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RESEARCH ARTICLE

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Genetic potential analysis of German mirror carp (*Cyprinus carpio* L.) using microsatellite markers

Abstract Using 30 microsatellite markers and combining quantifiable characteristics such as body weight, body length and body width, we evaluated the genetic potential of 3 German mirror carp (*Cyprinus carpio* L.) populations. Number of effective alleles (*Ae*), observed (*Ho*) and expected (*He*) heterozygosity values and polymorphic information contents (*PIC*) were all calculated. Two hundred and eighty-seven alleles and 559 genotypes were detected. The DNA fragment length was 109–400 bp. The Hardy-Weinberg Equilibrium was checked and the phenomenon of some disequilibrium was studied according to the χ^2 test. The results showed that the level of genetic variability was moderate, but genetic potential of Shuanglai population was much lower than that of Huanxin and Songpu breeding populations. *PIC* of the three populations of German mirror carp were between 0.08787 and 0.5377, both highly and moderately polymorphic markers were 13. The number of the *Ae* was between 1.1014 and 6.4665. The *Ho* and *He* heterozygosity values were 0.0968–0.9892 and 0.0926–0.8554, respectively. The linkage correlation was analyzed using the data of body weight, body length and body width, and 30 loci. The result showed that there existed 2 loci, *HLJ*319 and *HLJ*693, associated with body length. The *HLJ*693 locus was significantly correlated with body weight trait. The *HLJ*677 locus was linked with body width. And then the result was verified in Recombinant Inbred Lines (RIL) of common carp. It showed that the *HLJ*319 locus was significantly linked with body length, the same as the result of quantitative trait loci (QTL) location for common carp.

Keywords German mirror carp, economic characters, genetic markers, genetic potential

1 Introduction

German mirror carp originated from Bavaria of Germany, and it was the main fish of pool cultivation in Europe. It has been reared as a fine breed fitting for the north cultivation since it was introduced to China from Germany in 1984 (Li, 1983; Shen and Yan, 1987; Liu et al., 1995; Shen and Liu, 2000). German mirror carp grows fast and can be captured easily, but its resistance to disease is slightly weak. The hybrid progeny displays significant hybrid vigor when crossing with wild carp and red carp of Heilongjiang (Yin et al., 1995). German mirror carp has become one of the important freshwater aquaculture in the north of China. In recent years, the mirror carp breeding disease occurs more frequently and the germplasm quality declines sharply, the possible reasons are more artificial selection and increasing intensity of environmental degradation and inbreeding, but the exact cause and mechanism are unclear. Genetic recession caused by inbreeding is only theoretical speculation, which has not been validated by precise genetic testing. Therefore, it is necessary to estimate the genetic potential of mirror carp.

Microsatellites have some advantages over other markers and have been increasingly and widely used as molecular markers in recent years (Crooijmans et al., 1997). There were many researches related to applications, such as development of microsatellite markers (Aliah et al., 1999; Wei et al., 2001; Yue et al., 2004), establishment of genetic linkage map of carp (Sun and

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Liang, 2004), QTL of the cold resistance marker (Liang and Sun, 2003) and the carp population genetics research (David et al., 2001; Kohlmann et al., 2003; Lal et al., 2004; Quan et al., 2005; Liao et al., 2006), but there were not many reports about studying the German mirror carp using microsatellite or other molecular markers (Wang and Liu, 1994; Hu et al., 2006; Quan et al., 2006).

Our research estimated the genetic potential of three mirror carp populations by microsatellite markers, and explored genotypes frequency changes associated with the main economic characters after high-intensity artificial selection. Thus it could provide the basic data for the genetic structure optimization, avoiding declining of production and genetic recession. Furthermore, this paper verified the accuracy of QTL localization of the common carp (Zhang et al., 2007) through randomly grouping, which provided a technical way to link population economic characters with genetic markers.

2 Materials and methods

2.1 Materials

2.1.1 Experimental fish populations

According to the difference in their artificial selection intensity, a total of 432 individuals of the German mirror carp were collected from Shuanglai Nursery in Hulan District of Heilongjiang Province (SL), Huanxin National Grade Elite Farm (HX) and Songpu Experimental Station of Heilongjiang Fisheries Research Institute (SP). For Sample 1, we measured its phenotypic data such as body weight, body length and body width. Sample 1 (93 individuals) was one-year fish and artificial selection intensity of parents was about 300:2, Sample 2 (208 individuals) was obtained by extremely high artificial selection and selection intensity was about 100:2, Sample 3 (131 individuals) was the offspring of the early authorized national grade elite stock, and its selection intensity was about 60:2.

2.1.2 Primers and reagents

Thirty microsatellite polymorphic markers were screened from 187 primer pairs used for constructing a genetic linkage map and locating QTL of common carp. These markers were carp microsatellite sequences enriched by magnetic beads and searched on the internet. All the primers were synthesized by Shanghai Sangon Biological Engineering & Technology and Service Co. Ltd., Shanghai, China (Table 1). Biochemical reagents were provided by Promega, USA, and the other reagents were from China.

2.2 Methods

2.2.1 Extraction and purification of genomic DNA

All the rear fins of samples were kept in 70% ethanol during transportation, dipped in ion free water to wipe off ethanol, phenol, chloroform, and iso-amyl alcohol (25:24:1) extraction to obtain genomic DNAs using the method by Geng et al. (2006).

