

Analyses of Genetic Diversities in Chinese Fresh-water Fishes*

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ABSTRACT

A wealth of literature has been published in the studies of Chinese fresh-water fish chromosomes within recent years. In this paper, we review the inter- and intraspecific genetic diversities found in Chinese fresh-water fishes, and explore the characteristics and mechanisms that underline these diversities and variations. We also propose the hypothesis how the genetic diversities had evolved in the fresh-water fishes.

Key words fresh-water fishes, inter- and intraspecific genetic diversities

1 Chromosomal karyotypes of Chinese fresh-water fishes

The vast resources of natural water bodies in China harbor a variety of fishes that have evolved through a long history of diversification. Base on the estimation made by Li(1981), there are more than 800 species and subspecies of fresh-water fishes in China. Most of them belong to 32 families in 13 forms. The remaining (about 60) species are migratory fishes, belonging to 21 families in 9 forms. Approximately half of all the fishes are the cyprinids—the largest distribution comparing to other geographic regions in the world.

Since mid 1970s, fish chromosomes and genomes have been extensively studied in China by karyotype analyses, C-banding, Ag-NOR banding (silver staining of nucleolus organizer region), fluorescence staining and single cell DNA quantitation. To date, the chromosomes of 235 species and subspecies (about a quarter of Chinese fresh-water fishes) have been examined. Karyotypes of more than 170 species were reported by our laboratory (Yu X et al. 1987). Those studies unveiled the four basic characteristics on chromosomal diversities in Chinese fresh-water fishes (Yu X et al. 1989).

1.1 Varieties of the diploid chromosome numbers (2n)

The fishes known having the least chromosome number are *Leiobagrus marginatus* (Siluriformes) and *Monopterus albus* (Synbranchiformes). Both have $2n = 24$ chromosomes. However they differ in karyotypes significantly—the former has chromosomes all metacentric or submetacentric except for one or two pairs, and the latter has the chromosomes all acrocentric. The largest chromosomal number, $2n = 446$ chromosomes—more than any chromosome number known in vertebrate, was found in *Diptychus dipogon* (Schizothoraci-

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nae) collected from Lasa River in Tibet Autonomous Region. A total of 28 different $2n$ s have been documented in the fish karyotypes. Majority of these fishes (82%) have $2n$ s ranging from 40 to 60. Two peaks of the chromosomal numbers are located at $2n = 50$ and $2n = 48$, each representing 35% and 27% fishes studied (Fig. 1).

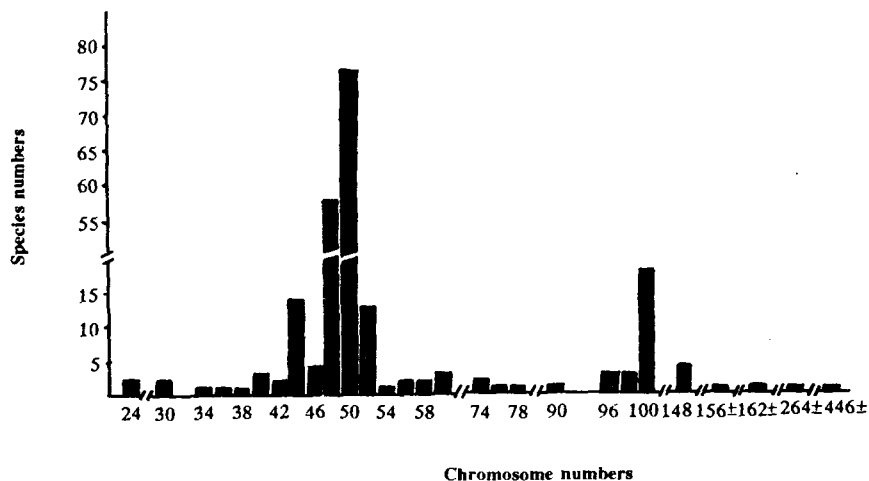


Fig. 1 Distribution of the fish chromosome numbers reported in China

1.2 Noncontinuous distribution of the chromosome numbers

Figure 1 clearly demonstrated the noncontinuous distribution pattern of the fish chromosomal numbers. Chinese fresh water fishes can be divided into seven groups based on several major gaps of their chromosomal numbers: $2n = 24$ chromosomes (group I), $2n = 30$ to 60 chromosomes (group II), $2n = 74$ to 78 chromosomes (group III), $2n = 96$ to 100 chromosomes (group IV), $2n = 148$ to 162 chromosomes (group V), $2n = 264$ chromosomes (group VI) and $2n = 446$ chromosomes (group VII). Since $2n = 48$ and $2n = 50$ represent the chromosome numbers of the common diploid fishes, the fishes in group IV were considered to be tetraploid fishes, such as *Cyprinus carpio*, *Carassius auratus* and *Myxocyprinus asiaticus*. Those in group V evolved through hexaploidization, such as schizothoracid fishes found in Chinese western plateau. *Diptychus dipogon* (Schizothoracinae) in group VII was also believed to be a polyploid.

