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Homologous Rearranged DNA can Change Phenotype and Genotype of the Host by Transgenic Method and a QTL Related to Weight was Obtained from it

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Abstract: The research study aim at looking for a simple way to obtain mutant while know what change in the genome of the host. We rearrange carp genomic DNA by digestion, ligation and addition of adaptor and then transferred the homologous rearranged DNA into carp eggs. The results showed that introduction of the homologous rearranged DNA slightly decreased the hatchability of fertilized eggs. PCR products with primers against adaptors amplified from offspring carps had different sizes compared with those amplified from the parent carps, indicating that shuffled genomic DNA has been incorporated into the genomes of offspring. Different size of PCR fragments were obtained after amplification of DNA from two small-size carps that has ceased to develop. Four clones of introducing DNA were sequenced and most of them were microsatellite DNA. Based on one of these sequences, we designed three forward and one reverse primer to amplify the genomic DNA from normal carps and we found that the amplified sequences were homologous rearranged DNA. Four transgenic fish with large body weight were selected as the father and hybridized with common female carp. We gained four groups of offspring. The muscle tissue was chosen as the sample for amplification of introducing DNA fragments. The separation of introducing DNA in three groups is confusing but clear in one group. Further analysis on the group with clear separation shows that the introducing sequence can make the weight of the host drift to the large direction and lower the differentiation between individuals with such sequence. The sequence has no coding function and no region similar to the known regulatory sequence. The study shown that the homologous rearranged DNA can be integrated into the genome of the host and make impact on the host both in genotype and phenotype.

Keywords: Common carp, homologous rearranged DNA, QTL, transgenes

INTRODUCTION

Transgenic technology is now widely used in plant and animal breeding. The genes operated and transferred in common transgenic technology was clearly defined in the function and the result can be predicted accurately. But there were still a lot of deficiencies in the technology. A lot of biological traits were quantitative ones and usually controlled by a lot of loci in the genome that the contribution of one of them to a certain trait was relatively small. How to improve such traits by transgenic technology was still a problem.

QTL location is another research focus, but also there were a lot of problems. How the located QTL can be applied directly into breeding in practice is one of them. Even a QTL was located in high-density genetic linkage group; it is still very difficult to find closely linked sequence because the region where the QTL was located cover large genomic regions (Pomp *et al*., 2004). In eukaryotic genome, coding sequences and corresponding regulatory sequences accounted for only a small part of the genome. Most of the genome is non-coding sequences with unknown function (Lynch and Conery, 2003). The functions of these sequences are now a hot research focus (Feng *et al*., 2009; Guttman *et al*., 2009; Zuckerkandl and Cavalli, 2007; Gregory, 2005). Many QTL may not locate in coding regions but in regulatory regions or other non-coding region. So how to find these sequences with regulatory function is another challenge in the study related to QTL.

Before this study, we carefully analyzed the method with which the heterologous DNA was introduced into plants and animals to improve some quantitative traits. In recent years, such research study s were reported in maize (Liu *et al*., 2008; Zhou and Gao, 2008), rice (Zhang *et al*., 2007), potato (Xiao *et al*., 2008), wheat (Chun-Lei *et al*., 2007) and so on in China. In animals, there were some applications, such as carp (Liu *et al*., 1991) and grass carp (Zhang *et al*., 1997), but no such research reports recently. By RAPD, some researcher found some difference bands which can distinguish them from the host (Wang, 2002; Zheng

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et al., 2002; Liu and Kang, 2006). But no specific sequence is identified in further research, so it is difficult to believe that these bands are surely the introduced heterologous sequences. This method has been widely questioned.