2.2.2 Processes of PCR

Amplification was performed with a reaction volume of 25 L, and the component referred to Geng et al. (2006). Thermal Cycling Profile for Standard PCR was as follows: 3 min at 94°C, followed by 40 cycles of denaturation at 94°C for 30 s, annealing temperature varied from 48°C to 54°C for 30 s, and extension at 72°C for 30 s, followed by an extra extension at 72°C for 5 min.

2.2.3 Amplification products detection

PCR products were separated by electrophoresis in 2% agarose gel (0.5 × TBE buffer at 5 V/cm) for 2 h with 0.02 × Gold View (SBS, Beijing, China) nucleic acid stain, and visualized under UV light on a Gene Genius Bio imaging system. Microsatellite alleles were identified by their size in base pairs using Gel works software package (Version 3.0, UVP, USA).

2.2.4 Data analysis

Microsatellite belongs to codominant inheritance, the individual genotype could be directly judged by the agarose gel electrophoregram. Allele Frequency (*P*), observed number of alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (*Ho*), and expected heterozygosity (*He*) were computed by using PopGene (Version 3.2). Polymorphism information content (*PIC*) was computed according to the following formula (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2,$$

where P_i and P_j are the frequencies of the ith and jth alleles at one locus; n is the number of alleles at one locus.

Linkage disequilibrium between pairwise loci was analyzed using GenePop (Version 3.4). Deviation from Hardy-Weinberg equilibrium (HWE) was estimated by χ^2 test. Genetic deviation index (d) directly indicates the absence or excess of heterozygote of one population. It can be described according to the following simple method:

Table 1 Thirty pairs of microsatellite maker sequence

locus	primer sequence (5′ → 3′)	repetitive sequence	renaturation temperature/°C	locus	primer sequence (5′ → 3′)	repetitive sequence	renaturation temperature/°C
HLJ041	F: AGACCACCGCAGTAACAA R: GACTCACTCAGCACCAGA	(CA)24	53	*HLJ392*	F: GGCTACAAGGCAACACTG R: TGCGGTTAATGAGGTCTG	(CA)22	54
HLJ044	F: GTACAGCGTGACAGCATT R: AAGTTCATCGGTGTCCTC	(CA)28	53	*HLJ393*	F: TGCGGTCATTACTCATTCG R: CCCAGCACCTGTTTCCAC	(CA)10	54
HLJ046	F: AACCCTGAACTCACAAAC R: CACGGAAACTGAGAAGAC	(GT)14	53	*HLJ400*	F: AAGAAGCCTCGGTCCTCC R: AAAGCCCAAAGCACATCA	(CA)22	51
HLJ049	F: GATTTGTGCTCCTCAACC R: CTGTCACTTCTCCTTCCA	(GT)28	54	*HLJ643*	F: CCGACTCAGTGTTCAATT R: GAAACCTAAGTCCCCAAC	(CA)7	50
HLJ057	F: GAATGTCATGCGGTTCAT R: TATTTGCTGGGTGTCCTC	(GT)23	51	*HLJ677*	F: ATACGGTATGTCTGGAAA R: CTGACTAGAGGAAAAGCA	(GT)28	50
HLJ058	F: CAGATGGCAGACAGGTAA R: GAGCAAGTGAGGGAACAG	(CA)21	53	*HLJ693*	F: GAGACCGCATGACTTCAA R: TAGCCATCTGTCCTAAACGA	(GT)16	50
HLJ133	F: TGGGTTGGTTCACAGACA R: TTCAGCGGATTTACAGAGC	(GT)34	51	*HLJ695*	F: AAGATGGAGGTCTGGTGT R: ATCACGGTTCTTTAGTGC	(TG)15	50
HLJ302	F: ACCTCATTTGAATCCCTG R: AATAGAGTTTGTTTGCTGA	(CA)23	50	*HLJ697*	F: AAGAATGGGTGAGTAAGA R: ACTAGGATTTGGAAGAGC	(AC)57	50
HLJ319	F: CAGTGGGATTGTGGGAGT R: CAGGGAGGGTCAAAGGTC	(CA)14	53	*HLJ699*	F: ACGTCATCAGACCCTTCT R: CTGGTGGTTTGTTATTGT	(AC)24	50
HLJ328	F: CCTGACACCTGCCGTTCT R: TCCTCTGTTCTGCCTCCC	(CA)30	51	*HLJ806*	F: GGTGTCAGGCTTTAGTCC R: CATCTGAGTTTTCTCCAAGT	(CA)48	48
HLJ338	F: GAAGAATGGGTGAGTAAGA R: ACTAGGATTTGGAAGAGC	(AC)58	51	*HLJ809*	F: ATCATCACAGCCAAAGAAGT R: TACGGACATAGTGCAGACAA	(CA)12	48
HLJ343	F: TCCTCACAACCCTCCGTAT R: CAAAGGCATCCCATCAGT	(TG)32	51	*HLJ821*	F: TGTTTAGAAGAGCCTGTTTG R: GCAGTTTATTTATTCTGGGAG	(GT)16 (GA)5	52
HLJ376	F: AAGAAGGACTACGAGGAGA R: TTCGGTTGCTTACTATGA	(CA)13	54	*HLJ845*	F: TCAGGTCAGGTGAGTCTT R: CTGCTGTTGTCTGGTTCT	(CA)18	51
HLJ379	F: GGGGAGACGAGAAGTGCA R: AGCAGGTCTGTGGGCAAG	(CT)13 CG(CT)5	54	*HLJ855*	F: CGACCGAACTCAGAACAC R: GAGCACCGCATTAACAGA	(AC)44	48
HLJ383	F: GGCTCCTCCTCATCCTCT R: GCACTTCTGCACCTTTCA	(CA)14	51	*HLJ856*	F: GACTGATGCAGCCCAGAG R: GCACAGACATTTCATAGCG	(CA)32	50

