Genetic Biodiversity of the Breeding Population of the Red and White Koi Carp

Dongjie Shi, Hua Zhu, Wentong Li, Saisai Wang and Jiangqi Qu*

Beijing Fisheries Research Institute, National Engineering Research Center for Freshwater Fisheries, Beijing 100068

*Corresponding author E-mail: quqi20122012@163.com

Abstract. Research on genetic biodiversity can essentially reveal the origin of species diversity, variation and evolution. The koi carp (Cyprinus carpio L) is well known for its vigorous body and gorgeous colour. However, there is still lack of knowledge for Koi carp genetic biodiversity. In this paper, the genetic biodiversity of 15 microsatellite loci in breeding population of red and white Koi carp was analyzed. Our results showed that 56 alleles were detected in the population by 15 microsatellite primers, and the number of allele at each locus ranged from 3 to 5, with an average of 3.73. The average observed heterozygosity was 0.6963 while the average expected heterozygosity was 0.6208. The average polymorphism information content (PIC) was 0.557. Out of 15 detected loci, 11 loci were in high polymorphism degree (PIC≥0.5), 4 locus was in medium polymorphism degree. The average Fis is -0.1251. The results indicated rich polymorphism information content and large genetic diversity in the population. This breeding population of the red and white Koi carp had a large selection potential and could be adopted as a base population for further selection.

1. Introduction

The koi carp (Cyprinus carpio L) is one of the five experimental species prescribed by the organization for economic cooperation and development (OECD)[1]. This fish belongs to the Cyprinidae (Cyprinus) of the order Cypriniformes. Koi carp is known for its vigorous body, gorgeous colours, gorgeous stripes, natural swimming posture, and docile habits[2]. After hundreds of years of biological differentiation, gene mutation and artificial breeding, this fish has formed more than 100 species of 13 major strains, including gorgeous body colour, rich stripes and different scales. It is the fish with the most abundant germplasm and genome resources of carp fish at present.

Microsatellite DNA, also known as simple sequence repeats (SSR), is one of the most widely used molecular markers in recent years[3]. It consists of 1-6 bp repeat sequences and flanking sequences. The conservative flanking sequence enables the microsatellite to be specifically located in a definite region of the chromosome, while the difference in the number of core sequence repeats results in the highly polymorphic microsatellite[4]. Due to its advantages of co-dominant inheritance, convenient and rapid detection, and highly polymorphic information content, it has been widely used in population genetic structure analysis, genetic map construction, QTL mapping and marker-assisted selective breeding [5]. There are many reports on studying other strains of carp using microsatellite technology, including genetic diversity, cold tolerance, growth and gender identification [6]–[8], but few reports on analyzing the genetic structure of koi carp. Therefore, in this paper, the genetic
structure of red and white koi carp population was analyzed to provide a genetic basis for further breeding.

2. Materials and Methods

2.1. Materials and DNA extraction
The 36 red and white koi carp used in the experiment came from the Beijing innovation team of Tongzhou ornamental fish industry technology system. The tail fins of the experimental fish were placed in a centrifugal tube containing anhydrous ethanol and brought back to the laboratory. DNA was extracted and the quality and concentration of DNA were tested according to the Promega genome kit and store at -20 °C until use.

2.2. PCR reaction system
15 pairs of microsatellite primers were synthesized by Shanghai Shenggong bioengineering technology services co., LTD. The sequence of microsatellite primers and the annealing temperature after screening are shown in Table 1. The total volume of the reaction system was 15μL, including 10×Buffer 1.5μL, 10mM dNTPs 0.2μL, 5U/ L Taq DNA polymerase 0.2μL, 10μM upstream and downstream primers 0.5 μL, and DNA template 0.2μL. The PCR amplification procedure was: predenaturation at 94° C for 3min; Denaturation for 30s at 94° C, annealing for 30s, extension for 1min at 72° C, a total of 32 cycles; Finally, it was extended at 72° C for 10 min and stored at 4° C.

