STUDIES ON GENETIC CHANGES IN COMMONN-AND ORNAMENTAL CARP(Cyprinus carpio),USING MICROSATELLITE DNA MARKERS(マイクロサテライトDNAマーカーによるマゴイおよびニシキゴイの遺伝的多様性の変化に関する研究)

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論 文 内 容 要 旨

STUDIES ON GENETIC CHANGES IN COMMON- AND ORNAMENTAL CARP (*Cyprinus carpio*), USING MICROSATELLITE DNA MARKERS

Common carp, *Cyprinus carpio* is probably the oldest cultures and most domesticated fish species in the world. This species has been recognized not only for human's food consumption but also as an ornamental fish. The numerous varieties of the common carp developed through a combination of forces including geographic isolation, adaptation, accumulation of mutations and naturals as well as human selection pressures.

Genetic study of common carp has been carried out by using several marker types, such as morphological traits (scalation and pigmentation), immunogenetic (histocompatibility antigens), and biochemical markers. However, the low level of polymorphism displayed by those markers in most common carp studied, has limited their uses.

Due to the low variability found in isozyme markers, a study based on more sensitive markers, such as microsatellite DNA, was seen as necessary. Microsatellites are segment of DNA with short repeats of one to four nucleotide sequences. To date, microsatellite markers in fish have been used in population studies, parentage and kinship analysis.

This study was investigated the use of microsatellite DNA markers to detect the genetic changes in wild population, cultured and ornamental strains of common carp, *Cyprinus carpio* with the aim of providing a means for developing proper management policies of this species in terms of conservation and genetic improvement.

1. Isolation of microsatellite DNA markers in common carp

Genomic DNA was extracted from blood samples and digested to completion with 3 restriction enzymes; *Rsa I, Hinc II* and *Hae III*. The DNA was ligated to pUC 18 SmaI/BAP and transformed into *E. coli*. The transformants were selected using ampicillin plates. The colonies were blotted onto hybond-N membranes and hybridized with $(GT)_{15}$ probes. The positive colonies were grown individually. Then their plasmid DNA was extracted, purified and sequenced.

Sixty-eight clones were sequenced, thirty-nine of which contained microsatellites. However, only 4 cloned contained microsatellites gave clear PCR amplification bands. They were *Cca-8*, Cca-17*, Cca-21*, and *Cca-30** (Table 1). The polymorphic of those loci was

tested in 50 individual fish. The number allele detected ranged from 5 to 11 while the heterozygosity ranged from 0.500 to 0.780 (Table 1).

2. The mode of inheritance of microsatellite DNA markers

Four loci, *Cca-8**, *Cca-17**, *Cca-21**, and *Cca-30** were used to detect the inheritance's mode of microsatellite DNA loci in normal progenies of nishikigoi. Furthermore, those loci were used to detect the non paternal inheritance in gynogenetic progenies of nishikigoi and parentage identification of Indonesian common carp.

The inheritance of microsatellite loci in six families of nishikigoi was shown to be codominant mendelian mode (Table 2 and Fig.2). The absence of paternal inheritance could be detected by using microsatellite loci in gynogenetic progenies. The recombination rates between loci and their centromere could be estimated by using gynogenetic fish. The rate was between 0.237 and 0.918 with an average 0.506, while the gene-centromere distance was between 12 cM and 46 cM with an average 25 cM (Table 3). Microsatellite markers were shown to be effective in parentage identification. The genotypes of parents were successfully expected from the alleles detected in their progenies.

3. Genetic variability in wild population of common carp

Three microsatellite loci, *Cca-17**, *Cca-21**, and *Cca-30** were applied to detect the genetic variability in the wild stock of common carp. Twenty-four individuals were collected from Shimanto River, Kochi Prefecture. The result showed that 6 alleles were detected and gave the heterozygosity (He) 0.778 (Table 5). Based on the genetic variability detected, it was shown that microsatellite markers were more sensitive than isozyme markers.

4. Genetic variability in recognized races of Indonesian common carp

Nine races of Indonesian common carp are recognized among fish farmers. They are Cangkringan, Mirror, Sinyonya, Jember, Sutisna, Majalaya, Punten, Rajadanu, and Wildan. Those samples were collected from the Research Agency of Freshwater Fisheries (RAFF), the Agency for Freshwater Aquaculture Development Center (AFACD), and government hatcheries at East Java province. Their genetic variability were analyzed by using four microsatellite loci, *Cca-8**, *Cca-17**, *Cca-21**, and *Cca-30**. The average number of alleles

detected was 5.19 and the average heterozygosity (He) was 0.656 (Table 5), while the genetic diversity was 0.292.

5. Genetic variability in hatchery stocks of Indonesian common carp

The genetic variability in hatchery stocks of Indonesian common carp from 6 locations were surveyed using four loci of microsatellite markers. Those locations were West Sumatra, North Sumatra, West Java (Bandung, Sukabumi, and Bogor), and East Java. Fish samples were obtained from both government and private hatcheries.