To make sure of if heterologous DNA can improve some quantitative traits after being transferred into the host, we must find what sequences have been introduced into the host genome. At the beginning, heterologous DNA was considered to be digested and added with a designed adaptor as the track sequence. Then we can know what sequences can be preserved in the host by track sequence. However, it is widely accept that most of heterologous DNA fragments will be degraded after being introduced into the host and produce negative effect on the host (Zhang *et al*., 1997; Zhou *et al*., 2006), so application of this approach will let us to detect a large number of offspring to find the suitable result. It is a great workload. So we abandon this idea and consider other way to reduce the degradation of exogenous DNA. Therefore, we assume that the host's own genome was digested with one restriction enzyme, ligated and digest with another restriction enzyme. Then the new DNA fragments were got. All the sequences of these fragments come from the host genome but not original sequence because the order has been disrupted after the second ligation. We think that these sequences could be apt to be kept in the host and make effect on the genome of the host and result in mutations. Homologous rearranged DNA mentioned in this study refers to such DNA fragments.

In this study, we will digest the genomic DNA from common carp with Msp I, ligation for genome rearrangements and then digest them with EcoR I, addition with adaptor as the track sequence, then transfer them into the egg cell by sperm. The effect on phenotype and genotype of the host will be observed and studied.

MATERIALS AND METHODS

DNA samples and carps were derived from Chinese Academy of Fishery Sciences, Freshwater Fisheries Research Center (Jiangsu, China). DNA was isolated from fresh blood samples. The parental carps were artificially inseminated and propagated.

Blood samples (50 μL) were collected from adult carps and added with 450 μL of STE buffer (150 mmol/L of NaCl, 50 mmol/L of Tris, 1 mmol/L of EDTA, 12.5 μL of 10% SDS and 10 μL of protease K (20 mg/mL) for overnight digestion at 55°C. After extraction with phenol and chloroform, equal volume of isopropanol was added to precipitate DNA (12 000 r/min for 30 min). Pellets were washed with 70% ethanol for two times and resuspended in 30 μL of water. The purity and concentration of DNA were analyzed by electrophoresis on 0.8% of agarose gel.

The extracted DNA was digested with Msp|in a 150 μL of reaction volume containing 5 μg of genomic DNA, 2 U Msp I and 15 μL 10×Tango buffer for 16 h at 37°C. Two volumes of ethanol were added to the digestion reaction to precipitate DNA before ligation overnight at 16°C. Ligation reaction was precipitated and re-suspended in water for the second digestion with EcoR I. After second digestion, DNA was added into 10 μL of ligation reaction containing 5×ligation buffers, 50 pmol of adaptor (cgagcaggactcatgatcctcgt agactgcgtacc; attggtacgcagtctacgaggatcatgagtcctgct) and 2.5 U of T4 ligase. Ligation efficiency was analyzed by electrophoresis in 0.8% agarose gel. Two volumes of ethanol were used to precipitate DNA. Precipitated DNA was re-suspended in 10 μL water for preservation.

Homologous rearranged DNA was mixed with lipidosome (4 μL of DNA, 4 μL of lipidosome and 62 μL sperm storing buffer containing 4% sucrose, 3% glycerol and 1% of DMSO) for 30 min at room temperature. Sperm cells (200 μL) and sperm storing buffer (150 μL) were added into the shuffled DNA mixture and incubated for 30 min at room temperature. Mixture of sperm cells and shuffled DNA were added into fish eggs for artificial fertilization. Two controls were included in this experiment. The first control was the artificial fertilization using sperm cells without shuffled DNA and eggs. The second control was using EcoR-digested DNA from tilapia for fertilization. Zygotes were incubated in a water bath and the hatched offspring fish was bred for two months to be observed for phenotypic changes. Fishes were sacrificed using 70% ethanol for DNA extraction.

