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High Proportion of Heterozygotes in Microsatellite DNA Loci of Wild Clonal Silver Crucian Carp, *Carassius langsdorfii*

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ABSTRACT—The silver crucian carp, *Carassius langsdorfii* has three reproductive characteristics: gynogenesis, polyploidy (triploid or tetraploid), and genetic homogeneity within a family. In natural water, the silver crucian carp populations consist of multiple clonal lines. In the present study, three microsatellite DNA loci were used to distinguish several clonal lines of the silver crucian carp sampled from natural water. Progeny and the maternal fish had the same genotype in the three loci. In 237 fish collected from the wild, nine alleles were observed in *GF1**, sixteen alleles in *GF17**, and nine alleles in *GF29**. Ten genotypes were observed in *GF1**, seventeen in *GF17** and eight in *GF29**. The proportion of heterozygotes was very high in each locus (1.000). Sixteen clonal lines were distinguished by the combined genotypes of three microsatellite loci. Two subtypes were also detected within the clonal line KOC-011.

INTRODUCTION

The silver crucian carp, *Carassius langsdorfii* (Matsubara and Ochiai, 1965) has three reproductive characteristics: gynogenesis, polyploidy (triploid or tetraploid) (Kobayasi, 1971; Kobayasi and Ochi, 1972), and clonal nature (Dong and Taniguchi, 1996; Dong *et al*., 1997; Umino *et al*., 1996). Sperms of other species initiate development of their eggs (Kobayasi, 1971; Kobayasi and Ochi, 1972). However, the introduced sperm nucleus does not transform into a male pronucleus and makes no contribution to the zygotic genome (Nakakuki *et al*., 1984; Yamashita *et al*., 1990, 1993). A cytological study concluded that a population in the Kanto district appeared to be comprised of both triploid and tetraploid types (Kobayasi *et al*., 1970). Thus it was suggested that the silver crucian carp is unisexual and produces unreduced polyploid eggs (Kobayasi, 1971).

Taniguchi and Sakata (1977) found that the silver crucian carp of western Japan has a specific band of muscle protein electrophoresis, which has been verified to be a creatine kinase in another study (Dong and Taniguchi, 1996). Taniguchi and Sakata (1977) also found that the silver crucian carp is independent from the gene pool of wild diploid crucian carp species. Murakami and Fujitani (1997) found that the repetitive DNA (Cal3nDr) are specific to polyploid silver crucian carp, and they suggested that this region is useful in the study of the genetic background of this species such as the origin of polyploidy and gynogenetic reproduction.

In terms of genetic homogeneity, several authors have observed that the progeny in this species are usually genetically identical to the maternal fish (Dong and Taniguchi, 1996; Dong *et al*., 1997; Umino *et al*., 1996). The wild silver crucian carp consist of several clonal lines (Dong *et al*., 1996; Umino *et al*., 1997; Ohara *et al*., 1998). These lines have been distinguished using tissue graft, isozymes, multilocus minisatellite DNA fingerprinting and mtDNA RFLP (Murayama *et al*., 1984; Dong *et al*., 1996; Umino *et al*., 1997; Ohara *et al*., 1998). Of these methods, tissue grafts, isozymes and mtDNA RFLP are time-consuming or lack the sensitivity to identify clonal lines. By contrast, multilocus minisatellite DNA fingerprinting is more sensitive method for the identification of clonal lines. This method, however, has limited application in population genetics because it is difficult to identify the zygotic condition (Takagi *et al*., 1997). Recently, a number of easily and highly variable genetic markers, known as microsatellite DNA, has been developed (Wright and Bentzen, 1994). Microsatellites are highly polymorphic, particularly in fish (Brocker *et al*., 1994).

In the present study, we used microsatellite DNA to examine the clonal nature of the artificially propagated silver crucian carp, and examined the zygotic condition of the loci of several clonal lines in this species collected from natural water in Kochi, Japan. As for the scientific name, we tentatively used *C. langsdorfii* for the triploid silver crucian carp while several other scientific names have been given to the silver crucian carp (Ginbuna), such as, *C. auratus langsdorfii* (Hosoya, 1993; Nakamura, 1969), *C. gibelio langsdorfi* (Miyadi *et al*., 1976) and *C. langsdorfii* (Matsubara and Ochiai, 1965).

