB_1/AB_3 (phenotype H) were observed in 29 polyploid ginbuna. Phenotype A was subdivided in six phenotypes based on the staining intensity of "a", "b" and "g" band.

In Clone IS, both ST4 and ST5 lacked "g" band (AA isozyme designated by BENDER and OHNO (1968)) (Fig. 3b). These specimens had only B subunits. This observation is inconsistent with OHNO 's hypothesis that both A and B alleles had been probably taken into a genome, which resulted in a permanent heterozygous state

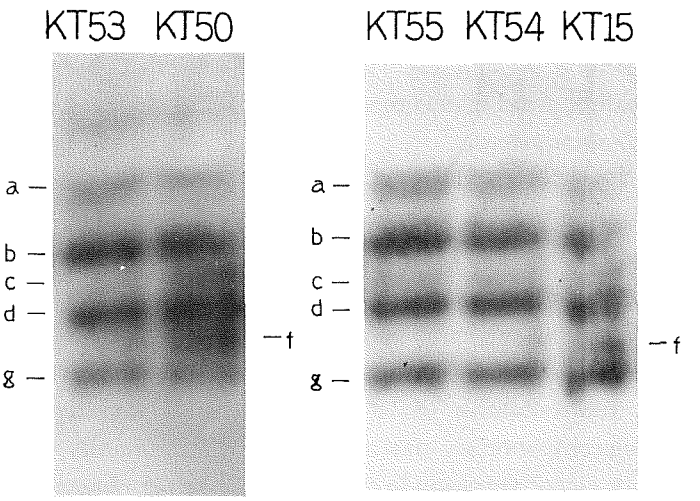

Figure 2. (a)-(b) Isozyme variations in 6-PGDH. (a) : Phenotypes observed in Clone IIK. (b) : AB_2/AB_3 phenotype observed in three specimens which were related in histocompatibility.

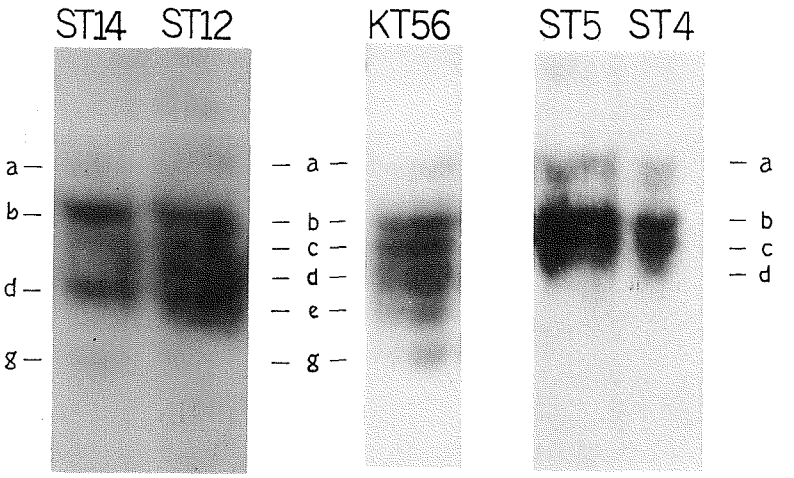

Figure 3. (a)-(b) Isozyme variations in 6-PGDH. (a) : Phenotypes observed in Clone IIS. COmpare with AB_1/AB_3 phenotype observed in KT56. (b) : Phenotype including only B subunits.

concerning 6-PGDH genes.

Intra-histocompatibility clonal variation was observed in Clone IIS and Clone IIK. In Clone IIS, ST14 showed phenotype A (AB_2/AB_3), but ST12 showed phenotype H (AB_1/AB_3). In Clone IIK, KT53 showed phenotype A but KT50 had the additional band "f" (phenotype B) (Fig. 2a and 3a). The "f" band was also observed in KT15 which exhibited the unidirectional histocompatibility to KT54 (Fig. 2b).