| Primers | Primer sequence (5' → 3') |
|---------|---------------------------|
| P1      | F:ACTGCTCCTCCTGTGTCTGG       R:TTTTTCATCCAGGCTTCAGTT |
| P2      | F:CCACCTCTACACGCAAATG        R:CAGCAGCAGCTGTCCACTAA |
| P3      | F:ATATAAGAAACTGAGTGTTGG     R:TGGAAATAGCGGTTGGTTAG |
| P4      | F:AGCGCTAATTGGACGGGCTGTG    R:TTCTTTATGATTTGCAAGT |
| P5      | F:AAGAGGAGAGCAGCGAACA       R:GTCAAAACCACAGCGCAGA |
| P6      | F:TTGCGCATATGGTATTGGG       R:CATGGTTATTTTGGAAGG |
| P7      | F:TCTGTTGTGCTTTTTGCTTGGCT  R:CAAGGTTTGCGACAGA |
| P8      | F:CGCGATCGACGATTTTTCGAG    R:TGTCTGACGGCTTTTGG |
| P9      | F:CATCTGAGTGCAAGGTTTTC    R:GCTTAGGTAGTGTTGATTGA |
| P10     | F:TGACAGCTGGCATATTGAG      R:CACTGAGACTGCAAGC |
| P11     | F:ACGTGGTGGTCTCCCAAGAG    R:TAAGTCAGTGCCAGCAGC |
| P12     | F:CTGAAAGTCACAAATCCTGACAA  R:CTCCATTCATTACCCC |
| P13     | F:GAAGATATGACGGACAC         R:CACTACCTATTCCAGTGG |
| P14     | F:GCATTCTTGAGTTGGAATCACA  R:TCGGCAGACGCAGTATTA |
| P15     | F:TTCTCTACCTGTGACATTTGCG   R:CAAGGCTGGCTTAT |

2.3. Genotyping of PCR products
PCR amplification products were detected by 8% non-denature-modified polyacrylamide gel electrophoresis (nPAGE), electrophoresis at 200V for about 3h, and then stained with GoldView nucleic acid dye (purchased from Shanghai SaiBaiSheng genomic technology co., LTD.) for 15min. UVP gel imager was used to observe and take pictures. The software UVP Vision Works TM LS Image Acquisition and Analysis in the gel imager system was used to determine the genotype of the
individual from the electrophoretogram. Each band in the electrophoretogram was denoted as A site, and the allele nearest to the sample hole was coded as A, followed by B and C.

2.4. Data statistical processing
Using PopGene software, MEGA4.0 software (Version 3.2) statistical microsatellite loci alleles number (A), allelic gene fragment length, observed heterozygosity (observed heterozygosity, Ho), expected heterozygosity (expected heterozygosity, He), coefficient of group internal fixation (Fis), polymorphism information content (polymorphic information content, PIC), etc. The polymorphic information content was calculated according to Botstein\[9\] formula (1).

\[
\text{PIC} = 1 - \sum_{i=1}^{n} P_i \sum_{j=1}^{n-1} \sum_{i=j+1}^{n} 2(P_i P_j)
\]

(1)

Where, Pi and Pj are the I and j allele frequency in the population, and n is the number of alleles.

3. Results

3.1. PCR amplification
Genomic DNA was amplified by PCR and detected by electrophoresis with 15 pairs of microsatellite primers, and stable and clear DNA bands were obtained, showing different degrees of polymorphism among individuals (Figure 1).

![Figure 1. The electrophoresis pattern amplified by P7 primers](image)

M: DNA marker (100bp DNA Marker), 1-36: 36 individuals of Koi carp

3.2. Population genetic structure and biodiversity
As shown in Table 2. A total of 56 alleles were detected at 15 microsatellite loci in the population of red and white koi, and the number of alleles at each site was 3-5, with an average of 3.73. Among them, GM558 and U999 sites had the most alleles, a total of 5. Polymorphic information (PIC) ranged from 0.400 to 0.738, among which 11 loci were highly polymorphic (PIC≥0.5), and 4 loci were moderately polymorphic (0.5 > PIC > 0.25). The average polymorphic information content was 0.557.

3.3. Heterozygosity estimation and genetic balance
The observed value of heterozygosity ranged from 0.4722 to 0.8889, and the average observed value of heterozygosity was 0.6963. The expected heterozygosity ranged from 0.4444 and 0.7860, and the average expected heterozygosity was 0.6208. The results indicated that 15 microsatellite loci have high polymorphism in the breeding population of the Koi carp. In this study, the mean fixed coefficient was -0.1251, which was close to 0. And the mean observed heterozygosity was little
different from the mean expected heterozygosity, indicating that the genotype distribution in the population of red and white koi carp was relatively balanced.