The average number of alleles and heterozygosity detected were 7.54 and 0.720, respectively (Table 6), higher than those in nine recognized stock of Indonesian common carp. The genetic diversity, however, was found lower (0.138) than that of nine recognized stocks indicating that hatchery stock was consisted of mixed population while a recognized stock was to be a fixed population.

6. Genetic variability in nishikigoi stocks from fish farms at Niigata Prefecture

Genetic variability in the most popular varieties of nishikigoi, such as Kouhaku, Taisho and Showa were observed by using 4 microsatellite DNA loci. Fish samples were collected from fish farms in Yamakoshi village, Niigata Prefecture and Niigata Prefectural Inlandwater Fisheries Experimental Station.

The average number of alleles and heterozygosity were 4.08 and 0.440, respectively (Table 7), while the genetic diversity was 0.093. The excess of homozygosity was observed at several loci surveyed, indicating that inbreeding is taken places.

7. Genetic changes in wild population, recognized races, hatchery stocks and nishikigoi

Genetic changes in wild population, hatchery stocks, races and ornament of common carp were compared, based on three loci, *Cca-17*, Cca-21*,* and *Cca-30**. Based on allele and allele frequency detected in those loci, it was shown that wild population and ornamental strain were separated from Indonesian common carp (Fig. 3). The average number of alleles detected in wild population and hatchery stocks were higher than that of nine races and ornamental strain (Fig. 4). The average of heterozygosity (He) in wild population was higher than that of hatchery stocks and nine races, however, that of ornamental strain was

the lowest (Fig. 5). The genetic diversity of nine races (0.292) was found higher than that of hatchery stocks (0.138) and ornamental strain (0.093) (Fig. 6).

The genetic changes in 4 groups were well related with their genetic background (Table 8). The lower number alleles detected in wild population compared to hatchery stocks might be due to the small number of samples used in wild population. However, the random mating occurred and large founder in wild population cause the high heterozygosity. Both hatchery stocks and nine recognized had a large founder, however, non random mating resulted its heterozygosity lower than wild population. The low allele number found in nine races indicated that those races came from selection activities. The non random mating and small founder in ornamental strain made its heterozygosity was the lowest. The higher of genetic diversity found in nine recognized races indicating that they have a larger of based population size compared to hatchery stocks, and ornamental strain. Small based population size of ornamental strain might be caused of its originated from color mutant of common carp.

Considering the evaluation of their genetic variability, suggestions and recommendations were made for their proper management policies in terms for conservation and genetic improvement (Table 9). (1) Wild type of Japanese common carp probable has become to be an endangered species, based on their genetic variability evaluation. Therefore, the repeated genetic survey is suggested to be done with more samples and locations to provide a proper management policy of this species in the term for conservation of Japan inlandwater fisheries. (2) To improve the genetic variability in nine recognized stocks, out-breeding within race must be undertaken, besides of observation of their commercial traits. In the future, its possible to use those races as hatchery stocks. (3) To maintain the economic traits and to prevent the inbreeding depression of hatchery stocks, mass-selection should be done using low selection intensity because those stocks have already had a good performance. Furthermore, extension program of broodstock management should be given to hatchery managers. (4) The lack of precise genetic data about color inheritance in ornamental fish makes the out-breeding activities is difficult to be undertaken for improving its physiological traits and genetic variability. The mode of color inheritance could be solved if biotechnological approaches are used, such as mapping QTL (Quantitative Trait Loci) for controlling color pattern and chromosome manipulated fish.

