Genetic Characterization of Cultured Populations of Japanese Common Carp

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Julie M. MACARANAS, Junichi SATO, and Yoshihisa FUJIO

Department of Fishery Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Summary

Twelve cultured populations belonging to 2 races (Yamato and Asagi) of the Japanese common carp, *Cyprinus carpio*, were electrophoretically analyzed at 24 gene loci. Nei's D values revealed substantial genetic differentiation of 2 populations of the Yamato race which originated from a small effective number of parents, and which could be attributed to random genetic drift. Deviations from Hardy-Weinberg expectations indicate a general trend of excess heterozygotes in the cultured populations which may also be an effect of random genetic drift or of heterosis in the process of artificial selection.

The culture of the common carp dates as far back as 3,000 to 4,000 years in the Far East and at least 600 years in Europe (1). Carp culture began to be introduced in various areas of Japan around 1850 after the Meiji period (2). Several Japanese races of the common carp are known, those with the greatest productivity being Yamato, Shinshu, and Asagi (3) which demonstrated facility of crossing with the European and Chinese races and had high fertility. Comparison among various races, i.e., mirror carp, German scaly carp, Yamato carp, Asagi carp, and Japanese wild carp yields significant differences in growth and survival rates under running water and standing water ponds (4). Moreover, meristic characterization of interracial hybrids of the Japanese, Chinese, and European races revealed heterosis in their F1 hybrids (5).

Isozyme markers in carp culture have been used to identify species differences from other cyprinids (6, 7, 8) as well as suitable gene markers for breeding purposes (9, 10, 11, 12, 13). To identify or characterize the genetic relatedness between the Japanese and German races, Kosaka *et al.* (14) investigated 11 isozyme systems coded by 30 gene loci. One of the great potentials of biochemical markers is their applicability in the quantitative estimation of the amount of genetic differentiation between populations at the intraspecific level. Electrophoretic data provide an understanding of the genetic variability patterns within

the populations. Using isozyme markers, this study attempts to measure the genetic differences among cultured populations of the Japanese common carp and to discuss their population structure as presumably influenced by artificial selection.

Materials and Methods

Twelve cultured populations of Japanese common carp, claimed to be of the Yamato or Asagi race, were sampled in 1980 and 1985 (see Table 1).
Five populations came from fisheries experimental stations while the rest are from private farms. The samples were analyzed electrophoretically for allelic variation at 9 enzyme systems encoded by at least 24 gene loci. Pair-wise genetic distance values (D) among the 12 cultured populations were calculated using Nei' s formula (15) and a dendrogram was constructed from the D values using the UPGMA method of Sokal and Sneath (16).

To measure genetic variation within each population, observed heterozygosity (Ho) was calculated by direct count of heterozygous phenotypes per locus and averaging them over all loci per population. Expected heterozygosity (He) was calculated from the equation: $h=1-\Sigma x_i^2$, where x_i denotes the frequency of the ith allele, also averaged over all loci per population. The proportion of polymorphic loci (P) is the ratio of polymorphic loci over the total number

TABLE 1. *Collection Data on Cultured Populations of Japanese Carp, Cyprinus carpio*

Race	Cultured population	Date of birth	No. of fishes	Origin
Yamato	Yoshokuken	1978	29	maintained from the original Saku strain which has undergone intensive selection with a limited number of parents
	Miyagi(Gamoh)	1979	30	unknown parental strain ; some were claimed to have come from Lake Kasumigaura
	Fukushima	1979	60	origin unknown but all samples were derived from only one spawning female
	Niigata(Uonuma)	1979	50	originally cultured in Uonuma
	Nagano(Shioda)	1979	51	origin unknown but claimed to be of Saku strain
	Nagano(Saku)	1979	66	originally cultured in Tsuginoki, Saku city
	Nagano(Iida)	1979	63	cultured strain from Lake Suwa
	Shiga	1979	51	originally cultured in Ohtsu city ; parents were Ohmi strain males and Saku strain females
	Aomori	1984	25	origin unknown
	Akita	1985	49	origin unknown
	Yamagata	1983	30	origin unknown
Asagi	Niigata	1979	47	originally cultured in Muramatsu, Nakakamuhara-gun, Niigata