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MATERIALS AND METHODS

Sampling and DNA extraction

One silver crucian carp, *C. langsdorfii*, caught from the Monobe River in Kochi Prefecture, Japan, was used as the maternal fish. Sperm from several goldfishes were used to activate egg development without the effect of genetic contribution to the offspring (Kobayasi, 1971; Dong and Taniguchi, 1996). The fry were reared until 150 days after hatching, and 40 fish were used for the analysis.

Samples of silver crucian carp from natural waters were collected from two locations, the Niyodo and Monobe Rivers in Kochi Prefecture, Japan. The triploidy of these samples was determined by analyzing the red blood cell size using the methods detailed in Sezaki *et al.* (1977). In this study, we used the samples of triploid silver crucian carp that had previously been identified as 17 clones (designated as KOC-001 to 015 and 017) by minisatellite DNA fingerprinting in a previous report (Ohara *et al.*, 1998). The samples of KOC-016 were not used due to denaturation of DNA. Total DNA was extracted from the blood of each fish by standard SDS-phenol/chloroform procedures and stored at 4°C prior to PCR analysis (Takagi *et al.*, 1997).

Microsatellite analysis

Three microsatellite loci, *GF1**, *GF17**, and *GF29**, developed by Zheng *et al.* (1995), were used in this study. Nomenclature of loci and alleles follow Shaklee *et al.* (1990). Radiolabeling and chemiluminescence methods were used to visualize microsatellite DNA. The radiolabeling method was performed using the methods reported by Takagi *et al.* (1997). The reverse primer was 5` end-labeled with [γ^{32}P]ATP. The PCR was programmed for 7 cycles of 1 min at 94°C, 30 sec at 53°C, 30 sec at 72°C, 33 cycles of 30 sec at 90°C, 30 sec at 53°C, and 30 sec at 72°C, respectively. Following amplification, PCR products were mixed with denaturing stop dye, heated at 95°C for 15 min, and electrophoresed on 6% polyacrylamide gel. Alleles were sized according to an M13 sequence ladder.

The chemiluminescence detection method was performed according to the methods given in Perez-Enriquez *et al.* (1998). The reverse primer was 5` end-labeled with biotin. The PCR and electrophoresis conditions were the same as in the radiolabeling method. After electrophoresis, DNA was transferred to a nylon membrane by blotting, then the membrane was dried and UV crosslinked. DNA on the membrane was detected by using the Phototope™-Star Detection Kit (New England Biolabs). A sequence ladder obtained from the pUC19 plasmid was used as a size marker and was prepared with the CircumVent™ Phototope™ Kit (New England Biolabs).

Estimation of genetic distance

We estimated the genetic distance (*D*) among the clonal lines based on the band sharing index (*BSI*) at the three microsatellite loci in order to determine the genetic relationship among the clonal lines (Wetton *et al.*, 1987; Gillbert *et al.*, 1990). The *BSI* between clonal lines was calculated with the formula $BSI=2Nab / Na+Nb$, where *Na* and *Nb* are the number of bands (alleles) present in each clone, and *Nab* is the number of bands shared. The genetic distance (*D*) calculated with the formula $D=1-BSI$. The value varies from zero, when the two individuals are identical, to 1, when there are no bands in common. A dendrogram was drawn by the UPGMA method (Sneath and Sokal, 1973) based on *D* values, using a program in the PHYLIP Ver 3.5 software package (Felsenstein, 1994).