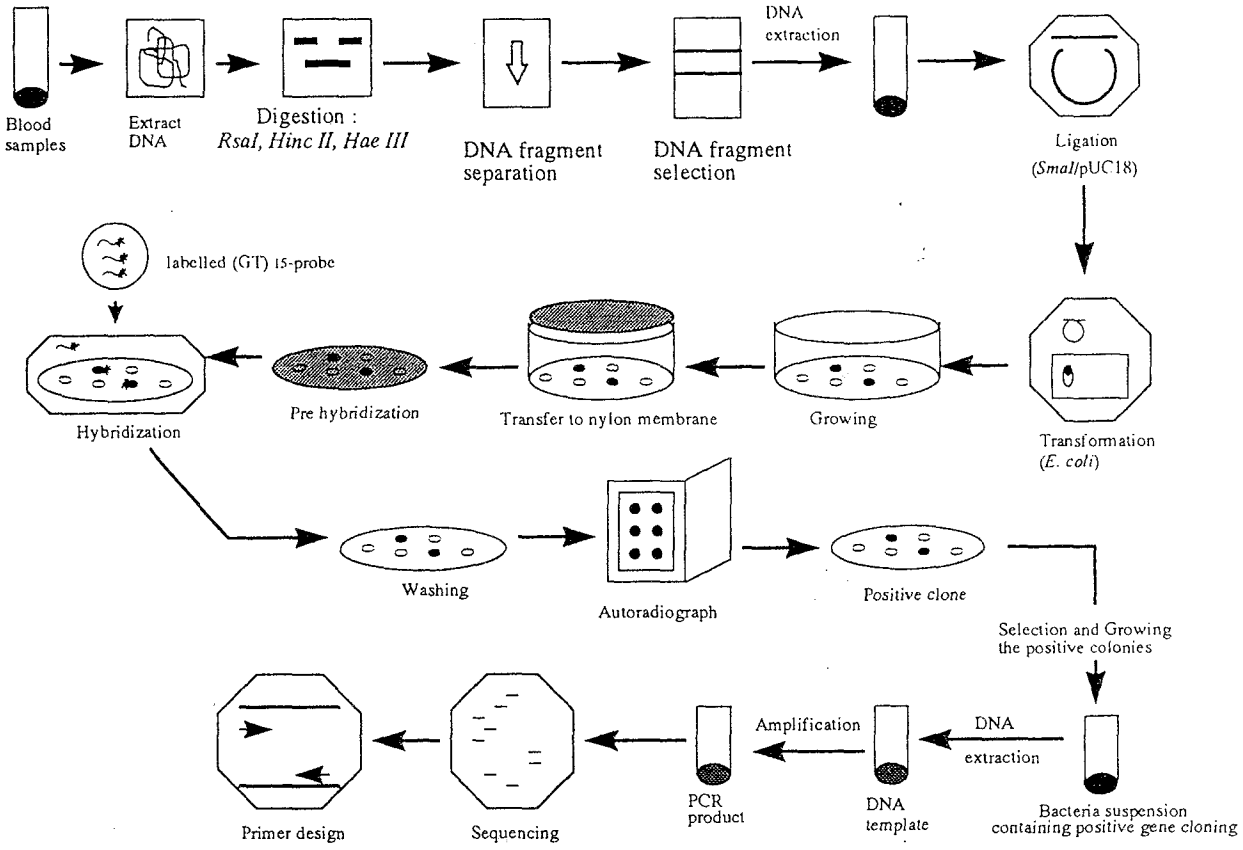

Fig. 1 Schematic illustration of isolation of microsatellite DNA loci.

Table 1. Nucleotide sequence of common carp microsatellites and primers for amplification of loci by PCR

Locus	Sequence 5' → 3'	Annealing temperature (°C)	No. of alleles	Size (bp)	Heterozygosity
*Cca-8**	$(GT)_{27}$				
	F*1 GGCTGTTTTACCTCTGTGAA	52	11	200 - 244	0.740
	R*2 AAATAACTTTGGACTGCT				
*Cca-17**	$(T)_{25}$				
	F*1 CAACACATAACCGCATTTCT	47	6	177- 187	0.620
	R*2 CTTTCTTGTGATTGACCTCC				
*Cca-21**	$(GT)_{11}$				
	F AAGGTGAGTGTGACGACGG	53	5	67 - 77	0.780
	R AGCACAAACACACGGTCAG				
*Cca-30**	$(GT)_{18}+(GA)_{20}$				
	F CTGCCTTCTTCTACTCTACAC	50	10	256- 290	0.500
	R TTGCCTCTATGCTTGATTTT				

*1 Forward primer. *2 Reverse primer.

Table 2. Genotypes of nishikigoi parents and their normal progenies at four microsatellite loci

Locus	Family	Parental Genotype		F1 offspring genotype				n
		Female	Male	Observed number *1(expected in each class)				
*Cca-8**	1	*230/230	*230/230	*230/230				
				22 (22)				22
	2	*220/220	*220/220	*220/220				
				22 (22)				22
	3	*228/242	*220/228	*220/228	*228/228	*220/242	*228/242	
				6 (5.5)	5 (5.5)	6 (5.5)	5 (5.5)	22
	4	*228/220	*242/242	*228/242	*220/242			
				14 (11)	8 (11)			22
	5	*232/232	*242/242	*232/242				
				22 (22)				22
	6	*222/222	*242/242	*222/242				
				22 (22)				22
*Cca-17**	1	*185/185	*185/185	*185/185				
				20 (20)				20
	2	*185/185	*185/185	*185/185				
				21 (21)				21
	3	*185/185	*185/185	*185/185				
				21 (21)				21
	4	*185/185	*185/185	*185/185				
				20 (20)				20
	5	*185/185	*185/185	*185/185				
				21 (21)				21
	6	*185/185	*185/185	*185/185				
				22 (22)				22
*Cca-21**	1	*75/79	*75/75	*75/75	*75/79			
				11(10)	9(10)			20
	2	*77/79	*79/79	*77/79	*79/79			
				9(11)	13(11)			22
	3	*79/79	*75/79	*75/79	*79/79			
				8(11)	14(11)			22
	4	*79/79	*77/77	*77/79				
				22(22)				22
	5	*75/79	*77/77	*75/77	*77/79			
				10(11)	12(11)			22
	6	*75/79	*77/77	*75/77	*77/79			
				13(11)	9(11)			22
*Cca-30**	1	*288/300	*288/318	*288/288	*288/318	*288/300	*300/318	
				6(5.5)	5(5.5)	5(5.5)	6(5.5)	22
	2	*288/318	*288/318	*288/288	*288/318	*318/318		
				3(5.5)	14(11)	3(5.5)		22
	3	*288/318	*288/318	*288/288	*288/318	*318/318		
				7(5)	9(10)	4(5)		20
	4	*288/288	*288/318	*288/288	*288/318			
				15(11)	7(11)			22
	5	*288/318	*288/318	*288/288	*288/318	*318/318		
				8(5.5)	7(11)	7(5.5)		22
	6	*288/288	*288/318	*288/288	*288/318			
				9(10.5)	12(10.5)			21

*1 Non significant at P<0.05.