One forward primer Gmprimer 2 (cagtctacgaggatcatgagtcctgct) was designed based on the adaptor sequences and three reverse random TRAP primers Ga5-800 (ggaaccaaacacatgaaga), Ga3-800 (tcatctcaaaccatatacac) and Odd 26-700 (ctatctctcgggaccaaac) were derived from reference (Hu and Vick). PCR reaction (25 μL) contained 1.0 μL of template DNA, 2.5 μL of 10×PCR buffer, 0.5 μL of TRAP primers (10 mmol), 0.5 μL of Gmprimer2 (10 mmol), 1.5 μL of dNTPs (2.5 mmol), 0.2 μL of Taq (5 U/μL) and 18.8 μL of H_2O. The parameters for PCR reaction includes 2 min of denaturing at 94°C followed by 5 cycles of 94°C for 30 s, 38°C for 45s and 72°C for 2 min and 30 cycles of 94°C for 30s, 55°C for 45s and 72°C for 2 min. After a final extension (72°C for 10 min), PCR reaction was analyzed by electrophoresis on 1.0% agarose gel.

Two fishes that were developed abnormally and had very small size of bodies were selected for genomic DNA extraction. Foreign DNA was amplified by two steps. PCR reaction of the first step contained 1.0 μL of template DNA, 2.5 μL of 10×PCR buffer, 1 μL of Gmprimer1 (actcatgatcctcgtagactgcgtacc) (10 mmol), 1.5 μL of dNTPs（2.5 mmol), 0.2 μL of Taq (5 U/μL)

and 18.8 μL of H_2O. The parameters for PCR reaction includes 2 min of denaturing at 94°C followed by 30 cycles of 94°C for 30s, 62°C for 45s and 72°C for 2 min and a final extension at 72°C for 10 min. PCR reaction of the second step contained 1.0 μL of template DNA, 2.5 μL of 10×PCR buffer, 1 μL Gmprimer1+2 (atcctcgtagactgcgtaccaattn) (10 mmol), 1.5 μL of dNTPs（2.5 mmol), 0.2 μL of Taq (5 U/μL) and 18.8 μL of H_2O. The parameters for PCR reaction includes 2 min of denaturing at 94°C followed by 30 cycles of 94°C for 30s, 60°C for 45s and 72°C for 2 min and a final extension at 72°C for 10 min. PCR products were quantified by electrophoresis on 1.0% agarose. Specific fragments were excised from the gel for purification and T-A cloning before sequencing.

The obtained DNA sequences were blasted on NCBI to search for similar DNA sequences in the database. TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) was used to determine if there were regulatory DNA sequences. Finally, software (DNAMAN) was used to analyze the coded sequences. Primers were designed by using Primer Premier5.

Based on one sequenced DNA, we designed 4 primers for amplification of genomic DNA from normal carps. These four primers were: forward primer1 (gtactgtacaatgtgactgcacact), forward primer2 (gagggctgtgtaggtcaggttt), forward primer3 (tccacct gacaacagaaagaaaga) and (reverse primer tgggac gattatcgcctctcct). PCR reaction of the second step contained 1.0 μL of template DNA, 2.5 μL of 10×PCR buffer, 0.5 μL of forward primer (10 mmol), 0.5 μL reverse primer (10 mmol), 1.5 μL of dNTPs (2.5 mmol), 0.2 μL of Taq (5 U/μL) and 18.8 μL of H_2O. The parameters for PCR reaction includes 2 min of denaturing at 94°C followed by 30 cycles of 94°C for 30s, 58°C for 45s and 72°C for 1 min and a final extension at 72°C for 10 min. PCR reaction was analyzed by electrophoresis on 1.0% agarose gel.

Transgenic carps are cultivated for one year. At the end of March in 2009, four male carp with big body size were selected as father fish and hybridize with one female carp. Four groups of F1 hybrids were obtained and cultivated to July. First, five individuals from each group were taken as the sample to test if the introduced sequences were still into existence. Then one group of them was selected and 70 individuals from it were chosen as the sample. The body weight of every individual was recorded and some muscle tissue was taken from them for genomic DNA extraction.

The method to obtain introduced homologous rearranged DNA and furtherly analyze of them referring the method provided in 2.7 and 2.8.

The data of weight and amplified result were input into SSPS software for further analysis. The mean, maximum, minimum, standard deviation and correlation were calculated.