of loci, where a locus was considered polymorphic if the frequency of the most common allele was no greater than 0.95. The populations were tested for goodness of fit to Hardy-Weinberg's proportions by Chi-square test and deviations from Hardy-Weinberg's expectations obtained from the equation: $d=(Ho-He)/He$. A positive value for d would mean an excess of heterozygotes for any population.

Results

Out of the 24 gene loci scored, 7 were found to be polymorphic, namely, *Aat-1*, *αGpd-2*, *Gpi-2*, *Gpi-4*, *Ldh-B1*, *Ldh-C1*, and *Pgm*. The allelic expressions of the polymorphic loci are shown in Fig. 1. The enzymes AAT, αGPD, GPI, IDH, LDH, MDH, 6-PGD, and SOD had duplicated loci. PGM was strongly expressed as a single locus in muscle but may possibly be duplicated as evidenced by the presence of a slower, lightly-staining zone which did not co-migrate with any of the other enzymes stained. AAT was expressed as 2 loci in muscle, the anodal locus being polymorphic for 2 alleles. αGPD in muscle consisted of the duplicated loci, *αGpd-1* and *αGpd-2*, with the monomorphic locus (*αGpd-2*) sandwiched between alleles *A* and *B* of *αGpd-1*; the formation of heterodimers between both loci resulted in 3-banded patterns for the homozygotes and 6-banded patterns for the heterozygotes. GPI in muscle had the most number of loci and also the highest number of alleles in one locus. The more anodal duplicated loci *Gpi-1* and *Gpi-2* consisted of one monomorphic locus (*Gpi-1*); *Gpi-2* was polymorphic for 2 alleles. The coexpression of heterodimers between the 2 loci resulted in 3-banded patterns for homozygotes and 6-banded patterns for heterozygotes. In the same manner, the other duplicated loci, *Gpi-3* and *Gpi-4* consisted of a monomorphic locus (*Gpi-3*) and the other (*Gpi-4*) polymorphic for 4 alleles; heterozygotes were 6-banded while the homozygotes were 3-banded but not all expected genotypes were observed. Muscle IDH stained as 3 bands, presumably of 2 loci with their heterodimer. LDH was expressed as heteropolymers between 3 types of sub-units, A(muscle-type), B(heart-type), and C(liver-type), both B and C having duplicated loci (7). *Ldh-B1* and *Ldh-C1* were likewise polymorphic for 2 alleles. MDH stained as 6 bands which are presumably duplicated loci of the mitochondrial forms (*mMdh-1* and *mMdh-2*) and 2 loci of the supernatant forms (*sMdh-A* and *sMdh-B*) with their respective heterodimers. 6-PGD stained as 2 monomorphic loci with no heterodimer. *Pgm* was polymorphic for 2 alleles, *B* and *C*. Allele *A* which was included here is a diagnostic allele for the mirror and German scaly carps (14) which also displayed the *B* but not the *C* allele. Liver SOD stained as 3 bands, presumably of 2 loci with their heterodimer.

Table 2 presents the gene frequency data of 12 cultured carp populations. Severe fluctuations in allele frequencies were observed at *Gpi-2* in the Fukushima sample, *Gpi-4* in the Yoshoku-ken sample, and *Pgm* in the Asagi race which

