SRAP Markers - Based Genetic Biodiversity and Differentiation of Three cultured Goldfish Strains

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Abstract. The genetic biodiversity and differentiation of three representative goldfish species in Beijing were studied using SRAP molecular markers. A total of 122 sites were amplified from 10 primer combinations with good polymorphism, including 99 polymorphic sites. The average polymorphism rate was 80.47% and the average polymorphism information content was 0.264. Diversity indexes of Nei's genes in short-tailed Bubble-eye goldfish, Redhead goldfish and Black Dragon-eye goldfish were 0.225, 0.208 and 0.238, respectively. The Shannon information index of the three groups was 0.342, 0.299 and 0.363, respectively. The proportions of polymorphic loci in the three populations were 69%, 65% and 72%, respectively. The genetic distance indicated that genetic divergence existed between the three groups. The genetic relationship between Bubble-eye goldfish and Redhead goldfish was far, while Redhead goldfish and Black Dragon-eye goldfish was close. This study is of great importance for the research of molecular marker-assisted biodiversity conservation of goldfish.

1. Introduction

Goldfish originated in China, evolved from "gold crucian carp", also known as the "grass goldfish"[1], [2]. At present, various colourful goldfish varieties are formed through a long period of artificial breeding, crossbreeding and selection breeding. Chinese goldfish germplasm resources showed rich genetic diversity. Body colour and morphological changes are the main basis for classification of Chinese goldfish. According to the variety characteristics of goldfish, it can be roughly divided into four categories, respectively as egg species, wen species, dragon species and grass species[3]. However, this classification is based on the internal strains of goldfish, and is completely different from the concept of "species" in biological classification. Therefore, morphological classification is not fully or truly reflects the genetic relationship of goldfish. From the perspective of genetic material DNA to analyze the genetic biodiversity, genetic variation and relationship among varieties of goldfish are of great importance to the germplasm resources and new strains breeding[4].

Sequence related amplified polymorphism, also called SRAP markers, have been widely used in the study of genetic diversity [5], [6], the germplasm resources identification [7] and the genetic linkage maps construction [8], [9]. However, there are few studies on the genetic diversity and relationship of goldfish by molecular genetic markers so far[10]. Therefore, in this study, the most representative Chinese royal goldfish Bubble-eye goldfish, Redhead goldfish and Black Dragon-eye goldfish were selected as experimental materials, aiming to study the genetic biodiversity, classification status and variation relationship by SRAP markers.
2. Materials and Methods

2.1. Materials and DNA extraction
Select each the 30 individuals of Bubble-eye goldfish, Redhead goldfish and Black Dragon-eye goldfish, and cut out their fresh tail fins for experiment. Follow the instructions in the Promega Genome Kit for DNA extraction and store in a refrigerator at ~ 20 ° C until use.

2.2. PCR reaction system
After preliminary experiments, 10 pairs of SRAP primer combinations with good polymorphism were selected (Table 1). In a 15 μl PCR reaction system, Mg$^{2+}$ concentration was 2.5 mmol / L, dNTPs concentration was 0.3 mmol / L, upstream and downstream primer concentrations were 0.8 pmol / L, DNA template was 60 ng, and Taq DNA polymerase was 1.0 U. The amplification reaction was performed on a Takara PCR amplifier. Amplified products were separated by 8% PAGE gel, electrophoresis was performed at 170 V for 2 h, and after electrophoresis, they were stained with GoldView dye (Shanghai Saibaisheng Biotechnology Co., Ltd.) for 15 min, photographed and stored in UVP gel imaging system.

2.3. Data statistical processing
SRAP is a dominant marker, and electrophoretic bands are converted to data in the form of 1/0. Clearly identifiable electrophoretic bands of 100 - 1000 bp are used for statistical analysis. The banded value is "1" and the bandless value is "0". The percentage of polymorphism bands and polymorphism information content (PIC) of each primer pair are calculated. PIC calculation formula: PIC = 1 - Σ fi^2
fi refers to the number of alleles that can be detected by a marker and its distribution frequency, where fi is the allele frequency at a single locus. Using Popgene32, Nei's gene diversity index, Shannon information index, population polymorphic loci ratio, genetic distance and genetic similarity were calculated.