RESULTS AND DISCUSSION

The size of carp's genomic DNA extracted by phenol and chloroform was approximately 21 kb. After digestion with Msp I and ligation, the size the main band of the shuffled DNA was also ~21 kb. Diffused DNA bands were obtained after EcoR I digestion and addition with the adaptor. There were two types of DNA. The first type is genomic DNA that has adaptor but has not been rearranged. The second type is the rearranged genomic DNA with the adaptor.

Sperm cells with or without foreign DNA were used to fertilize 6000 carp eggs for three times (Table 1). The results showed that there was no direct relationship between the fertilization rate and the existence of foreign DNA. Some of the eggs were not developed normally after fertilization with sperms carrying rearranged DNA. However, the percentage of these abnormally developed eggs was much less than that after transferring heterologous DNA from tilapia into the carp eggs (Table 1).

Different PCR product patterns were obtained with template DNA isolated from the offspring compared to the template DNA isolated from parental or other unrelated normal carps (Fig. 1, compare lane 1-6 with lane 7-20). Some of the PCR products were not amplified from the genome of the parental or other normal carps (Fig. 1). These results indicated that the shuffled DNA sequences were not degraded and most possibly incorporated into the genome of the offspring. The adaptor sequences were artificially designed and were not identified in the NCBI database. Some fragments can be amplified from normal carp's genomic DNA with primers against adaptor and TRAP random primers (Fig. 1). This is possibly due to non-specific binding of the primers during the annealing step at 38°C.

We next tried to amplify the introducing foreign DNA directly. Since the introducing DNA has low-copy number, it is difficult to obtain enough PCR products for sequence analysis. Therefore, we designed two primers. The first primer is used for enriching and the second primer is used for amplification. We selected two small-size carps for PCR analysis. The

Table 1: Fertilization and hatch rate with sperm cells with or without homologous rearranged DNA

		No of carp eggs	Rate of fertilization (%)	Rate of hatching (%)
First time	NF	2152	95	86.5
	HR	2098	95	63.2
	ET	2108	94	1.5
Second time	NF	2332	92	78.5
	HR	2345	93	54.1
	ET	2179	93	0.9
Third time	NF	1987	56	32.8
	HR	2019	57	22.2
	ET	1874	56	0.0

NF: Normal fertilization; HR: Homologous rearranged DNA from carps; ET: EcoR-digested DNA from tilapia

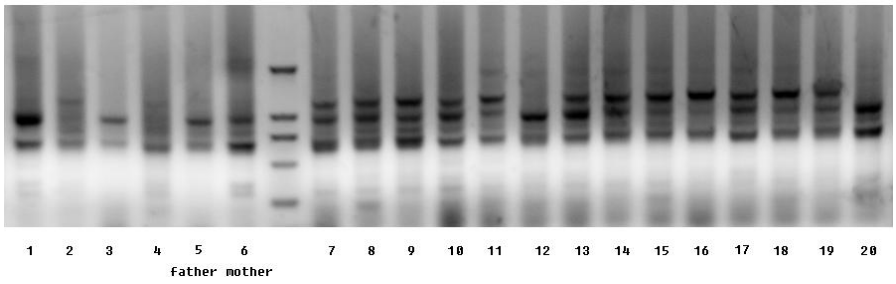

Fig. 1: PCR product pattern using different combinations of TRAP primers Ga3-800 and Gmprimer 2
Lane 1~4: PCR product amplified with template DNA from normal carps; Lane 5: PCR product amplified with template DNA from father carp; Lane 6: PCR product amplified with template DNA from mother carp; Lane 7~20: PCR product amplified with template DNA from offspring carps