$$d = (H_O - H_e)/H_e.$$

The data from quantitative traits such as body weight were treated using Excel 2003.

3 Results

3.1 PCR results

Thirty microsatellite markers were found to produce well-amplified and reproducible electrophoretic bands in 3 German mirror carp populations (432 individuals) and showed different degrees of polymorphism among individuals. *HLJ*041 locus displayed low polymorphism in SL population. The number of alleles detected in each locus was 2–10; the number of average alleles was 5.5141. The amplified fragments ranged from 109 bp to 400 bp. The result of agarose gel electrophoregram at locus *HLJ*643 of partial samples of 3 German mirror carp populations is shown in Fig. 1.

3.2 Analysis of genetic potential

Two hundred and eighty-seven alleles and 559 genotypes were observed at the 30 microsatellite loci among the 432 German mirror carps sampled from 3 populations. The effective number of alleles (*Ae*) per locus varied greatly from 1.1014 to 6.4665. The observed heterozygosity (*Ho*) also varied sharply among loci from 0.0968 to 0.9892, the expected heterozygosity (*He*) ranged from 0.0926 to 0.8554. *PIC* of the 3 populations of German mirror carp varied from 0.08787 to 0.5377, the number of highly ($PIC \geqslant 0.5$) polymorphic markers were 13, the same as the number of moderately polymorphic loci ($0.25 \leqslant PIC \leqslant 0.5$). The four genetic potential statistics parameters (*Ae*, *Ho*, *He* and *PIC*) of SL population were 2.0961, 0.4731, 0.4815 and 0.4084, respectively. These values were lower than those observed in HX population, whose *Ne*, *Ho*, *He* and *PIC* were 2.7063, 0.5031, 0.5478 and 0.5133, and the values of SP population were 2.9213, 0.5898, 0.5910 and 0.5377, respectively (not significant by analysis of variance, ANOVA). It could be concluded the genetic potential of the three populations was moderate; the genetic potential of SL was lower than that of HX or SP according to the statistical results (Table 2).

3.3 Linkage disequilibrium between microsatellite loci

The pairwise genotypic disequilibrium values were computed at each polymorphic locus in each population by GenePop software (Version 3.4). It's found that *HLJ*133, *HLJ*338 and *HLJ*343, *HLJ*338 and *HLJ*697 showed close linkage. The linkages of other loci were not significant ($P > 0.05$) and there was no locus linked to the main economic characters (data not shown).

