Genetic Basis of Unique Dual Modes of Unisexual and Sexual Reproduction in Gibel Carp

Sex is one of important propositions in life sciences, and is "the queen of problems in evolutionary biology" (Bell, 1982, *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*). Since the first unisexual fish, the Amazon molly *Poecilia formosa*, was found in 1932 by Hubbs & Hubbs, about 90 all-female unisexual complexes have been reported in primitive vertebrates, including fish, amphibians and reptiles, and these unisexual species have been demonstrated to reproduce by gynogenesis, hybridogenesis or parthenogenesis. According to traditional evolutionary genetics, there are two major problems for the unisexual animals. One problem is the Muller's ratchet effects of deleterious mutation accumulation because of the absence of genetic recombination and crossovers, and the unisexual species were thereby thought to be short-lived on an evolutionary timescale. The other problem is that the genetic uniformity of the unisexual offspring leads to a much lower genetic diversity, which inevitably makes it much more difficult to adapt to changing environments. However, most of the unisexual vertebrates have been revealed to have an ancient origin, and high genetic diversity has been also demonstrated. The late evolutionary geneticist J. Maynard Smith, Fellow of the Royal Society, thought them as one of the biggest 'scandals' in evolutionary biology, and suggested that studies on them might lead to a deep understanding of the origin and maintenance of sex.

Gibel carp (*Carassius auratus gibelio*), also called silver crucian carp or Prussian carp, is one of polyploid fishes and all-female unisexual populations can be reproduced by gynogenesis. In comparison with other unisexual vertebrate species, gibel carp is more interesting and unique. In natural habitats, there exists a minor but significant portion (approx. 1-10%) of males in gibel carp. Since the 1980s, gynogenesis has been demonstrated using heterologous sperm from other fish species to activate egg and embryo development, and the propagated allogynogenetic gibel carp are widely used for aquaculture. However, there exist a lot of problems we are unable to answer. For example, what do the males contribute to the populations? Why are there abundant clones and genetic diversity in natural populations? Why does strong variety intermixing and fry death exist in aquaculture practice? Do they provide new clues to reveal the puzzles of evolutionary genetics in unisexual animals? These questions and problems have activated our studies since 1995.

To answer the above questions, Prof. GUI Jianfang with the CAS Institute of Hydrobiology (IHB) suggested a systematic resaearch plan on reproduction mode and genetic basis in 1995, and Gu and his cooperators, including IHB Research Fellows ZHOU Li, YANG Lin, LIU Jingxia and ZHU Huaping, have made several new findings.

Firstly, they identified several significant molecular markers including transferrin, isozyme, RAPD, SCAR, microsatellites, AFLP and mitochondria DNA sequences to discriminate different gynogenetic clones. Then, intraclonal homogeneity, interclonal heterogeneity, and homogeneity between males and females of the same clone were revealed by these markers. Based on these efforts, artificial propagation experiments between different clones were performed and genetic recombination evidence in the mated offspring was obtained by cytological investigation and molecular marker analysis. Moreover, recombination evidence was also observed by comparing 10 transferrin allele-coding sequences, in which a total of 26 recombination events were detected, and partial mosaic sequences and breakpoints were identified by identity scanning and information site analyses. Thereby, they discovered dual modes of unisexual gynogenesis and sexual reproduction. When the eggs are inseminated by heterologous sperm from other fish species, the eggs produce clonal lineage of all females by unisexual gynogenesis. When the eggs are inseminated by homologous sperm from gibel carp males, the responding development mode is sexual reproduction, which produces female and male offspring (Figure 1).