RESULTS

Microsatellite DNA patterns of artificial propagated offspring

Microsatellite DNA markers of 40 offspring from one maternal fish were examined. Since both the offsprings and the maternal fish had the same genotype at the three polymorphic loci, they are of the same clonal line. The genotypes of the clonal line were determined as **307/* (**307* or **311*) /

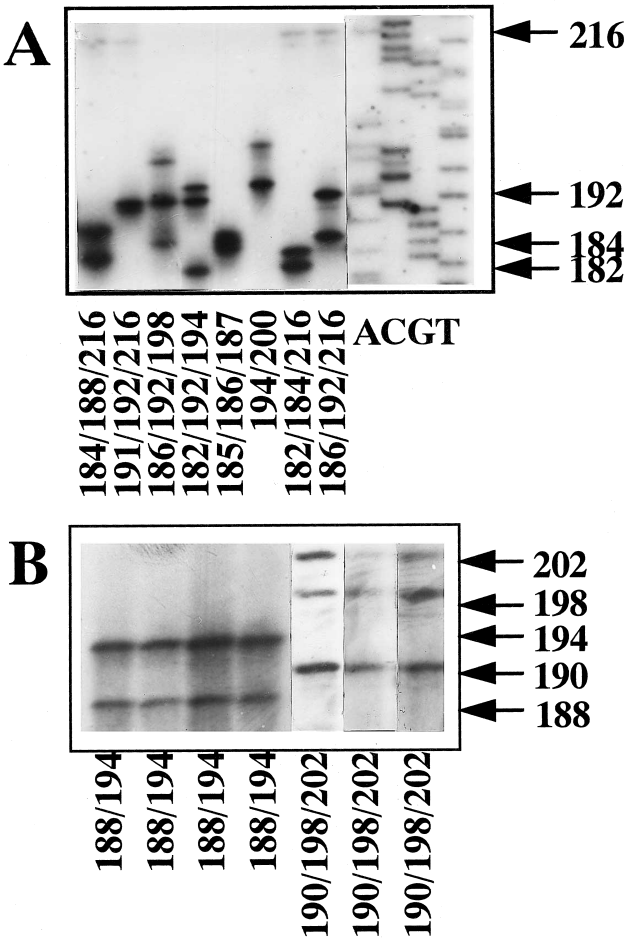

Fig. 1. Microsatellite banding pattern of Silver crucian carp, *Carassius langsdorfii*. (A) Microsatellite banding pattern of eight clones (left to right: KOC- 010, 006, 002, 011-1, 012, 013, 014, 015) in *GF17** obtained with the radiolabeling method. The size standard is a sequencing of M13 mp18. (B) Microsatellite banding pattern of two clones (KOC-002, 003) in *GF29** obtained with the chemiluminescence detection method.

Table 1. Numbers of alleles, genotypes and fragment size as well as proportion of heterozygotic individuals and allelic combinations of 17 clonal lines of silver crucian carp, *Carassius langsdorfii*

	*GF1**	*GF17**	*GF29**
No. of samples	237	237	237
No. of alleles	9	16	9
No. of genotypes	10	17	8
Fragment size(bp)	293–335	182–216	186–202
Proportion of heterozygotes*	1.000	1.000	0.992
Allelic combination			
Triallelic	0.253	0.734	0.376
Diallelic	0.747	0.266	0.624
Monoallelic	0	0	0

* Proportion of heterozygotic individuals

Table 2. Genotypes of three microsatellite DNA loci in 17 clonal lines of silver crucian carp, *Carassius langsdorfii*