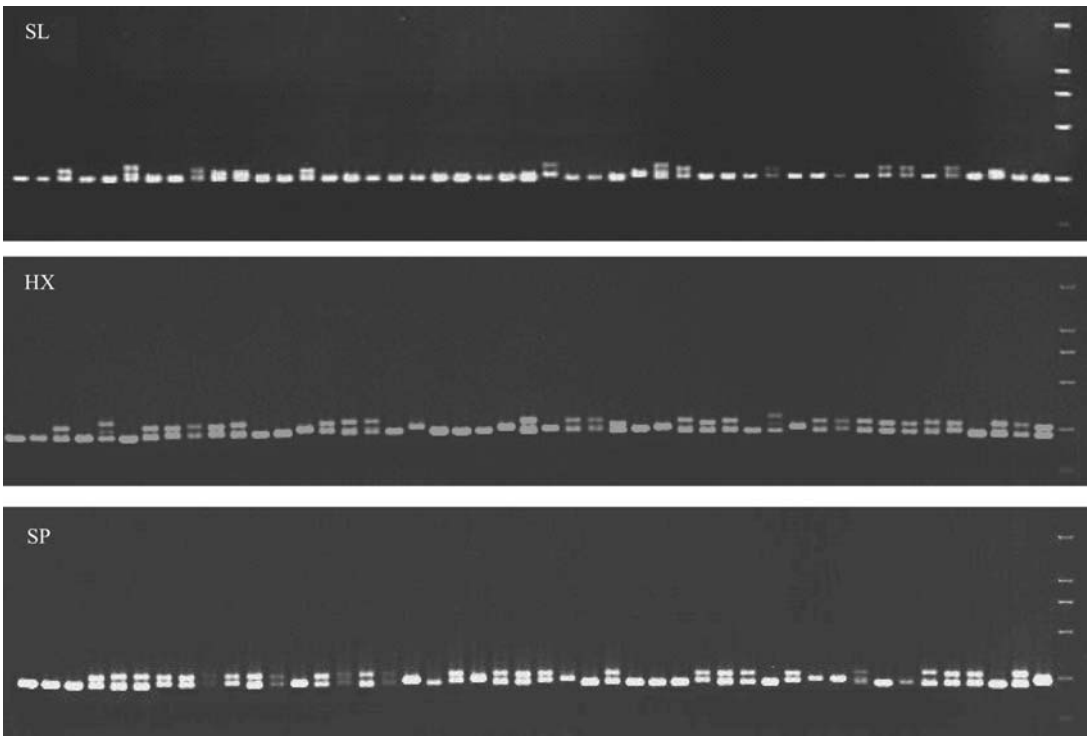

Fig. 1 Genetic diversity at locus *HLJ643* of partial samples of three German mirror carp populations
Note: SL refers to the population sampled from Shuanglai of Hunan; HX refers to the population sampled from Huanxin of Tianjin; SP refers to the population sampled from Songpu of Heilongjiang.

Table 2 The polymorphic information at 30 microsatellite loci of 3 German mirror carp populations

locus	SL				HX				SP			
	Ae	*Ho*	*He*	*PIC*	*Ae*	*Ho*	*He*	*PIC*	*Ae*	*Ho*	*He*	*PIC*
HLJ041	1.10	0.1	0.09	0.09	1.64	0.35	0.39	0.37	1.18	0.17	0.16	0.15
HLJ044	3.22	0.75	0.69	0.63	5.06	0.50	0.80	0.78	3.94	0.76	0.75	0.70
HLJ046	1.36	0.31	0.26	0.23	1.36	0.31	0.26	0.23	1.53	0.40	0.35	0.31
HLJ049	2.46	0.51	0.6	0.51	3.34	0.30	0.70	0.65	4.21	0.60	0.76	0.72
HLJ057	1.75	0.33	0.43	0.39	1.68	0.36	0.40	0.39	1.73	0.23	0.42	0.40
HLJ058	2.65	0.99	0.63	0.55	1.26	0.22	0.20	0.20	1.34	0.27	0.26	0.24
HLJ133	2.20	0.6	0.55	0.49	2.17	0.38	0.54	0.51	4.06	0.72	0.75	0.71
HLJ302	2.67	0.71	0.63	0.55	1.50	0.34	0.34	0.32	1.60	0.35	0.37	0.36
HLJ319	2.32	0.7	0.57	0.51	4.43	0.77	0.77	0.75	4.04	0.73	0.75	0.72
HLJ328	2.86	0.63	0.65	0.58	4.49	0.95	0.78	0.75	5.01	0.73	0.80	0.77
HLJ338	2.68	0.12	0.63	0.56	3.89	0.49	0.74	0.70	3.82	0.69	0.74	0.70
HLJ343	2.95	0.14	0.66	0.6	2.33	0.37	0.57	0.54	3.43	0.49	0.71	0.68
HLJ376	1.25	0.23	0.2	0.18	1.31	0.20	0.24	0.23	1.27	0.24	0.22	0.20
HLJ379	1.72	0.6	0.42	0.33	2.35	0.98	0.57	0.48	2.60	1.00	0.62	0.54
HLJ383	1.83	0.26	0.46	0.39	1.85	0.38	0.46	0.43	2.44	0.36	0.59	0.56
HLJ392	1.41	0.35	0.29	0.25	1.87	0.42	0.46	0.43	2.13	0.56	0.53	0.48
HLJ393	2.0	0.97	0.5	0.37	1.97	0.56	0.49	0.45	1.60	0.41	0.38	0.33
HLJ400	3.29	0.6	0.7	0.64	2.64	0.46	0.62	0.60	4.38	0.70	0.77	0.74
HLJ643	3.06	0.59	0.68	0.62	5.84	0.82	0.83	0.81	6.33	0.96	0.84	0.82
HLJ677	2.61	0.33	0.2	0.54	6.47	0.58	0.85	0.83	3.11	0.81	0.68	0.64
HLJ693	1.81	0.33	0.45	0.35	2.86	0.22	0.65	0.60	1.71	0.15	0.42	0.38
HLJ695	1.97	0.54	0.5	0.37	1.44	0.25	0.31	0.29	3.45	0.34	0.71	0.66
HLJ697	2.03	0.38	0.51	0.39	2.11	0.53	0.54	0.47	2.25	0.80	0.56	0.49
HLJ699	1.9	0.54	0.47	0.36	1.91	0.53	0.49	0.45	2.27	0.93	0.57	0.46
HLJ806	1.97	0.46	0.49	0.37	3.08	0.60	0.69	0.61	3.44	0.77	0.72	0.66
HLJ809	1.32	0.28	0.24	0.21	1.43	0.37	0.30	0.25	1.90	0.77	0.48	0.36
HLJ821	1.33	0.29	0.25	0.22	3.28	0.90	0.71	0.64	3.67	0.70	0.74	0.68
HLJ845	1.88	0.48	0.47	0.36	1.64	0.33	0.40	0.31	2.88	0.40	0.66	0.59
HLJ855	1.71	0.59	0.42	0.33	3.51	0.63	0.73	0.66	3.73	0.93	0.74	0.68
HLJ856	1.56	0.47	0.36	0.3	2.44	0.90	0.60	0.52	2.53	0.83	0.62	0.53
mean	2.10	0.47	0.48	0.41	2.71	0.50	0.55	0.51	2.92	0.59	0.59	0.54

Note: SL: the population sampled from Shuanglai of Heilongjiang; HX: the population sampled from Huanxin of Tianjin.; SP: the population sampled from Songpu of Heilongjiang; *Ae*: the effective number of alleles per locus; H_0: the observed heterozygosity per locus; *He*: the expected heterozygosity per locus; *PIC*: Polymorphism information content per locus.