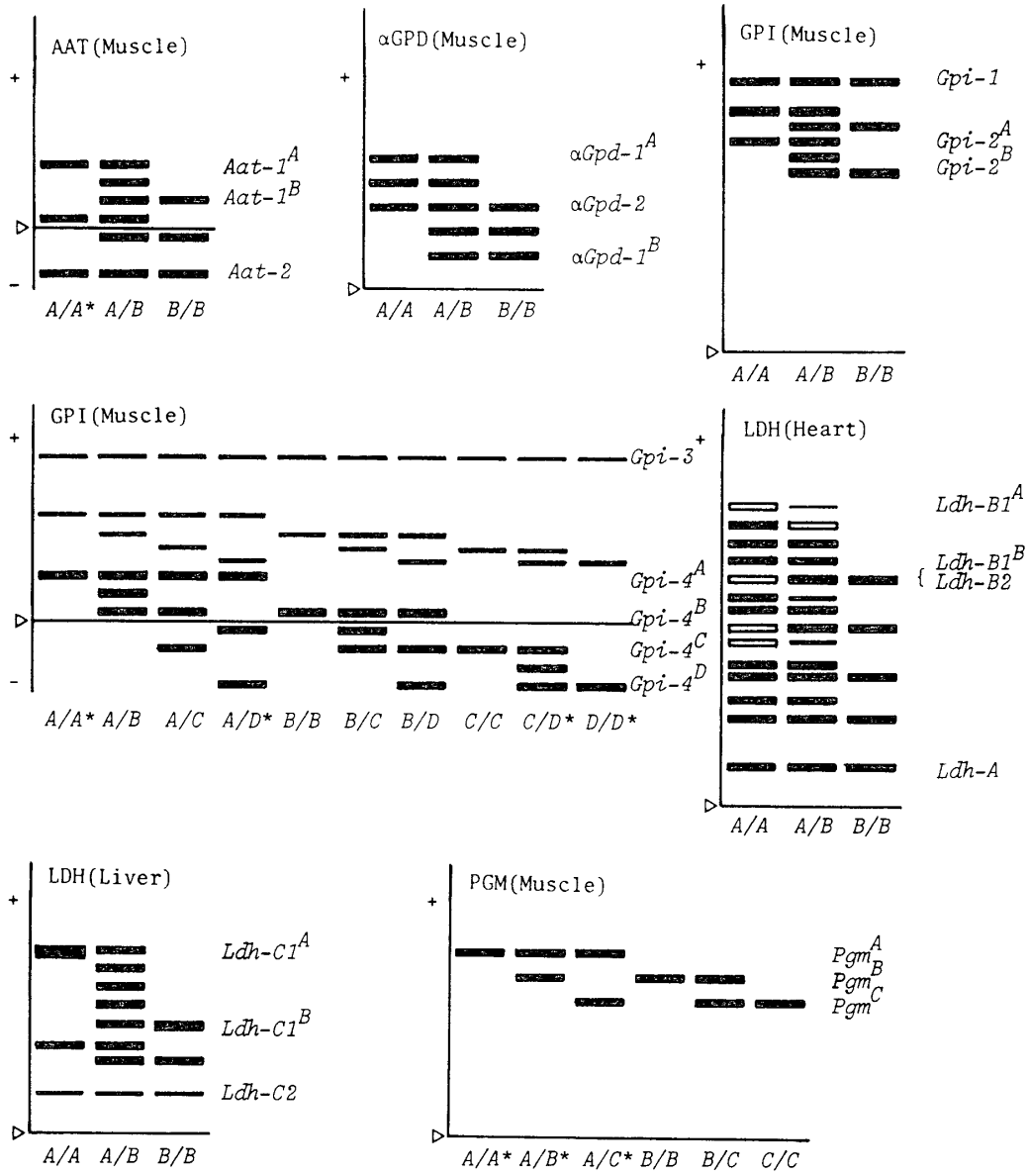

FIG. 1. Allelic expressions at 7 polymorphic loci in common carp, *Cyprinus carpio*. Some genotypes were not observed (*).

contributed to the substantial genetic differentiation from the other populations. From the gene frequency data, the largest genetic distance value obtained ($D=0.0352$) was between Fukushima and Yoshoku-ken while the smallest ($D=0.0012$) was that between Yamagata and Aomori populations. The average D value among the 12 populations was 0.0115 ± 0.0011. As shown by the dendrogram presented in Fig. 2, all the samples were clustered except those of Fukushima and Yoshoku-ken.