1.3 Some correlations of the chromosome numbers, fundamental numbers (NF of chromosome arm number) and genomic DNA content to the taxonomy of the fishes

The fishes in lower taxonomic groups usually have more variable and larger $2n$ s than those in higher taxonomic groups do. For examples, the carps in Cypriniformes (a lower taxonomic group compared to Perciformes) have $2n$ s widely spread in groups II to VII, showing a trend of increasing chromosomal number by polyploidization. In contrast, the $2n$ s of the fishes in Perciformes are limited within a narrow range from 42 to 48.

The statistics of NFs and measurement of genomic DNA content demonstrated two apparent trends: 1) the higher taxonomic group that the fishes evolved to, the larger NFs or the more bi-arm chromosomes they have; 2) genomic DNA content increases from the lower to the higher taxonomic groups.

1.4 Differentiation of sex chromosomes present only in a few species

Among the 235 species of fishes, 11 species were found having morphologically different

sex chromosomes. These 11 species are involved sporadically in various evolutionary groups.

2 *The chromosomal changes that lead to genetic diversities in fresh-water fishes*

2.1 Polyploidization

Polyploidization is a genetic change through which the genome size doubles or increases multiple folds, judged by the increase of chromosomal numbers and cellular DNAs. Among the 235 species examined, about 16% (42 species) are tetraploid or hexaploid. A special polyploid form was found in *Diptychus dipogon*.

Ohno (1969) suggested that during vertebrate evolutionary course, polyploidization underwent in the early stage when they lived in water. As land animals evolved, differentiation of sex chromosomes occurred between the two genders, forming the obstacle to polyploidization. Therefore, polyploidization is a primary mechanism during fish chromosomal evolution. Based on Li's analyses (Li Shushen 1981), low temperature is one of the main factors to induce polyploidization in fishes. For an instance, *Schizothoracid* in Yungui and Qinzhang Plateaus of China have evolved from primitive diploid toward tetraploid, hexaploid and higher polyploid under the pressure of chilly weather and poor living environment, and become today's species of fishes that are adapted to the plateau environment. Following polyploidization, the chromosomal complement is fixed through functional diploidization, i. e. only two sets of the genes remain to be functionally expressed. For example, tetraploid fish *Cyprinus carpio* functions as a diploid in gene expression. It has two NORs instead of four. It was hypothesized that polyploid fishes were genetically equipped to gain new functional genes. Such evidence may be found in many tetraploid carps that gained broad geographical distribution and divergence.

Polyploidization also plays a major role in the genetic diversification among populations within the same species. A typical example is *Misgurnus anguillicaudatus* widespread in vast area of China and east Asia. The samples collected from Tai Lake, Shenlongjia mountain, Helongjiang River, Sichuan and Guangxi Provinces all have a diploid karyotype including 50 chromosomes; whereas the populations resided in Hubei and Guangdong Provinces have a tetraploid karyotype comprising 100 chromosomes. Similarly, *M. anguillicaudatus* collected in Japan bears the karyotypes with a various number of chromosomes ($2n = 48, 50, 75$ and 100) among different populations (Ojima Y 1985). It is apparent that the genomic complexity within *M. anguillicaudatus* was derived through polyploidization based on the haploid karyotype ($2n = 25$). Similarly, *Cobitis sinensis* has at least three intraspecific populations with different chromosomal numbers: $2n = 40$ and $2n = 90$ (Guangxi Province), and $2n = 76$ (Shenlongjia Mountain). Obviously, the *C. sinensis* populations evolved through polyploidization and chromosomal rearrangement. In addition, we have found a natural sample of *Ctenopharyngodon idellus* from Wuhan (Hubei Province) that bears triploid (72) chromosomes by using karyotype and restriction endonuclease G-banding analyses.