6. *Aspartate aminotranspherase*

Phenotype A had two bands of "a" and "b" at anodal side. Phenotype B possessed the additional band "c", which was observed in only five specimens from the L. Suwa. Intrahistocompatibility clonal variation was not observed.

7. *Creatine kinase*

Maximaly five bands appeared on the gel. By the staining intensity of "d" and "e" bands and the existence of "b" and "c" bands, 22 specimens were classified in six phenotypes. As the mobilities of "d" and "e" bands were consist with that of hemoglobins, electrophoretic patterns were occasionally distorted. Intra-histocompatibility clonal variation in this enzyme was not observed.

8. *Phosphoglucomutase*

Liver PGM was highly variable. On the gel, eight kinds of isozyme were detected. By the existence and the staining intensities of six bands ("a", "c", "d", "e", "g" and "h"), 22 specimens were divided into 15 phenotypes. The "b" and "f" bands existed in

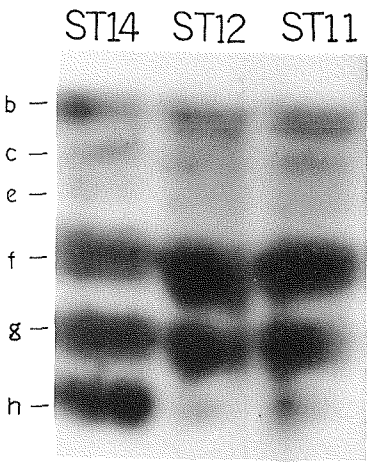

Figure 4. Isozyme variations in PGM. ST11 which was related in histocompatibility to Clone IIS showed the identical phenotype as observed in ST12.

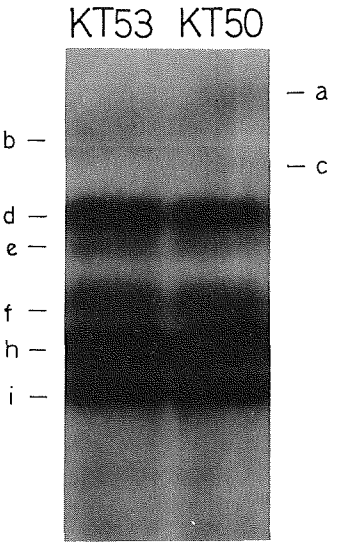

Figure 5. Isozyme variations in esterase observed in CLone IIK.

each phenotype.

In Clone IS, Clone IK and Clone IIK, intra-clonal variation was not observed. Although in Clone IIS, "h" band in ST14 was apparently more intense than in ST12 (Fig. 4).

9. *Non-specific esterase*

Liver esterase was separated in nine bands on the gel. This enzyme is not a single enzyme, but the mixture of several kinds of enzymes. Minor bands at anode side were observed in the following four combinations: "a", "b", "a"-"b" and "a"-"c". It is considered that the "a" and "b" bands may be controlled by the codominant alleles. Heterozygous "g" and "h" bands and homozygous "h" band were observed, but homozygous "g" band was not found in this study. Electrophoretic patterns of esterase were classified into 10 phenotypes. In clone IIK, KT50 had "a" and "c" bands, but KT53 had only "b" band at anode side (Fig. 5). Except Clone IIK, intra-clonal variation was not found.

10. *Muscle protein*

Muscle protein were separated in 5 to 7 bands on the acrylamide gel. Moderate-migrating bands of "c", "d" and "e" were observed in three combinations : only "d", "d"-"e" and "c"-"d"-"e". By the staining intensities of these bands, muscle protein from 29 specimens were divided in seven phenotypes electrophoretically.

In only Clone IIK, intra-clonal variations was observed. KT50 had three bands of "c"-"d"-"e", and "c" band was stained most deeply. KT53 lacked "c" amd "e" bands. ST7 which accepted the graft from ST2 had three bands of "c", "d" and "e", amd "d" band was stained most deeply. ST2 lacked "c" band (Fig. 6).