The discovery on dual reproduction modes revealed the role of males in maintaining clone diversity of gibel carp, and

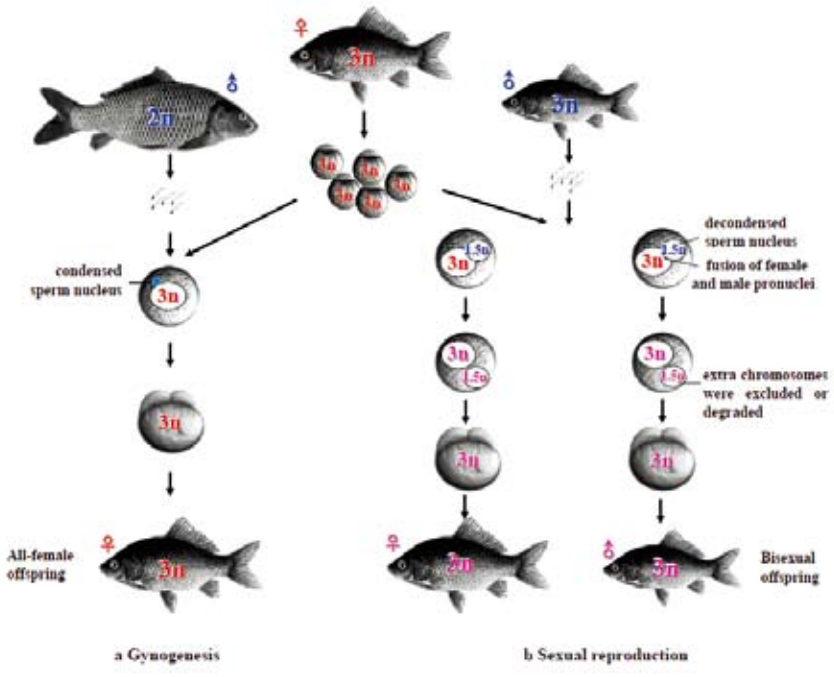

Figure 1: A schematic diagram of the dual modes of gynogenesis and sexual reproduction in gibel carp.

firstly explained the reproduction mechanism of high genetic diversity and long-lived on an evolutionary timescale of unisexual species. The unique dual reproduction mode is the first discovery in vertebrates and provides a special paradigm to resolve the puzzles of evolutionary genetics and to study sex evolution in unisexual animals.

What's more, polyploidy origin of gibel carp was suggested referring to the relative species goldfish with 100 chromosomes. It was known that tetraploidization have occurred in the goldfish, so the gibel carp should be hypertriploid or evolutionally hexaploid. Diverse karyotypes with 156 or 162 chromosomes were revealed from the selected natural clones, and genome reshuffling, chromosome and chromosomal fragment incorporation were demostrated to occur by manipulation or mating between various clones. Its triploid origin was ascertained by 5S rDNA fluorescence in situ hybridization (FISH) and individual chromosome painting. Several unusual allopolyploids with 212 chromosomes were discovered from the gynogenetic offspring of gibel carp activated by red common carp sperm. Moreover, the evidence of one chromosome set of common carp incorporation into the whole chromosomes of gibel carp in the allopolyploids was revealed through genomic *in situ* hybridization using DIG-labeled total common carp genome DNA as one probe and Biotin-labeled total gibel carp genome DNA as another probe.

Besides, there is anonther major breakthrough. Owing to the polyploid background and dual reproduction modes of gynogenesis and sexuality, gibel carp has become a promising model organism for evolutionary developmental (Evo-Devo) biology in vertebrates. A research system was established to identify differential expression of genes in fully-grown oocytes between the gibel carp and sexual crucian carp by using suppression subtractive hybridization, from which a lot of functional genes related to reproduction, such as cyclin A2, C-type lectin, CNBP, and H2A variant, were identified. For example, a novel oocyte-specific core histone H2A variant in gibel carp was identified and its intrinsic ability to modify chromatin properties was revealed by fluorescence recovery after photobleaching (FRAP). Two different cyclin A2 expression patterns during oocyte maturation between the gibel carp and sexual crucian carp were observed. A Ca^{2+}-dependent C-type lectin from the mature oocytes was purified, which associated and translocated with cortical granules during oocyte maturation and became phosphorylated on threonine residues upon induction of exocytosis by fertilization. Studies on CNBP expression pattern and developmental behaviour revealed that it played important roles in folliculogeneis and oogenesis.

Based on these achievements, a criterion procedure of large-scale production of fries was proposed and adopted by the national fishery technical extension center, which has brought significant social and economic benefits since its application. Prof. GUI and his collaborators were awarded for their innovative discoveries and worldwide influence in the field.