2.2 Robertsonian translocation

Robertsonian translocation is defined as the reciprocal process, through which two acrocentric chromosomes fuse, or a metacentric chromosome breaks up at centromere. Resulting from the translocation, the $2n$ changes, but the NF remains constant. According to the literature published to date, it seems that the fusion happens more frequently than fission.

Robertsonian translocation was widely observed in fishes. Abundant examples were found in other cyprinid fishes, specially in *Leuciscinae*. It is the major chromosomal recombination identifiable by karyotype analysis. In addition, in *Acheilognathinae*, the 2ns of three closely-related species decrease, in parallel to their evolutionary relationship, from 48 chromosomes (*Ophiocephalus argus*) to $2n = 46$ and 44 chromosomes (*Channa asiatica*), and last, to $2n = 42$ chromosomes (*C. maculata*.) the serial Robertsonian fusion that caused the chromosomal number changes was supported by C-banding and electronmicroscopy techniques. It is worth to note that Robertsonian translocation was only observed among closely-related species/subspecies. Thus, it plays an important role in small scale evolution.

The intraspecific Robertsonian translocation was best demonstrated in *Channa asiatica* (Channidae). The fish collected from Guangzhou (south of Guangdong Province) have $2n = 46$ chromosomes; whereas those obtained from Shashi (Hubei Province) and Shaoguan (North of Guangdong Province) have $2n = 44$ chromosomes. However, these two complements share an equal number of chromosomal arms, suggesting that the variation in chromosomal number between the intraspecific populations was derived through Robertsonian translocation.

2.3 Chromosomes rearrangement

It refers to the chromosomal recombinations including inversion, duplication, translocation and loss except for Robertsonian translocation. The large chromosome rearrangements may be identified by karyotype analyses and the small ones may only be detected by chromosomes banding, *in-situ* hybridization and restriction mapping. We have used Ag-NOR staining technique to resolve the chromosomal rearrangements taken place in *Erythroculter ilishaeformis* and *Culter erythropterus*. The two closely related species of fishes have the karyotypes indistinguishable by Giemsa-staining. However, they differ in NOR numbers and locations significantly. *E. ilishaeformis* has six NORs all located on small submetacentric chromosomes, whereas *C. erythropterus* has eight NORs all located on large or mediumsized submetacentric chromosomes, indicating that chromosomal rearrangement took place during the evolutionary course of these two species. Similar situation has been found in *Cyprinus carpio* and *Carassius auratus*, *Hypophthalmichthys molitrix* and *Aristichthys nobilis*, *Ctenopharyngodon idellus* and *Mylopharyngodon piceus*.

2.4 Heterochromatin amplification

The fishes in the genus *sarcocheilichthys* (Cyprinidae) share a special marker chromosome—a pair of large metacentric chromosomes. The C-banding demonstrated that the short arm of the marker chromosomes comprised of heterochromatin, indicating the heterochromatin amplification accompanied in the evolution of the genus *sarcocheilichthys*. In fishes, heterochromatin not only forms around centromeres, like that in mammal, but also appears at the tip and the middle of chromosomal arms. Obviously, the heterochromatin amplification is an important genetic event involved in fish evolution. In addition, when we examined the DNA base composition in fish heterochromatin, both AT-rich and GC-rich heterochromatin were found in evolutionarily more advanced forms of fishes, but little GC-rich heterochromatin was found in evolutionarily more primitive forms of fishes.

2.5 Supernumerary chromosomes (B-chromosomes)

It represents a special type of genetic diversity. For instance, a various number of B-chromosomes have been found in high-back silver carp (*C. auratus*) and in *Paramisg-grunus dabryanus*.

The five types of chromosomal changes described above have interplayed in a complex manner during fish evolution, resulting in a great extent of genetic diversities.

3 *Genetic diversities and dynamic evolution of fishes*

Genetic diversification of fishes took place at three levels: among the individuals, the populations and the species. The dynamic feature of the genetic complexes provided abundant alternatives for natural selection. Dobzhasky proposed that there were three evolutionary stages in the speciation that all related directly with the genetic diversification and adaptation to environment (Dobzhasky T 1953). In the first stage the genetic diversities are accumulated among individuals through gene mutation and chromosomes variation, which offers genetic varieties for evolutionary selection. The second stage involves in the expansion of the genotypes with adaptive advantage under selective pressure, forming new populations. In the third stage the genetic diversities evolved from the first two stages are so advanced that the new species forms, and interspecific genetic diversities are further developed.

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