Lotus carp

This was first obtained by crossing scattered mirror carp and Xingguo red carp, first conducted in 1975. This carp may reach 6kg after the first year of cultivation.

Jian carp

The varieties of carp we mentioned before are all hybrids. The traits of the offspring of the hybrid carps will be segregated. So new hybrids should be made every year and at least two varieties of parental fish must be conserved in order to obtain the hybrid carps. This can be inconvenient in practice. Jian carp is the first variety of common carp that was produced by artificial breeding in China. The technique applied in its breeding procedure includes hybridization, family selection and gynogenesis. Jian carp has several advantageous characteristics, for example, a high growth rate and fine body shape. The growth advantage of Jian carp is significant. The body weight increment outperforms purse red carp, Yuanjiang carp and Heyuan carp by a factor of 141-250 %, 80-96 % and 40-42 %, respectively. The gross production of this carp may be more than 30% greater than other varieties of common carp.

Molecular studies on the mechanism of heterosis

It takes several years for fish to reach maturity and selecting heterotic crossbreeds from hybrid combinations often takes a lot of selection work, time and effort. Therefore, a study looking for a stable and practical index to predict heterosis is a highly desirable aid for fish breeding. Fisheries scientists in China also have tried to study the mechanism and prediction of heterosis at a molecular level. Random amplified polymorphic DNA (RAPD) technique was applied to analyze the genetic relationship within and between Feng carp and the parental fish. The genetic distance between parental fish and genetic similarity indices between Feng carp and parental carps were calculated. The results showed that the genetic distance between Xingguo red carp and scattered mirror carp was farthest in the experimental fish. The genetic similarity indices between Feng carp and two parental fish were almost same, which indicated that Feng carp inherited equal genetic material from maternal and paternal fish. The same technique was also used to examine the heterozygosity of Jian carp. The result revealed that Jian carp has a higher ratio of polymorphic loci and higher average allelic heterozygosity compared to other carp populations. The advantageous traits of Jian carp may come from the greater heterozygosity of these fish.

Suggestions on the utilization of heterosis

The major factors influencing heterosis include the genetic relationship and the purity of the parents. We know that within a certain range, hybrids generated by varieties with a farther 'genetic distance' (ie. that are less closely related) have a greater heterosis. So in practice, we should use varieties with a farther genetic distance as the parental stock to generate hybrids with high heterosis and superior characteristics. But how can we know which varieties have a greater genetic distance? Molecular biological methods are highly accurate but are not available to farmers. Fortunately, there are some general guidelines that farmers can use to select broodstock varieties with a greater genetic distance:

1. Varieties whose native habitats lie in widely separated geographic regions usually have a greater genetic distance and can be used as parents to produce heterotic hybrids. For example, the crossbreeds of purse red carp (native habitat in Jiangxi, mid-east of China) and Yuanjiang carp (native habitat in Yunan, southwest of China) have a high heterosis.
2. Varieties with greater differences in morphology/body shape or physiology can be used as parental stock to produce crossbreeds of high heterosis. Evidence of this comes from Feng carp and lotus carp whose parental fish are Xingguo red carp (red color and all scales) and scattered mirror carp (caesious color and scattered scales).
3. Another factor influencing heterosis is the genetic purity of the parental fish. Usually, more pure the parent fish are, higher the heterosis of their crossbreeds. It is necessary to select broodstock every year in order to get the hybrids with higher heterosis.

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Progress of fish gene technology research in China

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Research on the genetic modification of fish (transgenic and nuclear transplantation) has been undertaken in China for over two decades, since the first successful human growth hormone gene transfer in goldfish[1]. To date, most research in this area has focused on improving the growth rate of species or other useful traits such as cold-tolerance and resistance to disease. Most of the Chinese research can be categorized into three main groups: 1) Growth hormone gene transformation, 2) anti-freeze protein gene transformation, or 3) disease-resistance gene transformation.

To date, the Chinese researchers have engineered more than 20 species of fishes. Some of these are cloned[2]. However, the first engineered fish to enter commercial production is likely to be a carp developed by the Zhu research group. In this fish the carp beta-actin promoter and grass carp growth hormone have been fused using a combining of gene cloning with nuclear transplantation.

Progress of studies on engineering fish

Growth hormone gene transformation

In the Wuhan Hydrobiology Institute, researchers have conceived and developed an "all fish" growth hormone model. They have cloned and sequenced the grass carp and common carp carbonic anhydrase (CA) gene and growth hormone gene. The grass carp CA gene (beta-actin) promoter has been linked to a grass carp growth hormone cDNA to form a high efficiency expression vector called pCAZ. Using the CAT gene as a reporter gene, a pCA grass carp growth hormone recombinant was microinjected into fertilized, non-activated common carp eggs via the micropyle, generating "all fish" transgenic carp. The presence of the transgene was detected by reverse-transcriptase PCR and Northern blotting. A number of these transgenic fish showed dramatic increases in their growth rate (137% of the control). The growth rate in the first generation offspring is up to 120% of the control[3,4,5]. The transgenic fish from this group has successfully passed field-testing and safety assessment and commercialization has sanctioned.