3.4 Analysis of Hardy–Weinberg equilibrium

Unbiased Measures of the Hardy-Weinberg test on multi-locus based on the Markov chain method showed that the three populations were in genetic equilibrium, but *HLJ*379 and *HLJ*856 loci showed significant genetic disequilibrium ($P < 0.01$) in all populations. Besides, there were 4, 3, 2 loci displaying Hardy–Weinberg equilibrium deviations across SL, HX, SP populations, respectively ($P < 0.05$). Hardy-Weinberg genetic deviation index (*d*) directly indicated absence or excess of heterozygote. The statistics showed that most loci and populations were in Hardy–Weinburg equilibrium, but 73.33% loci appeared absence of heterozygote in HX population (Table 3).

3.5 Correlation analysis associated loci genotypes with dominating economic characters

Body weight, body length and body width data of Sample 1 were given statistics analysis contrasting to all loci and genotypes. The average body weight, body size and body width of SL population (93 individuals) were 239.84 g, 18.97 cm and 8.29 cm, respectively. *HLJ*319 and *HLJ*693 loci showed significant linkage with body size, *HLJ*693 locus was also significantly correlated with body weight, *HLJ*677 locus had significant correlation with body width. There was linkage between genotype 310/285 of *HLJ*693 locus and body length and body weight, two genotypes of *HLJ*319 (280/220 and 220/220) linked to body length, and genotype 290/290 of *HLJ677* showed correlation with body width. The detailed results of correlation analysis of loci genotypes and dominating economic traits are displayed in Table 4.

3.6 Comparison with the results of QTL analysis

Genetic markers linked to dominating economic traits were checked in recombinant inbred lines of common carp. Results showed that *HLJ*319 locus correlated with the body length of German mirror carp and fundamentally coincided with the result of QTL analysis. QTL

Table 3 Hardy-Weinberg equilibrium analysis of threea German mirror carp populations

locus	SL		HX		SP	
	P	*d*	*P*	*d*	*P*	*d*
HLJ041	0.6447	0.0454	0.1374	−0.1002	0.3822	0.0797
HLJ044	0.1853	0.0860	1.0000	−0.3769	0.2942	0.0126
HLJ046	0.0807	0.1784	0.0026**	0.1850	0.0070**	0.1650
HLJ049	0.9018	−0.1529	1.0000	−0.5743	0.9985	−0.2193
HLJ057	0.6702	−0.2251	0.1758	−0.1202	0.9962	−0.4566
HLJ058	0.7043	0.5812	0.1900	0.0567	0.8375	0.0676
HLJ133	0.7834	0.0993	0.9742	−0.2871	0.6280	−0.04812
HLJ302	0.9043	0.1285	0.3475	0.0033	0.8305	−0.0627
HLJ319	0.4054	0.2196	0.6912	−0.0063	0.9610	−0.0262
HLJ328	0.2938	0.0292	0.0001**	0.2245	0.8397	−0.0940
HLJ338	0.0000**	0.8124	1.0000	−0.3464	0.6469	−0.0695
HLJ343	0.6789	−0.7897	0.8951	−0.3512	1.0000	−0.3101
HLJ376	0.2321	0.1212	0.9988	−0.1445	0.1334	0.0989
HLJ379	0.0000**	0.4233	0.0000**	0.6986	0.0000**	0.6260
HLJ383	1.0000	−0.4354	0.9990	−0.1731	1.0000	−0.3922
HLJ392	0.1410	0.2089	0.4524	−0.0878	0.5127	0.0511
HLJ393	0.000**	0.9254	0.6896	0.1312	0.9999	0.0986
HLJ400	0.4457	−0.1392	0.6127	−0.2574	0.3966	−0.0899
HLJ643	0.0000**	−0.1270	0.9544	−0.0138	0.0000**	0.1423
HLJ677	1.0000	−0.4622	1.0000	−0.3176	0.9936	0.1933
HLJ693	0.5616	−0.2600	1.0000	−0.6672	1.0000	−0.6325
HLJ695	0.4116	0.0845	0.3477	−0.2013	1.0000	−0.5271
HLJ697	0.0326*	−0.2629	0.4978	−0.0185	0.5003	0.2500
HLJ699	0.2181	0.1286	0.5233	0.0816	0.5309	0.6667
HLJ806	0.5306	−0.0645	0.8817	−0.1304	0.2593	0.0694
HLJ809	0.1252	0.1563	0.3265	0.2333	0.4527	0.6042
HLJ821	0.1089	0.1635	0.0056**	0.2676	0.8516	−0.0540
HLJ845	0.7120	0.0313	0.9153	−0.1750	0.9898	−0.3940
HLJ855	0.7060	0.4121	0.8354	−0.1370	0.6052	0.2568
HLJ856	0.0030**	0.3026	0.0000**	0.5000	0.0022**	0.3387

Note: * represents significant difference; ** represents high significant difference.

interval mapping located it in the interval *HLJ*190-*HLJ*497 of the second linked group. Correlation analysis indicated *HLJ*677 locus was related to body width; however, QTL map located it in the linkage group of body length (Zhang et al., 2007). Whether *HLJ*693 locus correlated with body length or body weight needed to be further tested as QTL map of body weight in common carp was not performed. The PCR result of *HLJ*319 in recombinant inbred lines is shown in Fig. 2. Mapping QTLs of body length trait in common carp and *R* ratio curves for evidence of QTL are shown in Fig. 3.