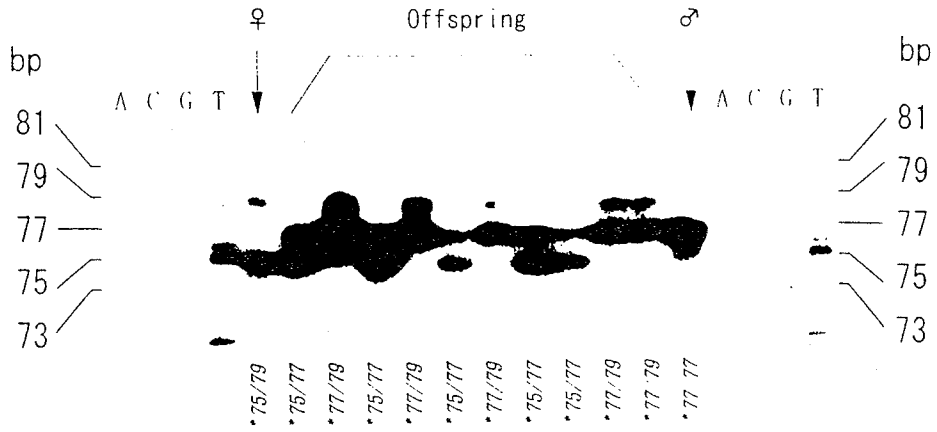

Fig. 2 Microsatellite alleles of nishikigoi and its F1 offspring at locus Cca-21* (lane 1 : female parent, lane 2 - 11 : F1 offspring, lane 12 : male parent). The size standard is a sequencing ladder of M13 mp18.

Table 3. Progeny genotypes of meiotic and mitotic gynogenetic nishigoi at 3 microsatellite loci

Locus	Family	Maternal genotype	No. of fish observed	Progeny genotypes[*1)] 11	12	22	y[*2)]	SD	G-C map distances (cM)
*Cca-8**	3 - G2NA	**228/242*	144	50	41	53			
	4 - G2NA	**220/228*	139	48	26	65	0.285		
				98	67	118		0.07	12
							0.187		
	3 - G2NB	**220/242*	32	16		6			
	4 - G2NB	**220/228*	13	6		5	0.236		
*Cca-21**	5 - G2NA	**75/79*	104	7	95	2			
	6 - G2NA	**75/79*	66	2	61	3	0.913		
				9	156	5		0.01	46
							0.924		
	5 - G2NB	**75/79*	13	5		8			
							0.919		
*Cca-30**	3 - G2NA	**288/318*	144	36	59	49			
	5 - G2NA	**288/318*	104	34	31	39	0.410		
				70	90	88		0.08	18
							0.298		
	3 - G2NB	**288/318*	32	15		17			
	5 - G2NB	**288/318*	13	6		7	0.354		

[*1)] 11 and 22 are homozygote for the first and second maternal alleles, respectively
[*2)] post-reduction rates/gene-centromere recombination rates

Table 4. Genetic variability of three microsatellite loci in the wild type of common carp

	*Cca-17**	*Cca-21**	*Cca-30**	Mean
No. of samples	24	23	24	
No. of alleles	5	6	7	6.0
Heterozygosity (Ho)	0.638	0.933	0.978	0.850
(He)	0.629	0.797	0.719	0.715
(Ho/He)	1.014	1.171	1.360	1.189

Table 5. Genetic variability of 4 microsatellite loci, *Cca-8**, *Cca-17**, *Cca-21**, and *Cca-30** in nine recognized stocks of Indonesian common carp