**Table 2.** Summary of statistics for Koi carp population with microsatellite analysis

| Primers | No. of allele | Ho (He)          | PIC      | Fis       | allele frequency |
|---------|---------------|------------------|----------|-----------|-----------------|
|         |               |                  |          |           | A     | B     | C     | D     | E     |
| P1      | 4             | 0.7778 (0.6968)  | 0.634    | -0.1319  | 0.2083 | 0.2500 | 0.0972 | 0.4444 |
| P2      | 4             | 0.6389 (0.6616)  | 0.589    | 0.0207   | 0.4583 | 0.1528 | 0.0556 | 0.3333 |
| P3      | 3             | 0.6111 (0.6088)  | 0.520    | -0.0180  | 0.1389 | 0.5000 | 0.3611 |
| P4      | 4             | 0.8889 (0.7046)  | 0.636    | -0.2793  | 0.2222 | 0.3472 | 0.0694 | 0.3611 |
| P5      | 3             | 0.5000 (0.4444)  | 0.400    | -0.1408  | 0.1111 | 0.7222 | 0.1667 |
| P6      | 5             | 0.4722 (0.5309)  | 0.480    | 0.0980   | 0.0556 | 0.2083 | 0.6528 | 0.0556 | 0.0278 |
| P7      | 4             | 0.5556 (0.6553)  | 0.590    | 0.1403   | 0.1389 | 0.4861 | 0.3056 | 0.0694 |
| P8      | 3             | 0.4722 (0.5149)  | 0.440    | 0.0699   | 0.0833 | 0.2778 | 0.6389 |
| P9      | 3             | 0.7222 (0.5869)  | 0.500    | -0.2480  | 0.3611 | 0.5278 | 0.1111 |
| P10     | 4             | 0.8056 (0.6948)  | 0.632    | -0.1757  | 0.1667 | 0.1111 | 0.4444 | 0.2778 |
| P11     | 3             | 0.8611 (0.5794)  | 0.485    | -0.5071  | 0.0972 | 0.3750 | 0.5278 |
| P12     | 5             | 0.7500 (0.7860)  | 0.738    | 0.0324   | 0.1111 | 0.2500 | 0.2222 | 0.2917 | 0.1250 |
| P13     | 3             | 0.8889 (0.6397)  | 0.553    | -0.4092  | 0.1806 | 0.3889 | 0.4306 |
| P14     | 4             | 0.7778 (0.6244)  | 0.537    | -0.2632  | 0.1111 | 0.4167 | 0.4444 | 0.0278 |
| P15     | 4             | 0.7222 (0.6882)  | 0.622    | -0.0642  | 0.2222 | 0.0694 | 0.2639 | 0.4444 |
| average |               |                  |          |          |        |        |        |        |

4. Discussion

Research on genetic biodiversity can essentially reveals the origin of species diversity, variation and evolution[10]. Allele diversity is an important parameter of genetic diversity. The number of effective alleles is the reciprocal of gene homozygosity, reflecting the interaction of alleles, and can also be used as an indicator of population genetic variation [11]. In this study, the number of alleles ranged from 3 to 5, and the average number of alleles and effective alleles were 3.73 and 2.76, respectively. The polymorphic information content (PIC) based on the number of alleles is a better indicator of fragment polymorphism and can reflect the genetic information capacity contained in a genetic marker. According to Botstein et al. (1980)[9], when PIC≥0.5, the site is highly polymorphic, while when 0.25 <PIC<0.25, this site is a low-degree polymorphic site. Among 15 microsatellite loci selected in this study, the mean PIC value of the red and white koi population at the 15 polymorphic loci was 0.557(0.400-0.738). As can show in Table 2, 11 of them belong to highly polymorphic sites, while the
remaining 4 belong to moderate polymorphic sites. It can be seen that 15 sites in the population of red and white koi have higher PIC level and rich genetic diversity.

In addition, the degree of genetic heterozygosity (H) refers to the proportion of heterozygous genotypes at the detected microsatellite loci to all genotypes at the loci, which can reflect the level of genetic variation in the population at each loci. It is generally considered to be an optimal parameter for measuring population genetic variation [12], and its size can reflect the level of population genetic variation. The higher the heterozygosity, the greater the adaptability of the population to environmental changes. The greater potential for evolution and breeding, and the more conducive to the stability and continuation of species [13], [14]. In this study, the average observed heterozygosity of red and white koi carp was 0.6963 and the average expected heterozygosity was 0.6208, indicating the breeding population had a high level of genetic variation for further breeding.