4 Discussion

4.1 Analysis of genetic potential of three German mirror carp populations

Biological population variation and genetic potential are an important foundation for evaluating species resources. It is a base of the species to adapt themselves to various environments. It is a precondition to make persistent use of the species resources and to keep the highest level of genetic potential. *Ae*, *Ho*, *He* and *PIC* are all parameters

Table 4 Relational analysis between genotypes of genetic loci and dominating economic characters

dominating economic traits	genetic loci	genotypes	average values	percentage of mean values	*F* values	*Fα* values ($\alpha = 0.05$)
body weight/g	*HLJ693*	310/285	275.98	1.15	4.01*	3.10
body length/cm	*HJL693*	310/285	19.91	1.05	3.51*	3.10
	HLJ319	220/220 280/220	18.35	0.97	2.55*	2.31
body width/cm	*HLJ677*	290/290	7.79	0.94	2.37*	2.32

Note: * represents significant difference.

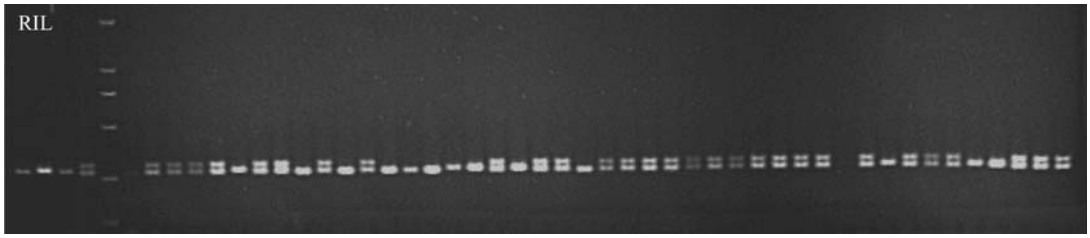

Fig. 2 Amplified result of *HLJ693* in partial samples of RIL
Note: RIL indicates the sample of recombined inbreeding lines.

of population genetic diversity and inherited potential. The value of these parameters varies with the abundance. On the basis of the present study, genetic potential of three German mirror carp populations was at a middle level ($Ae = 2.10$–2.92, $Ho = 0.43$–0.59, $He = 0.48$–0.59, and $PIC = 0.41$–0.54), but all showed a downward trend. SP population showed the highest genetic potential in the three populations. This was probably due to the fact that screening intensity of SL and HX populations were higher than that of SP population, the other reason was SP population was cultured earlier with some hybrid genotypes that originated from the initial breed, while SL and HX population were obtained by further selection, and genotypes related with selection pressure would be enriched. In other words, genotypes frequency that correlated with selection pressure would be increased, and some rare alleles would be lost.

Compared to the genetic potential of common carp (De Woody and Avise, 2000; Du et al., 2000), our results showed that the effective allele was less, and the other parameters of polymorphism were still at high levels. The possible reason is the special genetic background of the carp. David et al. (2003) found that common carp had doubled its genome through its long history of evolution. The doubling of genome would be conducive for the enhancement of heterozygosity, but this was the reason why the modern common carp kept higher polymorphism parameters and genetic variation. However, when compared with the research results of Quan et al. (2006) ($Ae = 4.75$–5.78, $He = 0.70$–0.78, and $PIC = 0.69$–0.75), the genetic potential of three mirror carp populations was significantly lower. The reduction of effective alleles might be on account of the diminishing effective population size and the loss of effective alleles caused by the growing deterioration of the ecological environment, high-intensity artificial breeding and inbreeding.

In order to maintain high genetic potential of mirror carp, we should ensure mirror carp population size exceeds the minimum effective population size. For most of the fish, $Ne = 50$ is the minimum population size (Taniguchit, 2003). *Ne* is related to *He* or *Ae* and can be described by the following equation: $Ne = (He/(1 - He))/4u$ (Kimura and Crow, 1964), where u is mutation rate of microsatellite and approximate reference value is