Clonal lines	Microsatellite locus			Number of individuals		
	*GF1**	*GF17**	*GF29**	Niyodo River	Monobe River	Total
KOC-001	*307*/(*307* or *311*)/*311*	*185*/*192*/*193*	*188*/(*188* or *194*)/*194*	7	3	10
KOC-002	*299*/*303*/*335*	*186*/*192*/*198*	*190*/*198*/*202*	10	6	16
KOC-003	*303*/*307*/*311*	*188*/(*188* or *192*)/*192*	*188*/(*188* or *194*)/*194*	2	3	5
KOC-004	*303*/(*303* or *305*)/*305*	*185*/*187*/*216*	*188*/(*188* or *194*)/*194*	25	1	26
KOC-005	*299*/(*299* or *303*)/*303*	*188*/*189*/*198*	*186*/*188*/*194*	0	4	4
KOC-006	*303*/*307*/*311*	*191*/*192*/*216*	*186*/*188*/*194*	10	10	20
KOC-007	*303*/(*303* or *311*)/*311*	*194*/(*194* or *204*)/*204*	*188*/(*188* or *194*)/*194*	2	2	4
KOC-008	*293*/(*293* or *303*)/*303*	*196*/(*196* or *204*)/*204*	*192*/*200*/*202*	36	0	36
KOC-009	*303*/(*303* or *311*)/*311*	*184*/(*184* or *216*)/*216*	*188*/(*188* or *194*)/*194*	6	0	6
KOC-010	*293*/*309*/*311*	*184*/*188*/*216*	*194*/(*194* or *196*)/*196*	10	0	10
KOC-011-1	*307*/(*307* or *311*)/*311*	*182*/*192*/*194*	*188*/(*188* or *194*)/*194*	65	0	65
KOC-011-2	*307*/(*307* or *311*)/*311*	*182*/(*182* or *194*)/*194*	*188*/(*188* or *194*)/*194*	1	0	1
KOC-012	*303*/(*303* or *305*)/*305*	*185*/*186*/*187*	*188*/(*188* or *194*)/*194*	3	0	3
KOC-013	*303*/(*303* or *305*)/*305*	*194*/(*194* or *200*)/*200*	*196*/(*196* or *202*)/*202*	11	0	11
KOC-014	*303*/(*303* or *311*)/*311*	*182*/*184*/*216*	*188*/*192*/*196*	11	0	11
KOC-015	*303*/*313*/*335*	*186*/*192*/*216*	*190*/*194*/*196*	2	0	2
KOC-017	*299*/*303*/*311*	*188*/*192*/*216*	*194*/(*194* or *196*)/*196*	7	0	7

311* in *GF1, **185* / **192*, / **193* in *GF17**, and **188* / (**188* or **194*) / **194* in *GF29**. This clonal line was identified as the clone KOC-001.

Microsatellite DNA polymorphism of wild crucian carp

Microsatellite polymorphism is shown in Fig. 1 as examples of the radiolabeling and chemiluminescence methods. A total of 237 silver crucian carp from natural water were analyzed. The number of alleles per locus, amplified product size range, proportion of heterozygotes, allelic combination and the number of genotypes for the three loci are summarized in Table 1. In the two bands types, the one allele was recognized to be duplicated to the one of two bands. The assumed genotypes of the silver crucian carp are given in Table 2. In *GF1**, **303* and **311* were predominant and appeared in 13 and 10 clonal lines, respectively. In *GF17**, **192* and **216* appeared in seven clonal lines. In *GF29**, **188* and **194* appeared in 11 and 13 clonal lines, respectively.

The observed number of alleles and genotypes were quite large in the three loci comparing with isozyme analysis (Simizu *et al*., 1993; Don *et al*., 1996). The proportion of heterozygotes in the wild population was very high over the three microsatellite loci comparing with gold fish (Zheng *et al*., 1995). The observed number of genotypes was quite small although a large number of alleles was observed. In the results of allelic combinations, the ratio of triallelic types were high in *GF17** (0.734). In *GF1** and *GF29**, the ratio of diallelic types were relatively high with values of 0.747 and 0.626, respectively, compared with isozyme analysis (Simizu *et al*., 1993; Dong *et al*., 1996). Yet, monoallelic type was not observed in this population.

The 16 clonal lines, which were identified by minisatellite DNA fingerprinting, are clearly distinguished based on the genotype in *GF17**. Based on the analysis of each locus, all individuals within each clonal lines except KOC-011 had the same genotype. However, among the 66 individuals of KOC-011, one fish had two alleles (**182* and **194*) while the others had three alleles (**182*, **192*, and **194*) in *GF17**. This fish had the same with the others in genotype of *GF1** and *GF29**.