	*Cca-8**	*Cca-17**	*Cca-21**	*Cca-30**	Mean (within population)
Cangkringan-1					
No. of samples	15	15	15	15	
No. of alleles	6	3	4	6	4.75
Effective no. of alleles	3.81	2.11	3.17	3.63	3.18
Heterozygosity (Ho)	0.467	0.400	0.800	0.667	0.583
(He)	0.738	0.527	0.684	0.724	0.668
(Ho/He)	0.633**	0.759	1.169	0.920**	0.873
Cangkringan-2					
No. of samples	18	18	18	18	
No. of alleles	7	4	4	6	5.25
Effective no. of alleles	3.16	1.92	3.58	2.50	2.79
Heterozygosity (Ho)	0.500	0.500	0.944	0.611	0.639
(He)	0.684	0.480	0.721	0.600	0.621
(Ho/He)	0.731**	1.042	1.310	1.018	1.029
Cangkringan (Pooled)					
No. of samples	33	33	33	33	
No. of alleles	8	5	4	7	6
Effective no. of alleles	3.97	2.20	3.41	3.03	3.15
Heterozygosity (Ho)	0.485	0.455	0.879	0.636	0.613
(He)	0.748	0.546	0.707	0.670	0.668
(Ho/He)	0.648**	0.833	1.243	0.949	0.917
Mirror					
No. of samples	18	18	18	18	
No. of alleles	5	3	5	7	5.00
Effective no. of alleles	2.59	1.63	4.53	2.47	2.81
Heterozygosity (Ho)	0.889	0.500	0.944	0.500	0.708
(He)	0.614	0.387	0.779	0.596	0.594
(Ho/He)	1.447**	1.291	1.212	0.839	1.192
Sinyonya					
No. of samples	18	18	18	18	
No. of alleles	5	3	5	9	5.50
Effective no. of alleles	3.66	1.71	2.68	5.89	3.49
Heterozygosity (Ho)	0.667	0.278	0.500	0.889	0.584
(He)	0.727	0.415	0.627	0.830	0.650
(Ho/He)	0.917	0.669	0.798	1.071	0.898
Majalaya					
No. of samples	18	18	18	18	
No. of alleles	4	5	4	6	4.75
Effective no. of alleles	2.53	2.31	3.62	4.18	3.16
Heterozygosity (Ho)	0.667	0.667	0.944	0.556	0.709
(He)	0.605	0.568	0.724	0.761	0.664
(Ho/He)	1.102**	1.174	1.304	0.731**	1.068
Jember					
No. of samples	13	13	13	13	
No. of alleles	5	5	5	6	5.25
Effective no. of alleles	3.76	1.81	3.98	3.76	3.33
Heterozygosity (Ho)	0.692	0.385	0.692	0.846	0.654
(He)	0.734	0.447	0.749	0.734	0.666
(Ho/He)	0.944	0.861	0.925	1.153	0.982

continued..... Rajadanu

Table 5. Cont.

Rajadanu					
No. of samples	13	13	13	13	
No. of alleles	4	2	3	3	3.00
Effective no. of alleles	2.10	1.99	2.18	2.00	2.07
Heterozygosity (Ho)	0.462	0.615	0.692	0.231	0.500
(He)	0.524	0.497	0.541	0.500	0.516
(Ho/He)	0.881	1.237	1.279	0.462**	0.969
Sutisna					
No. of samples	13	13	13	13	
No. of alleles	5	4	3	4	4.00
Effective no. of alleles	3.07	3.76	2.96	2.77	3.14
Heterozygosity (Ho)	0.385	0.615	1.000	0.308	0.577
(He)	0.675	0.734	0.663	0.639	0.678
(Ho/He)	0.570**	0.839	1.509	0.482**	0.851
Wildan					
No. of samples	13	13	13	13	
No. of alleles	6	5	5	5	5.25
Effective no. of alleles	3.84	4.69	2.86	2.77	3.54
Heterozygosity (Ho)	0.692	0.384	0.769	0.462	0.577
(He)	0.740	0.787	0.651	0.639	0.704
(Ho/He)	0.936	0.489**	1.182	0.722	0.820
Punten-1					
No. of samples	24	24	24	24	
No. of alleles	7	5	3	7	5.50
Effective no. of alleles	3.96	3.19	2.52	2.91	3.15
Heterozygosity (Ho)	0.625	0.333	0.667	0.625	0.563
(He)	0.747	0.688	0.603	0.656	0.674
(Ho/He)	0.836	0.484**	1.105	0.952**	0.835
Punten-2					
No. of samples	10	10	10	10	
No. of alleles	8	3	3	6	5.00
Effective no. of alleles	5.88	2.47	2.53	2.78	3.42
Heterozygosity (Ho)	0.700	0.500	0.800	0.700	0.675
(He)	0.830	0.595	0.605	0.640	0.668
(Ho/He)	0.843**	0.840	1.322	1.094	1.010
Punten-3					
No. of samples	23	23	23	23	
No. of alleles	6	4	5	8	5.75
Effective no. of alleles	5.02	3.36	2.78	3.83	3.74
Heterozygosity (Ho)	0.435	0.391	0.826	0.565	0.554
(He)	0.801	0.702	0.641	0.739	0.721
(Ho/He)	0.543**	0.557**	1.289	0.765**	0.768
Punten-4					
No. of samples	26	26	26	26	
No. of alleles	10	3	5	8	6.50
Effective no. of alleles	6.76	2.05	3.34	4.46	4.15
Heterozygosity (Ho)	0.615	0.308	0.846	0.565	0.584
(He)	0.852	0.512	0.700	0.776	0.710
(Ho/He)	0.722**	0.601**	1.208	0.728**	0.823
Punten (Pooled)					
No. of samples					
No. of alleles	83	83	83	83	
Effective no. of alleles	11	5	5	11	8
Heterozygosity (Ho)	6.01	2.92	2.91	3.78	3.91
(He)	0.578	0.361	0.735	0.605	0.570
(Ho/He)	0.834	0.658	0.656	0.734	0.721
	0.694**	0.550**	1.120	0.824**	0.791