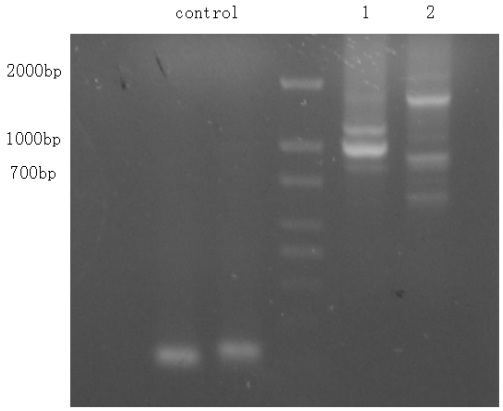

Fig. 2: PCR product after enriching and amplification steps with primer Gmprimer 1 + 2
Lane 1 and 2: PCR product with template DNA isolated from transgenic carp No. 1 and 2

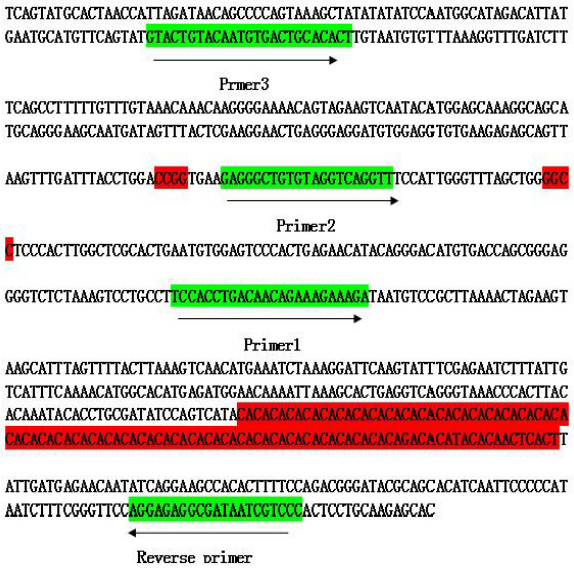

Fig. 3: Integration sequences with primer binding sites
Sequences highlighted in red indicate the satellite sequences and restriction enzyme sites; Sequences highlighted in blue indicate primer binding sites

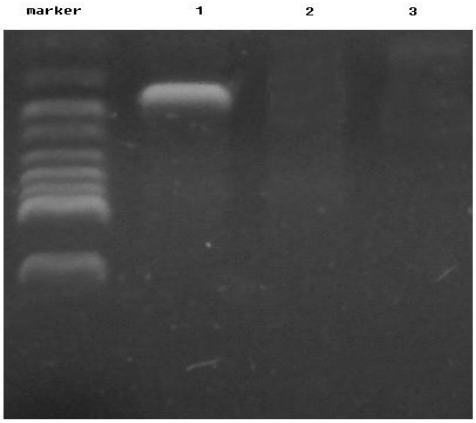

Fig. 4: PCR products amplified with primer Gmprimer 12
Lane 1 and 2: PCR product with template DNA isolated from 2 small-size transgenic carps No. 1 and 2

results showed that the introducing DNA in one carp was approximately 900 bp (Fig. 2, lane 1) while ~1800 bp PCR product was amplified from another small-size carp (Fig. 2, lane 2). These results indicated that different transgenic carps have different size and number of introduced DNA. Furthermore, these results also suggested that alterations in different genomic sites may result in the same phenotypic changes (Fig. 3).

The amplified DNA fragments from Fig. 4 were sequenced after T-A cloning. Two types of DNA sequence were obtained from carp No. 1. The first type of DNA sequence has a size of ~400 bp, which may come from the original carp's genomic DNA. The second type of DNA sequence has a size of 880 bp containing one microsatellite sequence and two CCGG sites. The second type of DNA sequence is most possibly derived from shuffled DNA. Two types of DNA were also obtained from carp No. 2. The first one is small (~600 bp) and contains two CCGG sites. The main DNA fragment cannot be sequenced because there was a microsatellite sequence with 201 repeats in the upstream and a microsatellite sequence with 102 repeats in the downstream, which may result in