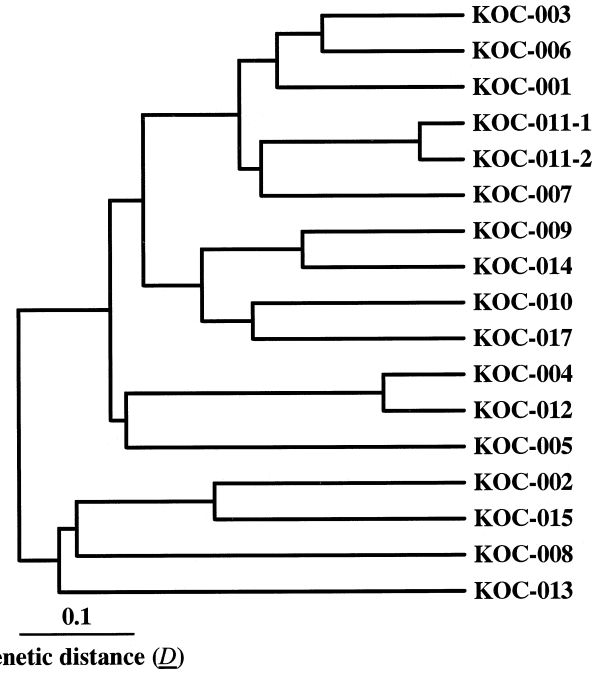

Fig. 2. UPGMA dendrogram drawn on the basis of the genetic distance of three microsatellite loci between each pair of 17 clonal lines in the silver crucian carp, *Carassius langsdorfii*, using PHYLIP ver 3.5.

Thus KOC-011 was subdivided into two clonal lines: one with three alleles (**182*, **192*, and **194*), and the other with two alleles (**182* and **194*), which were designated KOC-011-1 and KOC-011-2, respectively.

The average genetic distance (*D*) between clonal lines was calculated as 0.637±0.191. The *BSI* within clonal lines, except for KOC-011, was equal to 1.000, indicating genetic uniformity. The dendrogram drawn by the UPGMA method based on *D* values is given in Fig. 2. KOC-011-1 and KOC-011-2 had a very close genetic relationship compared to other clones.

DISCUSSION

The analysis of microsatellite DNA loci can be used as a fingerprinting method to distinguish clonal lines. In the loci, information of the zygotic condition of each locus can also be obtained. The maximum allele number of an individual at each microsatellite locus was three as observed in this study. This suggests that the silver crucian carp *C. langsdorfii* is a true triploid. Since the genotypes can be recorded by each locus, in the future, microsatellite DNA loci will be applied to establish a registration system of clonal lines of silver crucian carp.

The proportion of heterozygotes was very high over the three microsatellite loci. Similar results were observed in the electrophoretic analysis (Simizu *et al.*, 1993; Dong *et al.*, 1996; Ohara *et al.*, 1998). The fixed multilocus heterozygosity observed in this study is a characteristic of unisexual-hybrid vertebrates (Vrijenhoek, 1990). The unisexual vertebrates were generally originated from hybridization between congeneric species (Dawley, 1989). The silver crucian carp may be originated from hybridization (Simizu *et al.*, 1993). One explanation for the high proportion of heterozygotes is that during the oogenesis of silver crucian carp, the first meiotic division does not occur, but rather the oocyte undergoes a homotypic nuclear division as in somatic cells, which are called apomixis (Kobayasi, 1976; Yamasita *et al.*, 1993; Arai, 1997). Therefore, they can maintain an almost heterozygous state for each generation within a clonal population. The multilocus heterozygous condition may lead to an increase of fitness in the clonal line of silver crucian carp.

In this study, we detected two subtypes within the clone KOC-011 collected from natural water. Kojima *et al.* (1984) observed the partial pairing of homologue-like chromosomes at the zygote stage in silver crucian carp. Zhang *et al.* (1992) observed the putative recombinant in offspring from a female of the silver crucian carp based on the electrophoretic pattern of isozyme. Two different processes can be considered to explain the origin of the subtype: 1) Synapsis and recombination occurred between homologous chromosomes within the microsatellite locus existed. 2) The microsatellite DNA mutated to a new allele (size changed or null allele). Information on this mechanism is important to understand how genetic diversity is maintained within silver crucian carp populations. Further studies are needed to determine the developing processes of these subtypes.