The proportion of polymorphic loci (P) ranged from 0.125-0.250, with Asagi exhibiting the lowest and Gamoh and Shiga exhibiting the highest. Observed

TABLE 2. *Gene Frequencies of 12 Cultured Ppopulations of the Japanese Carp, Cyprinus carpio*

Locus	Allele	Yamato											Asagi
		Yoshoku ken	Miyagi (Gamoh)	Fuku-shima	Niigata (Uonuma)	Nagano (Shioda)	Nagano (Saku)	Nagano (Iida)	Shiga	Aomori	Akita	Yama-gata	Nii-gata
Aat-1	*A*	0	0.100	0	0	0	0	0	0.059	0	0	0	0
	B	1.000	0.900	1.000	1.000	1.000	1.000	1.000	0.941	1.000	1.000	1.000	1.000
Aat-2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αGpd-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αGpd-2	*A*	0.828	0.933	1.000	1.000	1.000	0.955	1.000	0.980	0.980	1.000	0.984	1.000
	B	0.172	0.067	0	0	0	0.945	0	0.020	0.020	0	0.016	0
Gpi-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-2	*A*	1.000	0.917	0.375	0.920	0.853	0.909	0.849	0.814	0.940	0.888	0.850	1.000
	B	0	0.083	0.625	0.080	0.147	0.091	0.151	0.186	0.060	0.112	0.150	0
Gpi-3	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-4	*A*	0	0	0	0.070	0.039	0.015	0.016	0	0.040	0.020	0	0
	B	0.069	0.900	0.692	0.660	0.510	0.606	0.405	0.765	0.760	0.806	0.900	0.731
	C	0.931	0.050	0.308	0.270	0.451	0.371	0.579	0.235	0.200	0.143	0.083	0.269
	D	0	0.050	0	0	0	0.008	0	0	0	0.031	0.017	0
Idh-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-A	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-B1	*A*	0.845	0.917	0.847	0.883	0.882	0.970	0.849	0.940	0.700	0.643	0.759	1.000
	B	0.155	0.083	0.153	0.117	0.118	0.030	0.151	0.060	0.300	0.357	0.241	0
Ldh-B2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-C1	*A*	0.586	0.587	0.617	0.459	0.431	0.386	0.500	0.520	0.591	0.429	0.621	0.404
	B	0.414	0.413	0.383	0.541	0.569	0.614	0.500	0.480	0.409	0.571	0.379	0.596
Ldh-C2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
sMdh-A	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
sMdh-B	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mMdh-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mMdh-2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Pgd-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Pgd-2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm	*A*	0	0	0	0	0	0	0	0	0	0	0	0
	B	0.517	0.400	0.267	0.250	0.520	0.409	0.389	0.363	0.540	0.245	0.517	0.066
	C	0.483	0.600	0.733	0.750	0.480	0.591	0.611	0.637	0.460	0.755	0.483	0.934
Sod-1	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sod-2	*A*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