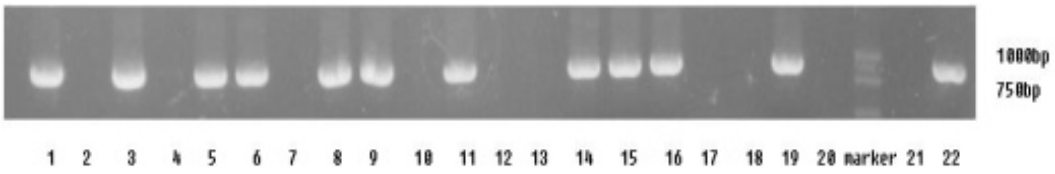

Fig. 5: Introduced sequence in the hybrid F1 generation
1-20: Offspring; 21: Mother; 22: Father

TATCCTCGTAGACTGCGTACCAATTGCTGCAAAACCAGACTCCCTCAGTCTGATGGTCT
TGCCAGGGGGGGCTCCACCTGCATTGTTGGTGCTGAGCTAGGGAGAGGCTGAGAGCC
TATAGGAAAAAATAAAACAGCTGGGTGAGGAAGAAGACGGCGGATGGAAGCAGACTT
GATGGAATAACCCACCGCTGTCCACAATCTCAACAGGATATTGCCATCAGGTATGTGAG
ATCTTGATTGTTGTTTGAGTGATTTATGTGTTTTAAAACCATTGTTTTTCATGTCTCTTAT
GATAATAAGGAGGGTGGTGCACTATGCCACTGTCTGGAAAGCCACAGAGAGGCTGTTG
AAATACAAAAGACTAAGCAGAAAATGGAAAAGATCTCGAAGAAGATGTTAAAAAAAC
GTTTATACAAAAAAAAAAAAAAAGAAAAAGATAGGCTATATGGAGAATTAAATAGGC
TACTGGAATTTGATCTTGTACTATCGTACTATCTAAAGACTTAGCCTATATACTATAAATG
TATTTATTATCATCATTATTGTTGATACCAAAAGAGATTTAGGCTAATGTTAAATTACTTAA
TTCGGTCTTATTTACGTGCATTGACAGTCTTGAGACTGGCTAGGTGCTGAAGGCTGCTG
TTTATGTTGGCAGGGTGAGGCCGTGTCATGAAATCTGGGCCTTTTTCGTAATTCAAA
TGTTTTCAGTTTCAAAAGCCAGACAGATTTAGACTGAAAATTTAGACGGCCAGACAG
TTCCAAAGGCCAGATAGATTTAGATAATAGGAGAGGGAAAGCGTCGTCTATGAGTATA
TAGAGCATTTTTTAAATTAGATGCGTTACTGAGGCAATTGGTACGCAGTCTACGAGGAT
AATCTCT

Fig. 6: Introduced sequence

Table 2: Statistics analysis of two groups

Group	Number of individuals	Mean	S.D.
1	37	2.5964	0.89017
2	33	2.2380	1.03931
Correlation	0.191		

S.D.: Standard deviation

formation of complex secondary structure. These sequences do not encode any proteins and have no similarities with any regulatory sequences. Blast analysis showed there were no similar sequences available in NCBI database.

Based on one sequenced DNA of carp No. 1, we designed 4 primers for amplification of genomic DNA from normal carps Forward primer 1 and reverse primer produced a 400 bp PCR product while forward primers 2 and 3 do not produce any PCR product (Fig. 4) indicating that DNA in the restriction site downstream of the forward primer 2 binding sequences was shuffled. No PCR products were obtained from normal carps.

We choose some fish with large body size and raise them until April 2009 (Fig. 5). As male fish become mature faster than female fish, so we selected four mature male fish for semen. One female jian carp was chosen for eggs. The eggs were divided into four groups and fertilized with the semen from four male transgenic fish respectively. The offspring of four groups were raised to July. Five individuals from each group were taken as the sample for extraction of genomic DNA. Then introduced DNA fragments were amplified. The amplified result in three groups was very confusing and difficult to interpret. We took seventy individuals again for further analysis from the only group which has a clear separation result. In Fig. 6, it is the result of amplification from 70 individuals. We can find that there are paternal-specific bands in 11 individuals while 9 individuals have not amplified products. The result is closely in line with the separation of 1:1.