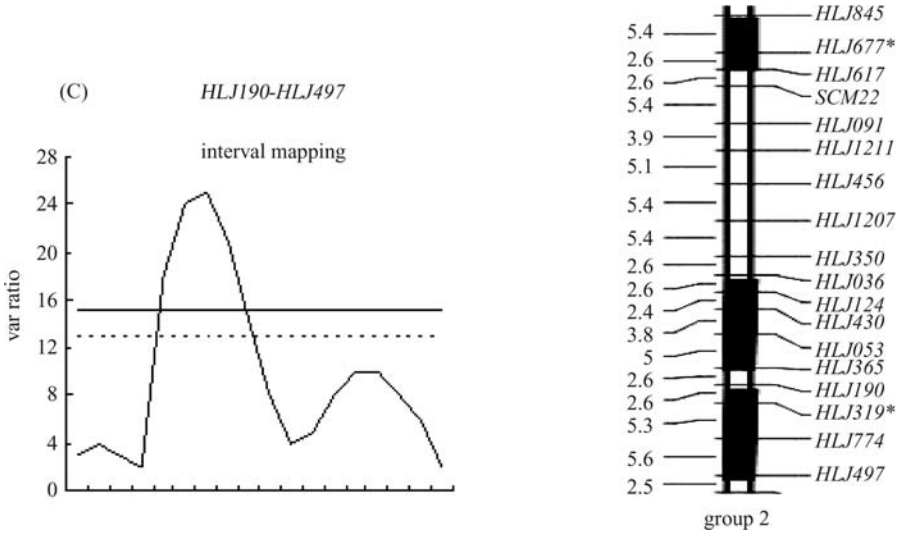

Fig. 3 Mapping QTLs of body length trait in common carp and *R* ratio curves for evidence of QTL
Note: The X-axis indicates the relative position on the linkage map; the Y-axis represents the *R*-ratio; (……) and (———) represents 5% and 1% chromosome-wise significance, respectively; (C) represents a QTL graph for body length on the 2nd linkage group; * represents QTL loci of verification.

$u = 4 \times 10^{-4}$ (Garcia et al., 1997). It can be calculated that the average effective population size of three German mirror carp populations is 734. Usually, mirror carp breeding population size cannot achieve the effective population size calculated by the above equation, small population is vulnerable to suffering gene loss and fixing caused by genetic drift. Therefore, when studying germplasm resource protection and genetic breeding of mirror carp population, the required number of effective groups should be considered to maintain the level of genetic diversity and genetic potential.

4.2 Correlation analysis and verification of QTL map

One important characteristic of quantitative traits is the vulnerability of environmental impact (Reid et al., 2005); accordingly, the three genetic markers linked with the dominating economic characters obtained from our research were verified and compared with QTL map of common carp. The analysis showed that *HLJ*139 was linked with body length, the average ratio was 0.97 and the additive effect direction was negative. The QTL map of common carp located it on the 2nd linkage group, which had a likelihood ratio of 16.4, additive effect of 0.31, explanation variation coefficient of 31% and contribution rate beyond 20.00% to the body length character ($P < 0.001$). Meanwhile, in the research of QTL interval mapping, locus *HLJ319* was included in the interval *HLJ*190–*HLJ*497 which has achieved a significant level of linkage group (Zhang et al., 2007). This result of study was the same as the result of common carp QTL location, both of them showed that the *HLJ319* locus was significantly linked with body length and it was approved that *HLJ319* locus can be used as the first selective molecular marker to assist breeding application. Such test confirmed the accuracy of this study and verified QTL map of common carp through random populations. Because there are no QTL results of body weight and body width, the genetic markers *HLJ693* and *HLJ677* still need further analysis and verification.

Molecular markers have one potential application for fish breeding as there is linkage relationship between gene and economic characters, so they can be used as a genetic tool to choose the advantaged breed with strong economic characters. For this study, molecular markers and genotypes related to economic characters could be used in breeding of fresh mirror carp stock.

4.3 Limitations and solutions of linkage analysis using outbred population

Inbred populations were mostly used in correlation analysis and QTL study and can lead to a satisfactory result (Majumder and Ghosh, 2005). Outbred population was not as effective as inbred population, but there were successful examples (Knott et al., 1998; Yalcin et al., 2005). Using a random mating outbred group for research may result in errors or mistakes of correlation analysis because they have more alleles per gene locus and fewer individuals with the same genotype. Although this study used such groups, the sample size was larger and our lab has been building a high density genetic linkage map and locating the QTL in common carp, which provides a platform to verify the results.

Linkage analysis between economic traits and genetic markers in wild populations and cultured populations is a long-term unresolved issue, but it has great practical value to germplasm and breeding research of fish. In our study, a technical method was provided to associate economic traits with genetic markers using many co-dominant markers to analyze several large groups. As there are more alleles and genotypes per locus in the outbred group, there should be enough large sample size to overcome errors and reduce mistakes.

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References

Aliah R S, Takagi M, Dong S, Teoh C T, Taniguchi N (1999). Isolation and inheritance of microsatellite markers in the common carp (*Cyprinus carpio* L.). Fisheries Science, 65(2): 235–239

Botstein D, White R L, Skolnick M, Davis R W (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314–331

Crooijmans R P M A, Poel J J, Groenen M A M, Bierbooms V A F, Komen J (1997). Microsatellite markers in common carp (*Cyprinus carpio* L.). Animal Genetics, 28: 129–134

David L, Blum S, Feldman M W, Lavi U, Hillel J (2003). Recent duplication of the common carp (*Cyprinus carpio* L.) genome as revealed by analyses of microsatellite loci. Mol Biol Evol, 20(9): 1425–1434

David L, Rajasekaran P, Fang J, Hillel J, Lavi U (2001). Polymorphism in ornamental and common carp strains (*Cyprinus carpio* L.) as revealed by AFLP analysis and a new set of microsatellite markers. Molecule Genetic Genomics, 266(3): 353–362