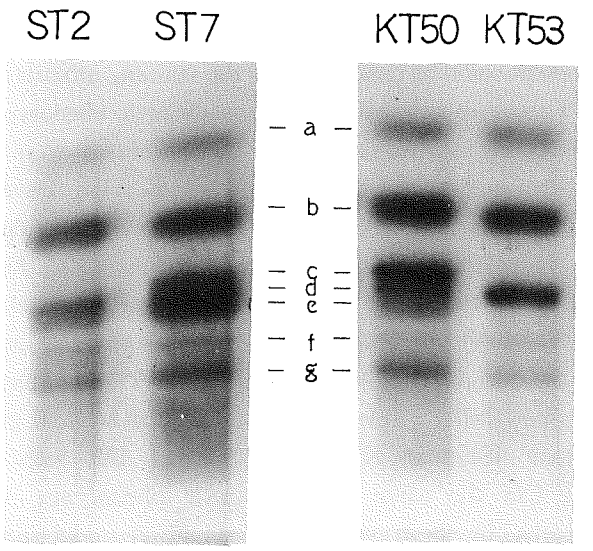

Figure 6. Phenotype variations in muscle protein.

§ 2. *Electrophoretic phenotype of enzymes and muscle protein in F_1 and F_2 progenies of four strains (Triploid ginbuna × Shubunkin).*

Precised investigations in these four strains were not carried out, because tissues of such young specimens were not sufficient to analyse many enzymes by electrophoresis. Furthermore, in 6-PGDH, PGM, and IDH, several apparently different phenotypes from adult specimens were observed. It is not clear whether these phenotypes are really new phenotypes or ontogenetic variations of a existent phenotype.

Intra-strain variation of ES, MDH, ADH, AAT and muscle protein was not observed. But there is the possibility that ontogenetic regulation may be involved.

DISCUSSION

If the triploid ginbuna, *Carassius auratus langsdorfii,* is evolved from a single origin and its reproduction is strictly maternal, all triploid ginbuna must be considered to be the identical genotype. In this study, it became apparent that such hypothesis is not available to be expected. Tissue incompatibility and electrophoretic variations were observed almost all of the triploid ginbuna from the L. Suwa and from the L. Kasumigaura. The results of tissue graft experiments indicate the hyper-variability of histocompatibility clones in triploid ginbuna.

By electrophoretic analyses of the genes coding ten proteins, triploid specimens from the L. Suwa and from the L. Kasumigaura could be divided into 12 and 13 electromorph clones, respectively. These results present a striking contrast to the results of other gynogenetic triploid fish, *Poeciliopsis 2 monacha-lucida,* in which only three electromorph clones were found in 172 specimens by the examination of 23 loci. (VRIJENHOEK, 1977). In triploid ginbuna, electrophoretic phenotypes of 6-PGDH, PGM, CK and muscle protein could be subdivided by the staining intensity of each component of such proteins. The hyper-variability of electrophoretic phenotypes of these proteins seems to be related to the hypothesis that the genus *Carassius* would have evolved from diploid species of cyprinid fish by total genome duplication (OHNO, 1970). The DNA value in the genus *Carassius* is approximately 2-fold of other diploid species of cyprinid fish (OHNO *et al.,* 1966, 1967). If a duplicated locus is functional, the gene dosage effect will be expected because tetraploid species must possess four allele in each duplicated loci. If the locus has multiple alleles, new genotypes may be produced by random incorporations of two original alleles into a genome. These hypothetical events may result in the hyper-variability of electromorph clones in triploid ginbuna.