3. Results

3.1. Polymorphism analysis
As shown in Figure 1 and Table 2, a total of 122 sites were amplified by 10 pairs of SRAP primer combinations, and an average of 12.2 bands were amplified per primer combination. The number of sites generated by each primer combination was distributed between 9 and 16. Among them, there are 99 polymorphic loci. On average, each primer combination provides 9.9 marker information.

Table 1. SRAP primers used in the current study

| primers combination | Forward primer (5′–3′) | Reverse primer (5′–3′) |
|---------------------|------------------------|-----------------------|
| F1                  | tgagtccaaacggtcc       | gactgcgtacgaattcca   |
| F2                  | tgagtccaaacggttaa      | gactgcgtacgaattcca   |
| F3                  | tgagtccaaacggagc       | gactgcgtacgaattcca   |
| F4                  | tgagtccaaacggtgc       | gactgcgtacgaattcag   |
| F5                  | tgagtccaaacggttaa      | gactgcgtacgaattcag   |
| F6                  | tgagtccaaacggagag      | gactgcgtacgaattgca   |
| F7                  | tgagtccaaacggagag      | gactgcgtacgaatttgga  |
| F8                  | tgagtccaaacggttaa      | gactgcgtacgaatttgga  |
| F9                  | tgagtccaaacggagac      | gactgcgtacgaatttgga  |
| F10                 | tgagtccaaacggttaa      | gactgcgtacgaattat    |
The number of polymorphic bands amplified by each primer combination is between 6 and 14. The ratio of polymorphic bands produced by each primer combination was 60% - 90.9%, with an average of 80.47%. The amount of polymorphic information (PIC) produced by each primer combination was between 0.195 and 0.326, and the average PIC was 0.264.

![Figure 1. The SRAP patterns of three cultured goldfish populations by F4 primer](image)

**Figure 1.** The SRAP patterns of three cultured goldfish populations by F4 primer

M:100 bp DNA marker. Lane 1-15: short-tailed Bubble-eye goldfish, Lane 16-30: Redhead goldfish, Lane 31-45: Black Dragon-eye goldfish.

**Table 2.** The amplification efficiency of different SRAP primer combinations

| Primer combination | Bands observed | Polymorphic bands | Polymorphism percentage (%) | PIC value |
|--------------------|----------------|-------------------|------------------------------|-----------|
| F1                 | 9              | 7                 | 77.8                         | 0.268     |
| F2                 | 10             | 6                 | 60                           | 0.195     |
| F3                 | 11             | 10                | 90.9                         | 0.280     |
| F4                 | 14             | 12                | 85.7                         | 0.266     |
| F5                 | 16             | 14                | 87.5                         | 0.275     |
| F6                 | 12             | 10                | 83.3                         | 0.274     |
| F7                 | 13             | 9                 | 69.2                         | 0.272     |
| F8                 | 10             | 8                 | 80                           | 0.263     |
| F9                 | 13             | 11                | 84.6                         | 0.326     |
| F10                | 14             | 12                | 85.7                         | 0.225     |

In total 122 99

Average 12.2 9.9 80.47 0.264

**3.2. Genetic diversity**

Nei's gene diversity index, Shannon information index and population polymorphic loci are all good parameters for measuring the degree of genetic variation. Diversity indexes of Nei's genes in short-
tailed Bubble-eye goldfish, Redhead goldfish and Black Dragon-eye goldfish were 0.225, 0.208 and 0.238, respectively. The Shannon information index of the three groups was 0.342, 0.299 and 0.363, respectively. The proportions of polymorphic loci in the three populations were 69%, 65% and 72%, respectively.