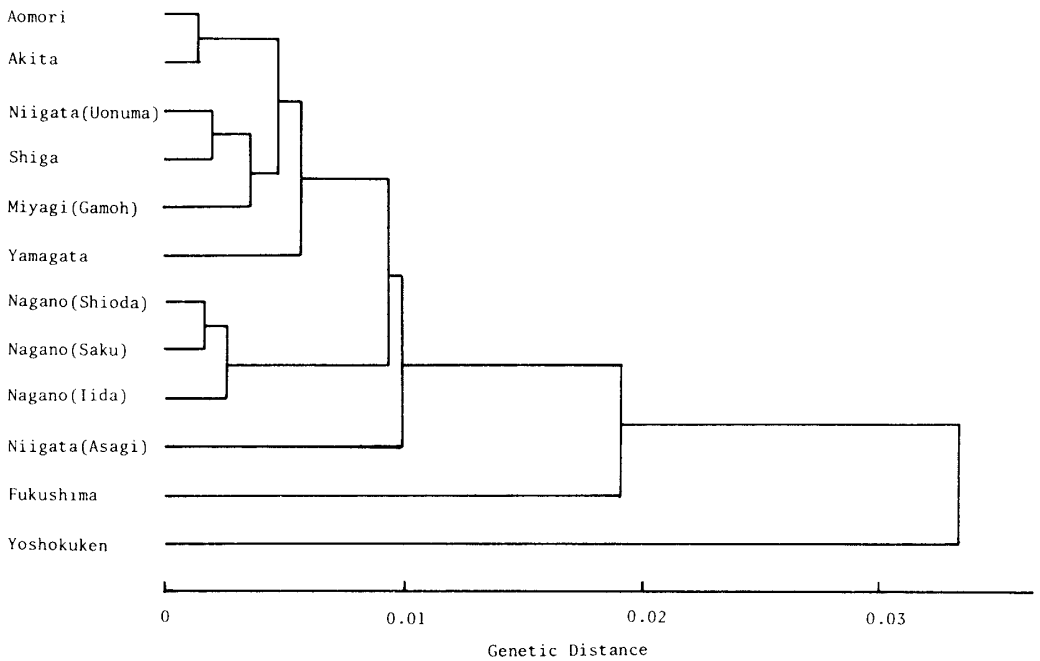

FIG. 2. Dendrogram (UPGMA, Sokal and Sneath 1963) constructed from pairwise genetic distances calculated from 24 gene loci.

heterozygosity (Ho) ranged from a low of 0.059 (Asagi) to a high of 0.092 (Fukushima) (see Table 3). Tests of Hardy-Weinberg proportions were performed at the 7 polymorphic loci and significant deviations were observed from Chi-square test analyses at *Gpi-2* ($\chi^2_1=11.074$; $P<0.005$) and *Gpi-4* ($\chi^2_1=6.777$; $0.005<P<0.010$) in the Fukushima sample, *Ldh-C1* in Gamoh ($\chi^2_1=5.271$; $0.010<P<0.025$) and Asagi ($\chi^2_1=16.463$; $P<0.005$), *Ldh-B1* in Akita ($\chi^2_1=15.124$; $P<0.005$) and Aomori ($\chi^2_1=4.593$; $0.025<P<0.050$). The deviation at *Gpi-4* in Fukushima is due to an excess of homozygotes while the deviations at *Ldh-C1*, *Gpi-2*, and *Ldh-B1* in the other populations are due to heterozygote excess. Deviations (d) from Hardy-Weinberg expectations showed a remarkable excess of heterozygosity in the Asagi population ($d=0.405$) and significantly in the other populations except for Uonuma and Shioda which showed a slight excess of homozygosity (see Table 3). Over all populations, there is an excess of heterozygosity ($d=0.097$).

Discussion

Genetic characterization of some races of carp have shown that the highest genetic distance value obtained between races is 0.040, that between mirror and Japanese fancy carp. Also, the genetic distance values obtained between the Yamato race and German scaly carps ranged from 0.012-0.017 while that between the Yamato race and mirror carps ranged from 0.012-0.015. Moreover, mirror and German scaly carps were separated only by a D value of 0.002 (14). It is therefore

TABLE 3. *Genetic Variation in 12 Cultured Populations of Japanse Carp, Cyprinus carpio*