**departure from Hardy-Weinberg equilibrium ($P<0.05$)

Table 6. Genetic variability of 4 microsatellite loci, *Cca-8**, *Cca-17**, *Cca-21** and *Cca-30** in hatchery stocks collected from 6 locations

	*Cca-8**	*Cca-17**	*Cca-21**	*Cca-30**	Mean (within population)
West Sumatra					
No. of samples	66	66	66	66	
No. of alleles	7	5	6	14	8.00
Effective no. of alleles	4.20	1.93	2.39	6.49	3.75
Heterozygosity (Ho)	0.394	0.182	0.591	0.591	0.440
(He)	0.762	0.482	0.581	0.846	0.668
(Ho/He)	0.517**	0.378**	1.018	0.699**	0.658
North Sumatra					
No. of samples	58	58	58	58	
No. of alleles	8	4	4	11	6.75
Effective no. of alleles	4.31	1.89	2.94	3.60	3.19
Heterozygosity (Ho)	0.586	0.397	0.690	0.466	0.535
(He)	0.768	0.470	0.660	0.722	0.653
(Ho/He)	0.763**	0.844**	1.062	0.645**	0.820
Sukabumi					
No. of samples	34	34	34	34	
No. of alleles	9	6	6	8	7.25
Effective no. of alleles	5.05	3.11	4.65	4.50	4.33
Heterozygosity (Ho)	0.647	0.529	0.882	0.706	0.691
(He)	0.790	0.668	0.773	0.767	0.750
(Ho/He)	0.819**	0.792**	1.141**	0.920	0.921
Bogor					
No. of samples	38	38	38	38	
No. of alleles	10	6	4	10	7.50
Effective no. of alleles	6.29	3.86	3.55	5.26	4.74
Heterozygosity (Ho)	0.632	0.711	0.896	0.710	0.738
(He)	0.830	0.731	0.709	0.799	0.767
(Ho/He)	0.761**	0.971**	1.264**	0.890	0.962
Bandung					
No. of samples	39	39	39	39	
No. of alleles	7	6	6	11	7.50
Effective no. of alleles	5.88	4.20	3.18	5.32	4.65
Heterozygosity (Ho)	0.821	0.539	0.641	0.410	0.603
(He)	0.819	0.752	0.677	0.802	0.762
(Ho/He)	1.002	0.717**	0.947	0.511**	0.791
East Java					
No. of samples	97	97	97	97	
No. of alleles	12	5	5	11	8.25
Effective no. of alleles	5.62	3.09	2.85	3.83	3.85
Heterozygosity (Ho)	0.598	0.361	0.784	0.567	0.578
(He)	0.822	0.676	0.649	0.739	0.722
(Ho/He)	0.727**	0.534**	1.208**	0.767**	0.809

** departure from Hardy-Weinberg equilibrium ($P<0.05$)

Table 7. Genetic variability of 4 microsatellite loci, *Cca-8**, *Cca-17**, *Cca-21** and *Cca-30** in nishikigoi.

	*Cca-8**	*Cca-17**	*Cca-21**	*Cca-30**	Mean (within population)
Kouhaku					
No. of samples	46	46	46	46	
No. of alleles	7	1	5	5	4.5
Effective no. of alleles	3.04	1	2.99	2.10	2.28
Heterozygosity (Ho)	0.630	0	0.630	0.565	0.456
(He)	0.671	0	0.665	0.525	0.465
(Ho/He)	0.939	-	0.947	1.077	0.981
Taisho					
No. of samples	53	53	53	53	
No. of alleles	6	1	4	7	4.5
Effective no. of alleles	1.96	1	2.74	2.04	1.94
Heterozygosity (Ho)	0.245	0	0.660	0.453	0.340
(He)	0.490	0	0.636	0.511	0.409
(Ho/He)	0.500**	-	1.039	0.887**	0.831
Showa					
No. of samples	30	30	30	30	
No. of alleles	5	1	3	4	3.25
Effective no. of alleles	2.77	1	2.23	2.44	2.11
Heterozygosity (Ho)	0.433	0	0.467	0.400	0.325
(He)	0.637	0	0.551	0.591	0.445
(Ho/He)	0.680**	-	0.847	0.677**	0.770
Miscellaneous					
No. of samples	25	25	25	25	
No. of alleles	9	1	4	7	5.25
Effective no. of alleles	4.64	1	3.15	2.84	2.91
Heterozygosity (Ho)	0.525	0	0.440	0.640	0.401
(He)	0.785	0	0.682	0.648	0.529
(Ho/He)	0.668**	-	0.645**	0.988	0.758

Departure from HWE (P<0.05), locus *Cca-17 was not included in the HWE test.