In this study, we used three microsatellite loci, yet, it is not enough to fully discuss the genetic relationship of the silver crucian carp. It is necessary to investigate many other microsatellite loci. Nonetheless, we found that the microsatellite loci used in this study are very valuable due to their application not only to identify clonal lines of this species, but also to study the origin of the silver crucian carp.

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REFERENCES

Arai K (1997) Biodiversity in fish chromosomes and its applicable potential as genetic resources. Fish Genetics Breed Sci 24: 3–20 (In Japanese)

Brocker AL, Cook D, Bentzen P, Wright JM, Doyle RW (1994) Organization of microsatellite differs between mammals and cold-water teleost fishes. Can J Fish Aquat Sci 51: 1959–1966

Dawley RM (1989) An introduction to unisexual vertebrates. In "Evolution and Ecology Unisexual Vertebrates" , Ed by R M Dawley, J P Bogart, Bull 466, New York State Museum, Albany, NewYork, pp 1–18

Dong S, Taniguchi N (1996) Clonal nature of offspring of ginbuna *Carassius langsdorfii* by RAPD-PCR and isozyme patterns. Nippon Suisan Gakkaishi 62: 891–896 (In Japanese)

Dong S, Taniguchi N, Tsuji S (1996) Identification of clones of ginbuna *Carassius langsdorfii* by DNA fingerprinting and isozyme pattern. Nippon Suisan Gakkaishi 62: 747–753 (In Japanese)

Dong S, Ohara K, Taniguchi N (1997) Introduction of sperm of common carp *Cyprinus carpio* into eggs of ginbuna *Carassius langsdorfii* by heat shock treatment and its confirmation by DNA markers. Nippon Suisan Gakkaishi 63: 201–206 (In Japanese)

Felsenstein J (1994) PHYLIP version 3.5. Department of Genetics, University of Washington, Seattle

Gillbert D, Lehman A, O'Brien SJ, Wayne RK (1990) Genetic Fingerprinting reflects population differentiation in the California Channel Island fox. Nature 344: 764–767

Hosoya K (1993) Fishes of Japan with pictorial keys to the species, Ed by T Nakabo, Tokai university press, Tokyo, pp 212–213 (In Japanese)

Kobayasi H, Kawashima Y, Takeuchi N (1970) Comparative chromosome studies in the genus *Carassius*, especially with a finding of polyploidy in the Ginbuna *C. auratus langsdorfii*. Japan J Ichthyol 17: 153–160

Kobayasi H (1971) A cytological study on gynogenesis of the triploid ginbuna (*Carassius auratus langsdorfii*). Zool Mag 80: 316–322 (In Japanese)

Kobayasi H and Ochi H (1972) Chromosome studies of the hybrids, ginbuna *C. auratus langsdorfii* × kinbuna *C. auratus* subsp., and ginbuna × loach *Misgurnus anguillicaudatus*. Zool Mag 81: 67–71

Kobayasi H (1976) A cytological study on the maturation division in the oogenic process of the triploid ginbuna, *Carassius auratus langsdorfii*. Japan J Icthyol 22: 234–240

Kojima K, Matsumura K, Kawashima M, Kajishima T (1984) Studies on the gametogenesis in polyploid Ginbuna *Carassius auratus langsdorfii*. J Fac Sci Shinshu Univ 19: 37–52

Matsubara K, Ochiai A (1965) Ichthyology Part II. Kouseisha-kouseikaku, Tokyo, pp 530–536 (In Japanese)

Miyadi D, H Kawanabe, N Mizuno (1976) Coloured illustrations of the freshwater fishes of Japan. Hoikusha, Osaka, pp 201–210 (In