In other work, an ocean pout anti-freeze protein promoter has been constructed and fused with salmon growth horomone (*Oncorhynchus keta*) (i.e. opAFP-GHc) and microinjected into Tangtufang (*Megalobrama amblycephala*). Carp metallothionein (MT) promoter has been fused with salmon growth hormone then microinjected into the carp. Similar growth-promoting effects were observed in both experiments and field tests.

Anti-freeze protein gene transformation

The ocean pout (*Macrozoarces americanus*) possesses an anti-freeze protein that enables it to reduce the effective freezing point of its blood and tissue. In contrast, the mud carp (*C. molitorella*) is a tropical species with poor cold tolerance that is of economic importance in south China. We tried to improve the cold tolerance of mud carp through gene technology. In our experiments, we microinjected genomic DNA from common carp and ocean pout. In the latter, four out of 73 samples (about 5%) were positive for the presence of the transgene[6]. Field assays of cold tolerance showed that the transgenic fishes could survive better than controls under average temperatures that are 4.0°C lower than normal winter temperatures. Expression of the ocean pout anti-freeze protein in transgenic goldfish (*Carassius auratus*) and its enhancement of cold adaptation has been successfully demonstrated in the Chinese Oceanology Institute. Ocean pout anti-freeze protein genes were microinjected into the oocytes of goldfish. Mature anti-freeze protein, detected by immunoblotting, was expressed in both first and second generation offspring demonstrating successful transfer and expression of the protein gene. Transgenic goldfish were significantly more cold tolerant than controls when challenged with low temperatures. The anti-freeze protein gene may have application in enhancing cold tolerance as well as freezing resistance for a variety of fish species.

Disease-resistance gene transformation

In the Chinese Wuhan Virology Institute and Ichthyology Institute, some researchers have piloted a gene contributing resistance to the grass carp haemorrhagic virus (GCHV). Eleven different gene fragments encoding protein were cloned and isolated from translation in vitro using GCHV genomic single gene fragments. A random library was constructed. Based on the information of capsid protein SP6 and SP7 gene cDNA, 3 oligonucleotides were synthesized and fused with SV40 MT promoter and transferred into grass carp cytokine-induced killer (CIK) cells via a constructed expression vector and transfected with GCHV. The results indicated that the mortalities were reduced by one order after challenge with the virus.

Another transgene anti-disease strategy trialed used a combination of electric fusion and nuclear transplantation methods. Somatic cells from grass carp liver cell strain (GLA), which are resistant to GCHV, were transferred into unfertilized eggs of grass carp by means of electric fusion and microinjection. Morphological studies have shown no distinguishable differences between engineered fish and donor grass carp, which suggests that the nucleus of the engineered grass carp may originate from somatic cells of the GLA. This technique has the potential to provide cell-engineered fish for aquaculture and for basic biological research. Further study on disease–resistant properties of cell-engineered fish to GCHV has been carried out[2].

Other studies on transgenic fish related to basic scientific research

Genetic modification of fish has been carried out to study the relationship between the nucleus and the cytoplasm in terms of the controlling effects determined by nucleus or cytoplasm or both during the development, cell differentiation and phenotypic expression in developing animals. This has generally been carried out using nuclear transplantation (cloning) techniques to produce nucleocytoplasmic hybrid (NCH) adult fish between different varieties, species, genera and subfamilies that are capable of producing viable offspring. These experiments have revealed that while most phenotypic characteristics are controlled by the nucleus, a few are controlled by the cytoplasm or by a combination of both. Some were found to be superior in certain characteristics of economic importance such as growth rate, high protein or low fat content.

Some of this research has indicated that the cultured or uncultured adult somatic cells in fish can support the nuclear transplantation of eggs, which develop into viable adults. A seventeen-month-old goldfish was obtained by transplanting an adult erythrocytes into an enucleated eggs[7]. A sub-cultured kidney cell nucleus of Crucian carp (*Carassius auratus*) has also been transplanted into the enucleated egg of the same species, developing into a three-year old adult female[8].

Our own research with serial nuclear transplantation has shown that a NCH embryo can be developed into blood circulation stage through an inter-genus combination (nucleus of crucian carp and enucleated egg cytoplasm of common carp). A NCH embryo of heart beat stage was obtained in an interfamily combination (nucleus of mud carp and enucleated egg cytoplasm of common carp). A NCH embryo of muscular contraction stage has also been reported from an inter-order mix (nucleus of mouth breeder and enucleated egg cytoplasm of common carp)[9].

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