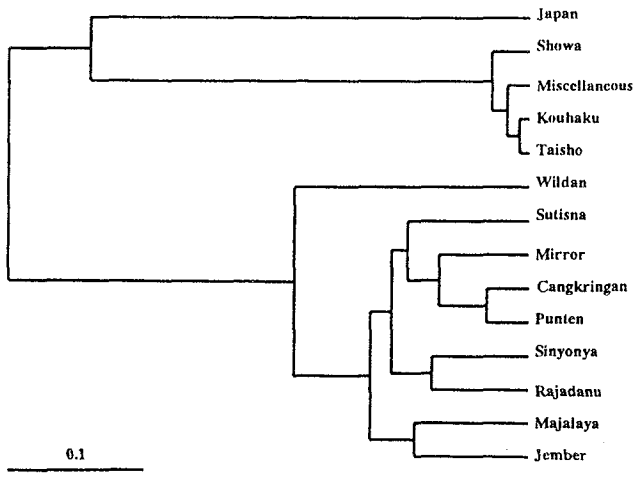

Fig. 3 UPGMA dendrogram based on Nei's genetic distance of 3 microsatellite loci, *Cca-17**, *Cca-21**, and *Cca-30** among Japanese wild stock, nishikigoi, and recognized races of Indonesian common carp.

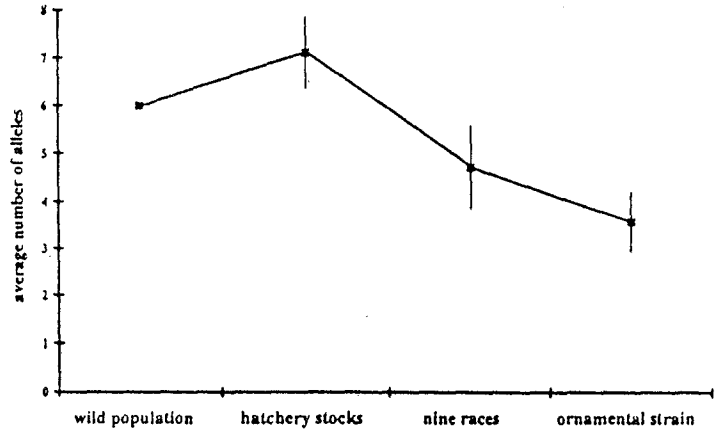

Fig. 4 Genetic changes in the average of alleles in wild population, hatchery stocks, nine races, and ornamental strain of common carp.

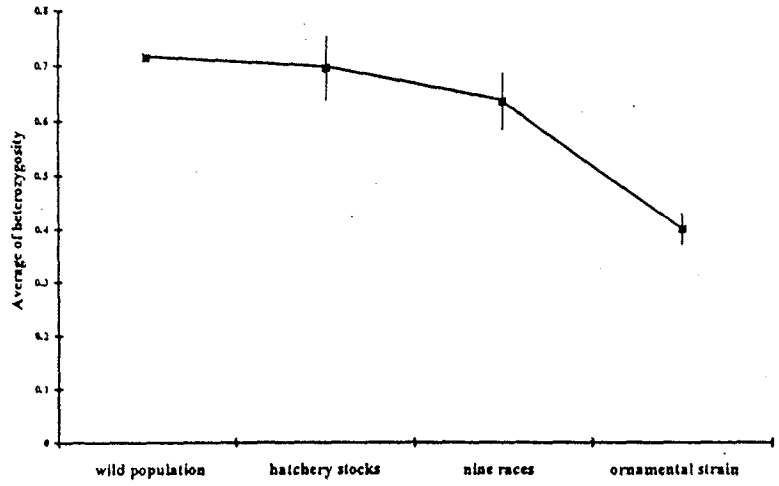

Fig. 5 Genetic changes in the average of heterozygosity in wild population, hatchery stocks, nine races, and ornamental strain of common carp.

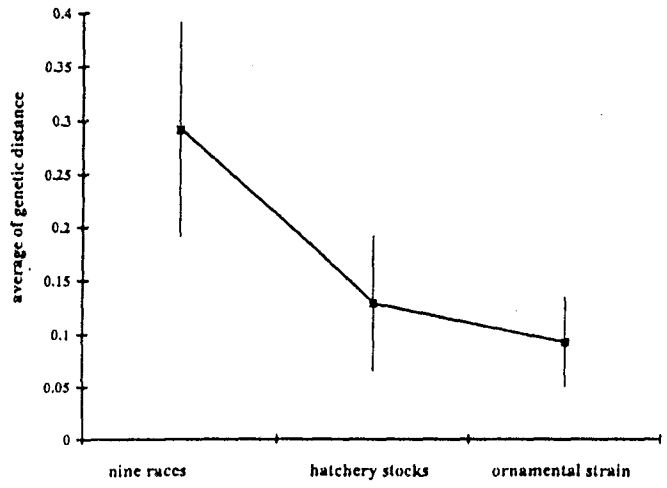

Fig. 6 Changes in genetic diversity of nine races, hatchery stocks, and ornamental strain.