All sequences obtained from father and offspring were cloned and sequenced. The result shown they were all the same sequence. The sequence was 886 bp long and contained four GGCC regions which may be rearranged sites. Further analysis of the sequence shown that it did not code for proteins and had no region similar to known regulatory sequences. Through the NCBI web site, we did not find any close sequences. The sequence is shown in Fig. 6.

In Table 2, group 1 was consisted of those from which introduced sequence can be amplified while group 2 was consisted of those from which no introduced sequence can be amplified. From the table, we can know that the group contains introduced sequence is higher in maximum, minimum and mean higher than the other group which does not contain introduced sequence, but smaller in standard deviation. It indicated that the group contains introduced sequence is larger in the shape than the other group, but also smaller differentiation within the group. Correlation analysis show the correlation between introduced sequence and body weight is 0.191.

DISCUSSION

The choice of Msp I for the first digestion was based on the following consideration. The cutting site of Msp I was in ccgg region, most of which were located in the coding region. For example, in completed sequences of the chromosomes 2 and 4 of Arabidopsis thaliana, the GC content of exons is 43.6 and 44.08%, respectively. In introns, these values drop to 32.1 and 33.08%, respectively (Lin *et al*., 1999; Bevan *et al*., 1998). So the first digestion by Msp I can be to get as much as possible the DNA fragments rearranged in coding region, it can rise mutation ratio. However, the result does not support this opinion. The obtained sequences were all located in non-coding region.

In this study, the rearranged DNA was introduced into carp's eggs by sperm cells. The method applied in the study is not widely used because of a lot of disadvantages. But if the method was good was not the

focus of this research. As a transgenic approach, it was proved to be effective in the study.

In this study, 4 uL DNA solution (about 2 ug genomic DNA) was mixed with 200 uL semen which contained approximately 5.9×109 sperm (normal carp sperm density of 29.4×109/mL) (Wang and Zhao, 2000). The genome of common carp was approximately 1.0×109 bp long (Crooijmans *et al*., 1997). If average molecular weight of one DNA base was 333 Daltons, 2 ug genomic DNA contained approximately 3.04×10-6 pmol DNA. The cutting site of EcoR I was consisted of six bases. If four kinds of bases were randomly distributed in the genome, the number of cutting sites of EcoR I in carp genome was probably 2.44×105. Two ug genomic DNA can produce 4.47×1011 fragments and roughly one sperm can carry 75 fragments. Because some fragments were lost during the precipitation by ethanol, but each sperm can still carry dozens of DNA fragments.

Many researchers can get mutants in phenotype by introduction of foreign DNA into the host (Liu *et al*., 2008; Zhou and Gao, 2008; Zhang *et al*., 2007; Xiao *et al*., 2008; Chun-Lei *et al*., 2007). There were a lot of such research reports in crop breeding and some reports in fish breeding. Zhang *et al*. (1997) reported that introduction of DNA fragments from carps into the zygotes of grass carps resulted in massive death of zygotes and high percentage of abnormal offspring. Our initial hypothesis is that introduction of rearranged DNA from carp genome into carp eggs may also result in massive death of zygotes and high percentage of abnormal offspring. However, our results showed that rearranged DNA only caused slight decrease in hatching rate and did not cause significant abnormal offspring. We can't find related research study in the last few years. The reason may be complex and need further research. It must be emphasized that different phenotypes such as size, weight, color occurred after introduction of rearranged DNA, indicating that the rearranged DNA may have certain effect on phenotype of the offspring.

In order to make sure if the results in 2008 can be repeated again, we did such experiment again in the April of 2009 and the offspring was cultivated for four months. We obtained three individuals whose scales were larger than others, three red individuals, one dark individual, one huge individual from nearly 700 descendants. This show that these introduced rearranged DNA was sure to make certain impact in the phenotype of offspring.