ST4-ST5 (Clone IS) and KT25-KT26 (Clone IK), which showed mutual histocompatibility, had the same electrophoretic phenotypes in eight and seven proteins, respectively. In these specimens, clonal reproduction was ascertained. However, unidirectional histocompatibility and protein variations in histocompatibility

clones were observed in gynogenetic ginbuna. That is, Clone IIS (ST14-ST12) had the isozyme variations in PGM and 6-PGDH and Clone IIK (KT50-KT53) had the isozyme or protein variations in ES and muscle protein; ST12-ST11 and KT15-KT54 had the same electrophoretic phenotypes, but unidirectional histocompatibilities were observed between the transplantation from ST11 to ST12 and from KT15 to KT54. These results seem to suggest the divergence of a clone. Because, considering the hyper-variabillity of histocompatibility clones and electromorph clones, the possibility that the clones which are not closely related share the identical genotype by chance is minute.

The divergence of a clone can be interpreted in several ways, and the one is mutations. Regarding to the histocompatibility genes, this assumption can not be abandoned (MOORE and EISENBREY, 1979). But as to the isozyme variations in histocompatibility clones, this probability is doughtful. Because all isozymes or protein components in histocompatibility clones were also found in other triploid specimens.

Unidirectional histocompatibility and protein variations in histocompatibility clones may be caused by the addition or loss of gene(s). These events occur when one has all of the genes possessed by the other additional genes lacking in the other.

Allografts transplanted between the F_1 progeny of 10S33 were rejected in several donor-host combinations. some of the offspring of this strain expressed the male-dependent transparent-scaled character. It is evident that the gametic fusions occurred and the chromosomes or genes from sperm were incorporated into the zygotes. Intra-strain tissue incompatibility and the presentation of paternal characters were also reported in other gynogenetic fish, *Poecilia formosa* (KALLMAN, 1964).

The DNA values of "a" and "c" were elevated to tetraploid state. This indicated the fact that the genome level incorporation into the zygote must have occurred. Such histocomaptibility antigens based on the sperm genome, presented in the graft of "a" and "c", and the grafts from "a" and "c" to "b" which remained triploid state were rejected acutely or chronically.

The graft from "b" to "c" showed chronic-type rejection. This fact seems to suggest the idea that the gene(s) or chromosome(s) must be incorporated into the zygote of "b". From these results, it was indicated that histocompatibilitydistinct clone may be evolved from a existent clone by the rare gametic fusion. If the native tetraploid fish also derived from a triploid fish, the grafts from the progenitorial triploid fish or clone to the tetraploid fish must be accepted. In this study, only one tetraploid fish (STe17) received the grafts from six triploid fish, but allografts were all rejected. Considering the hypervariability of histocompatibility clones in triploid fish, above hypothesis concerning to the origin of tetraploid fish can not be excluded.

In other strain, 10S22, the random reductions of DNA values were observed among F_1 progeny. It is still unknown why DNA values were reduced, because the meiotic processes in triploid ginbuna are still obscure. It has reported in *Carassius auratus*

gibelio that Meiosis I are omitted as the result of the formation of a tripolar spindle, which results in triploid egg (CHERFAS 1966). In *Carassius auratus langsdorfii,* the first polar body formation is skipped as the result of lacking of Meiosis I (KOBAYASHI, 1976). In F_1 progeny of 10S22, it seems probable that the several chromosome have been eliminated from triploid ova by the partial restoration of meiotic mechanism.

In spite of the reduction of DNA values, allografts transplanted among F_1 progeny of 10S22 were all succeeded. This strain may have been homozygous histocompatibility genes, or the chromosomes which does not link histocompatibility genes may have been eliminated.

In the four strains obtained from cross mating experiments with Shubunkin, clonal inheritance was ascertained by tissue transplantation experiments in two strains, Clone I and U29. Although, in F_1 progeny of 10S33, fusions of female and male pronuclei must have occurred, and the DNA values of some F_1 offspring of 10S22 apparently reduced to the diploid state. In present, it still remain unknown why the differences of clonal stability are took place, further investigations as to maturation mechanisms and fertilization mechanisms in triploid ginbuna are expected.

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