De Woody J A, Avise J C (2000). Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology, 56: 461–473

Du C B, Sun X W, Lou Y D, Shen J B (2000). The genetic heterozygosity analysis to wild carp and two cultivated strains of common carp using microsatellite technique. Journal of Shanghai Fisheries University, 9(4): 285–289 (in Chinese)

Garcia F J, Chikhi L, Bonhomme E (1997). Microsatellite polymorphism and population subdivision in natural populations of European seabass, *Dicentrarchus labrax*. Molecular Biology, 6: 51–62

Geng B, Sun X W, Liang L Q, Ouyang H S, Tong J G (2006). Analysis the genetic diversity of *Aristichthys nobilis* in China with 17 microsatellite markers. Heredita (Beijing), 28(6): 683–688 (in Chinese)

Hu X S, Li C T, Ma B, Shi L Y (2006). Preliminary studies on genetic variability of red mirror carp at six microsatellite loci. Journal of Fisheries, 19(2): 37–41 (in Chinese)

Kimura M, Crow J F (1964). The number of alleles that can be maintained in a finite population. Genetics, 49: 725–738

Knott S A, Marklund L, Haley C S, Andersson K, Davies W, Ellegren H, Fredholm M, Hansson I, Hoyheim B, Lundström K, Moller M, Andersson L (1998). Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. Genetics, 149(2): 1069–1080

Kohlmann K, Gross R, Murakaeva A, Kersten P (2003). Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. Aquatic Living resources, 16: 421–431

Lal K K, Chauhan T, Mandal A, Singh R K, Khulbe L, Ponniah A G, Mohindra V (2004). Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882). Journal of Applied Ichthyology, 20(2): 87–91

Li S F (1983). Application of genetic theory and technique to the propagation of cyprinide. Journal of Fisheries of China, 7(2): 175–184 (in Chinese)

Liang L Q, Sun X W (2003). Mapping cold tolerance strain on genetic linkage map of common carp. Journal of Dalian Fisheries University, 18(4): 278–281 (in Chinese)

Liao X L, Yu X M, Tong J (2006). Genetic diversity of common carp from two largest Chinese lakes and the Yangtze River revealed by microsatellite markers. Hydrobiologia, 568: 445–453 (in Chinese)

Liu M H, Bai Q L, Shen J B (1995). Choose breeding and application research of Germany mirror carp. Journal of Fisheries of Heilongjiang, 61(3): 4–10 (in Chinese)

Majumder P, Ghosh S (2005). Mapping quantitative trait loci in humans: achievements and limitations. Journal of Clinic Investion, 115(6): 1419–1424

Quan Y C, Li D Y, Cao D C, Sun X W, Liang L Q (2006). Population genetic variation and structure analysis on five populations of mirror carp *Cyprinus carpio* L. using microsatellites. Hereditas (Beijing), 28(12): 1541–1548 (in Chinese)

Quan Y C, Sun X W, Liang L Q (2005). Microsatellite variation among four breeding populations of common Carps. Zoological Research, 28(6): 595–602 (in Chinese)

Reid D P, Szanto A, Glebe B (2005). QTL for body weight and condition factor in Atlantic salmon (Salmo salar): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). Heredity, 94: 166–172

Shen J B, Liu M H (2000). Breeding Research of Common Carp. Harbin: Sciences Technology Press, 132–162 (in Chinese)

Shen J B, Yan Y Q (1987). Comparative studies on the inheritance of the major morphological traits of *Cyprinus pellegrini*, *C. carpio* (scattered), *C. carpio* (red carp) and their hybrid F_1. Acta Genetica Sinica, 4(1): 49–55 (in Chinese)

Sun X W, Liang L Q (2004). A genetic linkage map of common carp (*Cyprinus carpio* L.) and mapping of a locus associated with cold tolerance. Aquaculture, 238: 165–172

Taniguchi N (2003). Genetic factors in bloodstock management for seed production. Fish Biology and Fisheries, 13: 177–185

Wang Q, Liu M H (1994). Comparative analysis of isenzyme tries about Germany carp, Scatted carp and Songpu carp using electrophoresis. Chinese Journal of Fisheries, 7(2): 68–72 (in Chinese)

Wei D W, Lou Y D, Sun X W, Shen J B (2001). Isolation of microsatellite markers in the common carp (*Cyprinus carpio* L.). Zoological Research, 22(3): 238–241 (in Chinese)

Yalcin B, Flint J, Mott R (2005). Using progenitor strain information to identify quantitative trait nucleotides in outbred mice. Genetics, 171(2): 673–681.

Yin H B, Liu M H, Shen J B, Sun Z W (1995). Nucleontype research of Germany mirror carp. Biology Technology, 5(3): 16–18 (in Chinese)

Yue G H, Ho M Y, Orban L, Komen J (2004). Microsatellites within genes and ESTs of common carp and their applicability in silver crucian carp. Aquaculture, 234: 85–98

Zhang Y, Liang L Q, Chang Y M, Hou N, Lu C Y, Sun X W (2007). Mapping and genetic effect analysis of common carp (*Cyprinus carpio* L.) quantitative trait loci related to body length. Hereditas (Beijing), 29(10): 1243–1248 (in Chinese)