Japanese)
Murakami M, Fujitani H (1997) Polyploid-specific repetitive DNA sequences from triploid ginbuna (Japanese silver crucian carp, *Carassius auratus langsdorfii*). Genes Genet Syst 72: 107–113
Murayama Y, Hijikata M, Nomura T, Kajishima T (1984) Analysis of histocompatibility and isozyme variation in triploid fish, *Carassius auratus langsdorfii*. J Fac Sci Shinshu Univ 35: 125–138
Nakakuki M, Toya H, Sawano K, Kajishima T (1984) On the fertilization of the triploid ginbuna. J Fac Sci Shinshu Univ 19: 25–35
Ohara K, Dong S, Taniguchi N (1998) Identification and distribution of clonal lines detected by DNA polymorphism in silver crucian carp, *Carassius langsdorfii* collected from the Monobe and Niyodo rivers. Japan J Ichthyol 45: 21–27 (In Japanese)
Perez-Enriquez R, Takemura M, Taniguchi N (1998) Microsatellite DNA detection by chemiluminescence in red sea bream: a practical manual. Fish Genetics Breed Sci 26: 73–79 (In Japanese)
Sezaki K, Kobayasi H, Nakamura M (1977) Size of erythrocytes in the diploid and triploid specimens of *Carassius auratus langsdorfii*. Japan J Ichthyol 24: 135–140 (In Japanese)
Shaklee JB, Allendorf FW, Moritz DC, Whitt GS (1990) Genetic nomenclature of protein-coding loci in fish: proposed guidelines. Trans Am Fish Soc 119: 2–15
Simizu Y, Oshiro T, Sakaizumi M (1993) Electrophoretic studies of diploid, triploid, and tetraploid forms of the Japanese silver crucian carp, *Carassius auratus langsdorfii*. Japan J Ichtyol 40: 65–75
Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman & Co., San Francisco, pp 114–308
Takagi M, Taniguchi N, Cook D, Doyle RW (1997) Isolation and characterization of microsatellite loci from red sea bream *Pagrus major* and detection in closely related species. Fisheries Sci 63: 199–204
Taniguchi N, Sakata K (1977) Interspecific and intraspecific variations of muscle protein in the the Japanese crucian carp-II. Starch-gel electrophoretic pattern. Japan J Ichthyol 24: 1–11
Umino T, Arai K, Nakagawa H (1996) Growth performance in clonal crucian carp, *Carassius langsdorfii*. Effect of genetic difference and feeding history. Aquaculture 155: 271–283
Umino T, Arai K, Maeda K, Zhang Q, Sakae K, Niwase I, Nakagawa H (1997) Natural clones detected by multilocus DNA fingerprinting in gynogenetic triploid ginbuna *Carassius langsdorfii* in Kurose River, Hiroshima. Fisheries Sci 63: 147–148
Vrijenhoek RC (1990) Genetic diversity and the ecology of asexual population. In “Population Biology and Evolution”, Ed by K Wohrmann S Jain, Springer-Verlag Berlin, pp 195–197
Wetton JH, Caeter RE, Parkin DT, Walters D (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. Nature 327: 147–149
Wright JM, Bentzen P (1994). Microsatellite genetic markers for the future. In “Molecular Genetics in Fisheries”, Ed by GR Carvalho TJ Pitcher, London, pp 117–121
Yamashita M, Onozato H, Nakanishi T, Nagahama Y (1990) Breakdown of the sperm nuclear envelope is a prerequisite for male pronucleus formation: direct evidence from the gynogenetic crucian carp *Carassius auratus langsdorfii*. Devel Biol 137: 155–160
Yamashita M, Jiang J, Onozato H, Nakanishi T, Nagahama Y (1993) A tripolar spindle formed at meiosis I assures the retention of the original ploidy in the gynogenetic triploid crucian carp, ginbuna *Carassius auratus langsdorfii*. Develop Growth and Differ 35: 631–636
Zhang F, Oshiro T, Takashima F (1992) Chromosome synapsis and recombination during meiotic division in gynogenetic triploid Ginbuna, *Carassius auratus langsdorfii*. Japan J Ichthyol 39: 151–155
Zheng W, Stacey NE, Coffin J, Strobeck C (1995) Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus*. Mol Ecol 4: 791–792

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