3.3. Genetic similarity and genetic distance

It can be seen from Table 3, that the smallest genetic similarity index among the three goldfish populations was the Bubble-eye goldfish and Redhead goldfish (0.793), and the genetic distance is the largest (0.255). The results indicated that these two populations had the highest degree of genetic variation and the most distant relationship. However, the genetic similarity index of Redhead goldfish and Black Dragon-eye goldfish was the largest (0.916) and its genetic distance was the smallest (0.076). It can be concluded that the genetic relationship between these two groups of goldfish was closer.

| Table 3. Genetic identity (above diagonal) and genetic distance (below diagonal) among three populations of goldfish |
|---------------------------------------------------------------|
| **Genetic similarity** | **Genetic distance** |
|----------------------|---------------------|
| **Bubble-eye goldfish** | 0.793 | 0.816 |
| **Redhead goldfish**  | 0.255 | 0.916 |
| **Dragon-eye goldfish** | 0.224 | 0.076 |

4. Discussion

The characteristics of simple, stable and high yield of SRAP marker technology have been confirmed in the analysis of genetic diversity. Hu et al (2012) used the SRAP technique to identify the genetic difference between the Whole Red (WR) population and the Whole White (WW) population, which imply the SRAP method was suitable for discriminating different populations and conducting genetic analysis. Ding et al (2010) used the SRAP technique to analyze the gene differentiation between two cultured populations of grass carp. Their report suggested that some loci showed the SRAP markers attributable to artificial selection, and its fragments are putative markers of germplasm identification. Our study found that the SRAP target fragments of the goldfish population were mainly concentrated between 100 - 1,000 bp, and a total of 122 loci were amplified from 10 highly polymorphic primer combinations in the three goldfish populations, including 99 polymorphic loci, with an average polymorphism rate of 80.47%. It suggested that SRAP marker can detect more genetic sites in the population of goldfish, indicating that SRAP marker is a molecular marker technology with high detection efficiency, which is suitable for the analysis and research of goldfish genetics.

Genetic biodiversity is the foundation of the organisms adapt to the environment and evolution. It is the genetic basis for species to adapt to changing environments and maintain long-term survival and evolution. Mu et al [13] used the Random Amplified Polymorphic DNA (RAPD) technique to analyze the genetic diversity of genomic DNA in four species of goldfish. The results show that grass goldfish and Dragon-eye goldfish, Blisters-eye goldfish and White-head oval goldfish have closer relationships, respectively. Wu et al. [14] also used RAPD technique to analyze the genetic diversity of genomic DNA in four species of goldfish. Results of cluster analysis based on UPGMA indicated that the closest relationship was found between Red & White Moor with Butterfly Tail and Black Moor, then for Tiger head, and finally for Red & White Crown Pearlscale. The genetic distance between populations is a measure of genetic variation. The smaller the genetic distance, the closer the genetic relationship between them is. According to some reports, goldfish evolved from crucian carp around the Song Dynasty. Crucian carp first evolved into grass goldfish, and then grass goldfish differentiated into Red Dragon-eye goldfish and Red-head wen goldfish. Finally, Red Dragon-eye goldfish differentiated into Blisters-eye goldfish. Our results showed that the Bubble-eye goldfish and Redhead
goldfish had the highest degree of genetic variation and the most distant relationship. However, the genetic relationship between Redhead goldfish and Black Dragon-eye goldfish was closer.

5. Conclusions
At present, the species of goldfish are relatively abundant, but it should be noted that the character of some varieties is not stable. Some species still in the stage of preservation and rejuvenation, and some are in the stage of preservation or disappearance. Of course, this is certainly related to the artificial selection, but only to maintain the rich genetic biodiversity of goldfish can provide a variety of options for breeding in the future. SRAP can generate high polymorphic loci in the goldfish genome, which is helpful for the study of goldfish genetic marker-assisted biodiversity conservation in the future.

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