Fis is an important index to measure genetic dynamics of population, and also an important parameter to measure inbreeding recession and outbreeding recession[15]. The values of Fis range from -1 to 1. When Fis value is extremely significant positive value, indicating that the inbreeding degree within the population is serious. The closer the Fis value was to 0, the closer the genotype distribution was the equilibrium state, reflecting the equilibrium relationship between Ho and He. The mean value of Fis obtained in this experiment was -0.1251, which was close to 0, and the mean observed heterozygosity was little different from the mean expected heterozygosity (0.0755), indicating that the genotype distribution of red and white koi carp population was relatively balanced.

5. Conclusions
Our results of this experiment show that the breeding population of red and white koi carp had rich genetic biodiversity and still has great breeding potential, which can be used as the base population for further breeding in the future.

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References
[1] X. Tian et al, 2018. Dynamic regulation of mRNA and miRNA associated with the developmental stages of skin pigmentation in Japanese ornamental carp, Gene, 666: 32–43.
[2] X. Liu et al, 2018. Histopathology and Detection of Cyprinid Herpesvirus Infection in Two Koi Farms in Tianjin City (China), Isr. J. Aquac.-Bamidgeh, 70: 1532.
[3] T. R. H. Kerkhove, B. Hellemans, M. De Troch, A. De Backer and F. A. M. Volckaert, 2019.Isolation and characterisation of 14 novel microsatellite markers through Next Generation Sequencing for the commercial Atlantic seabob shrimp Xiphopenaeus kroyeri, Mol. Biol. Rep., 46(6): 6565–6569.
[4] E. Sawayama, H. Nakao, W. Kobayashi, T. Minami and M. Takagi, 2019.Identification and quantification of farmed red sea bream escapees from a large aquaculture area in Japan using microsatellite DNA markers, Aquat. Living Resour., 32: 26.
[5] S. Jayabalan et al, 2019. Analysis of genetic diversity and population structure using SSR markers and validation of a Cleavage Amplified Polymorphic Sequences (CAPS) marker involving the sodium transporter OsHKT1;5 in saline tolerant rice (Oryza sativa L.) landraces, Gene, 713:UNSP 143976.
[6] D.-D. Zhai, W.-J. Li, H.-Z. Liu, W.-X. Cao and X. Gao, 2019. Genetic diversity and temporal changes of an endemic cyprinid fish species, Ancherythroculter nigrocauda, from the upper reaches of Yangtze River, Zool. Res.,40(5): 427–438.
[7]  J. Xu, J. Feng, W. Peng, X. Liu, J. Feng and P. Xu, 2017. Development and evaluation of a high-throughput single nucleotide polymorphism multiplex assay for assigning pedigrees in common carp, Aquac. Res., 48(4): 1866–1876.
[8]  F. Andriantahina, L. Xiaolin and H. Hao, 2015. Using microsatellite markers to identify heritability of Pacific whiteleg shrimp Litopenaeus vannamei, Acta Oceanol. Sin., 34(6): 59–65.
[9]  D. Botstein, R. L. White, M. H. Skolnick and R. Davis, 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms, Am. J. Hum. Genet., 32: 314–316.
[10]  M. Agarwal, N. Shrivastava and H. Padh, 2008. Advances in molecular marker techniques and their applications in plant sciences, Plant Cell Rep., 27(4): 617–631.
[11]  I. Bbole, J.-L. Zhao, S.-J. Tang and C. Katongo, Genetic diversity of two Southern African cichlids (Oreochromis andersonii and O. macrochir) in the Zambezi and Congo River basins, J. Appl. Ichthyol, 13993.
[12]  B. Brinez R, X. Caraballo O and M. Salazar, Genetic diversity of six populations of red hybrid tilapia, using microsatellites genetic markers, Rev. Mvz Cordoba, 16(2): 2491–2498.
[13]  B. T. Thai, C. P. Burridge and C. M. Austin, Genetic diversity of common carp (Cyprinus carpio L.) in Vietnam using four microsatellite loci, Aquaculture, 269 (4): 174–186.
[14]  M. Morales et al, 2019. Genetic and morphological diversity and population structure of a polyploid complex of Mimosa (Leguminosae), Syst. Biodivers, 1696421.
[15]  P. L. Mwambene, M. Kyallo, E. Machuka, D. Githae and R. Pelle, Genetic diversity of 10 indigenous chicken ecotypes from Southern Highlands of Tanzania based on Major Histocompatibility Complex-linked microsatellite LEI0258 marker typing, Poult. Sci., 98(7): 2734–2746.