Table 8. Evaluation of genetic variability in 4 different groups

Population	Source	Selection methods	Selection intensity	Founder	Assumed of base pop. size	Genetic variability		Genetic diversity
						Av. no. of alleles	Average of heterozygosity	
1. Wild pop.	Japan	natural	natural	Jap.wild com. carp	Small	Med ?	High	-
2. Recog. races	Indonesia	mass selection	high	China, Dutch, Germany	Large	Low	Lower	Large
3. Hatchery stocks	Indonesia	cross-breeding	low	Majalaya, Sinyonya, Punten	Large	High	High	Relatively small
4. Nishikigoi	Japan	family selection by color mutant	extremely high	Jap.wild com. carp	Small	Low	Very low	Small

Table 9. Comments and suggestions based on the evaluation of genetic variability in 4 different groups

Population	Genetic variability		Genetic Diversity	Comments and suggestions
	Avg. no. of alleles	Avg. of Heterozygosity		
1. Wild population	Med. ?	High	-	- Endangered species. - Repeated genetic survey is suggested to be done with more samples and more locations to provide proper management policies of this species in the term of conservation of Japan inlandwater fisheries.
2. Recognized races	Low	Low	Large	- Genetic variability should be increased by out-breeding within a race without doing cross-breeding between races.
3. Hatchery stocks	High	High	Relatively small	- To maintain the economic traits and to prevent the inbreeding depression, mass-selection should be done using low selection intensity because of those stocks has already had a good performance. - Extension program of broodstock management should be given to hatchery managers.
4. Nishikigoi	Low	Very low	Small	- Mode of color inheritance should be cleared up before doing crossbreeding for improving physiological traits and increasing genetic variability. - Mode of color inheritance could be detected by using biotechnology approaches such as mapping QTL(Quantitative Trait Loci) those controlling color patterns and chromosome manipulated fish.

論文審査結果要旨

マゴイ(*Cyprinus carpio*)は世界で最も古い養殖種で，最も家魚化の進んだ魚種である。本種は食用魚としてだけでなく，鑑賞魚としても重要視されてきた。本種において，生産性や品質を高めるため多くの国において選択育種が行われてきたが，近交係数の上昇に伴う近交弱勢の発現がうたがわれている。

本研究は，マゴイ諸系統の遺伝的改良と保全のための適切な親魚集団の管理指針を提起することを目的としている。このため，高感度マーカーとして知られる核DNAのマイクロサテライト(MS)領域の検出法を開発し，マゴイ野生集団，養殖集団および観賞魚集団における集団内および集団間の遺伝的多様性のレベルを定量し，それらの変化の実態解明を試みたものである。

第1章では，マゴイのマイクロサテライトDNAマーカー開発のため，ゲノムDNAライブラリーから，68のクローンの塩基配列を検出し，39のクローンにMS領域を確認し，この領域の増幅が可能な4つのプライマーセット(*Cca-8*, Cca-17*, Cca-21, and Cca-30**)を設計している。第2章では，マイクロサテライトDNAマーカーの遺伝様式がいずれもメンデルの法則に従うこと，さらにこれらが，親子鑑定に応用可能であることを確認している。

第3章では，高知県西部の四万十川産のマゴイ野生集団の遺伝的多様性を，第4章では，インドネシア政府機関において継代保存されてきた諸品種の遺伝的多様性を定量評価している。ここでは，マイクロサテライトDNAマーカーにおけるマゴイの遺伝的多様性レベルが，他の淡水魚や海産魚にくらべ明らかに低いこと，さらには，継代保存諸品種の遺伝的多様性が野生集団のそれにくらべ低いことを示し，インドネシアには長年の継代飼育により品種として確立されたものが存在することを示した。第5章では，民間の養殖用種苗生産施設の親魚群における遺伝的多様性が，継代保存諸品種のそれらに比べ高いことを明らかにした。一方，養殖用種苗生産施設間の遺伝的距離の平均値は継代保存諸品種間の平均値にくらべ小さかったことから，ここで使われている親魚集団が複数の継代保存品種の遺伝的混合群である可能性を示唆している。

第6章では，新潟県のニシキゴイの主生産地である山古志村の養魚家および新潟県内水面水産試験場で採卵用親魚として保存されている紅白，大正三色，昭和三色からDNA検出用サンプルを採集し，遺伝的多様性を評価したところ，インドネシアの養殖品種にくらべ変異水準は明らかに低く，遺伝的距離の平均値は保存品種や種苗生産用親魚における集団間の平均値に比べかなり小さかった。このようにニシキゴイの遺伝的多様性が低いのは，本種の品種改良が色彩変異に限った特殊な家系選択により実施されてきた育種の歴史的背景に起因するものと考えられた。また，遺伝的変異性および系統間差が明らかに小さくなっていたことから，ニシキゴイ集団における近交係数の上昇および創始集団のサイズが小さかったことが示唆された。

本研究において，マゴイの野生集団，継代保存品種，種苗生産用親魚集団および観賞魚(ニシキゴイ)集団の遺伝的多様性の現状を明らかにしただけでなく，それらの利用と保全の立場から，それぞれの集団の親魚管理指針を提言した。また，水産生物の集団遺伝学的研究の意義を再確認させるとともに，種々のマゴイ養殖の発展に遺伝学的側面から寄与するものと考えられる。よって，審査委員一同は本論文の著者を博士(農学)の学位を授与するに値するものと判定した。