Race	Cultured population	Proportion of polymorphic loci (P)	Heterozygosity		d*
			Ho	He	
Yamato	Yoshokuken	0.208	0.079	0.069	+0.145
	Miyagi (Gamoh)	0.250	0.083	0.073	+0.137
	Fukushima	0.208	0.092	0.084	+0.095
	Niigata(Uonuma)	0.208	0.065	0.072	−0.097
	Nagano(Shioda)	0.208	0.075	0.083	±0.096
	Nagano(Saku)	0.167	0.083	0.073	+0.137
	Nagano(Iida)	0.208	0.083	0.083	0
	Shiga	0.250	0.081	0.079	+0.025
	Aomori	0.208	0.086	0.081	+0.062
	Akita	0.208	0.090	0.077	+0.169
	Yamagata	0.208	0.089	0.075	+0.187
Asagi	Niigata	0.125	0.059	0.042	+0.405
Average		0.205	0.080	0.074	+0.097

* d is the deviation from Hardy-Weinberg's expectations wherein a +value denotes an excess of heterozygotes while a-value an excess of homozygotes.

remarkable that cultured carps of the Yamato race, i.e., Fukushima and Yoshoku-ken, could be more genetically differentiated from other Yamato race carps than Yamato race is to the German carps, considering that the latter have undergone more years of selection and maintenance than the Japanese races. Another interesting observation was that the Asagi race clustered with the Yamato race except Fukushima and Yoshoku-ken. The Asagi race is easily distinguishable by its bluish color and is genetically separated from the Yamato race by an average D of 0.0098 ; this value estimates the genetic divergence level of a local carp race. The considerable differentiation of the Fukushima and Yoshokuken populations from the rest of the Yamato race carps is understandable from the information given on their parental origin which consisted of a very limited number of parents. The use of a small effective number of parents in the maintenance of stocks results in drastic allele frequency changes due to random genetic drift (17). It is apparent from our results that random genetic drift can result in genetic changes that exceed those arising from racial differences and can be effected in a very short period of time. In the case of the clustered Yamato race populations, drastic differences were not apparent and even samples taken 4 or 6 years later, namely, Aomori, Akita, and Yamagata populations, were still genetically close to the other Yamato samples obtained earlier. This observation can likewise be attributed to effective population size of stocks, which in these populations might have been appreciably large. Within the Yamato race cluster, the samples obtained from

the Nagano prefecture (Shioda, Saku, and Iida) formed a sub-cluster, which suggests a common origin of the three populations.

Of more applied interest is the observed general trend of heterozygote excess in the cultured carps. While the Asagi population exhibited the lowest degree of genetic variability, it had the highest excess heterozygosity. The Asagi race must be the population that has undergone the most intensive selection as evidenced by fixation of alleles at 3 polymorphic loci resulting in low genetic variability. The tendency that a decrease in over-all heterozygosity is accompanied by heterozygote excess may be one kind of mechanism of maintaining polymorphism during selection and inbreeding of cultured organisms. This hypothesis can be proven by an examination of the trend in heterozygosity over several generations of selection in a carp strain. A study with this objective has revealed an increasing frequency of heterozygotes at the *Est-4* locus in the process of producing inbred strains of Japanese quail (18).

Although heterosis is a well-established phenomenon in their interracial hybrids, the selective advantage of heterozygotes in cultured carps still has to be clarified. A positive correlation between average heterozygosity and growth rate was observed in the sunfish, *Micropterus spp.*(19), mosquito fish, *Gambusia affinis* (20), and herring, *Clupea harengus L.* (21). Marked differences in growth rate were also associated with an excess of heterozygotes at the *sIdh* locus in cultured populations of the plaice, *Paralichthys olivaceus* (22). A decrease in genetic variability accompanied by heterozygote excess was similarly observed in hatchery stocks of Atlantic salmon (23, 24). Stahl (24) attributed the excess heterozygosity to the very restricted number of males and/or females (homozygous for different alleles at *Aat-3*), used in producing the group of fish.

The underlying reason/s for the general trend of excess heterozygosity observed in cultured carp populations cannot easily be explained from our study and even from the other works mentioned here. Whether it is a simple result of random genetic drift caused by limited population size or an evidence of a more complex phenomenon, that of balancing selection of enzyme polymorphism (heterosis) remains to be investigated by complementing electrophoretic, morphological, and growth performance data.

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