One of the particular interesting observations in this study is that different genotypes were identified in all the collected 30 offspring carps, indicating that most of adaptor sequences and introduced sequences were not degraded. The result was repeated in 2009. The F1 hybrid between transgenic male carp and common female carp in 2009 also shown a lot of rearranged DNA sequences were kept in the genome of transgenic fish. Because each sperm can carry dozens of DNA fragments, if all the rearranged DNA sequences were kept in the host cell or some have been degraded needs further research.

Because the foreign genes in this study were derived from the genome of the same species and most of them were not degraded by the host, furthermore, these foreign genes do not have significant effect on the expression of host genes. Therefore, we think these rearranged DNA is not recognized as real foreign genes by the host.

Adaptor and TRAP random primers were used to amplify the upstream and downstream sequences of the introducing DNA. Normally, there are 4 types of introducing DNA within the host. First of all, introducing sequences can be free DNA that is not integrated into host genomes. Secondly, the introducing DNA can be "head-tail" ligated and existed as episome. Thirdly, "head-tail" ligated introducing DNA can be integrated into the genome. Finally, introducing DNA can be individually integrated into the host genome. In theory, adaptor and TRAP random primers will not amplify any PCR products for the first three situations. Our PCR results indicated that TRAP random primers have some stable binding sites in the genome and some introducing DNA have been individually integrated into the host genome. The separation of introducing DNA in F1 hybrid between transgenic male carp and common female carp in 2009 made sure of the result that most introducing DNA has been individually integrated into the host genome

The key issue of molecular breeding is the integration of foreign DNA into the genome of offspring. However, it remained questionable since there was no direct evidence available to demonstrate if some foreign DNA integrated into the genome. In this study, we introduced rearranged DNA containing a 35 bp-adaptor into the host and the results showed that these rearranged DNA were not degraded, suggesting that hosts can tolerate long-term existence of a few less homologous DNA sequences within the nucleolus. Capecchi (1989) and Hunger-Bertling *et al*. (1990) invented gene targeting put forward a new method SFHR (Small Fragment Homologous Replacement) as an improvement of gene targeting. They all originated from homologous recombination. The 400-800 bp single-stranded DNA (single-strand DNA, ssDNA) or double-stranded DNA (double-strand DNA, dsDNA) homologous with the target gene sequence were introduced into cells and these DNA fragments contained specific mismatched bases or the absence or insert of one or a few bases for correcting the genome sequence with the original one or more nucleotide mutations (Gruenert, 1998; Yanez and Porter, 1998). We think the homologous rearranged DNA may make the similar effect on the host as the small fragments in

SFHR. Because there are a lot of similarities among the genomes of different species, these similar DNA sequences are not recognized as foreign genes by the recipients and are maintained in the genome of the recipient for long time. In addition, these foreign genes may interfere with the assembly of the recipient genome, leading to the occurrence of new phenotypes.

Because transgenic female fish didn't become mature within one year, so in this study, four transgenic male fish were used for hybridization with one female common carp. Four groups of F1 generation of hybrids were obtained. The results of amplification in three groups of F1 hybrids were confusing and difficult to explain only based on the classic theory of the separation and recombination. Possible reasons included:

- Genomic DNA was extracted from muscle tissue. Since most of the first generation of transgenic animals is chimeric individuals (Cui and Zhu, 1998), so the rearranged DNA fragments in muscle tissue are not the same as those in testis.
- Because each sperm carried dozens of pieces, these introduced fragments separated and recombined in F1 hybrid and become different genotypes.
- Because these introduced fragments came from religation among different homologous sequences located in different chromosomal region or different chromosome, during meiosis, some mistakes can occur in autosynapsis. An unknown repair mechanism may lead to generate new genotypes. The specific reasons need further study.

We obtained rearranged microsatellite sequences from 2 small-size, non-developing carps. Particularly in the carp No. 2, religation of the two microsatellite sequences caused changes in the secondary structure, which makes us unable to know what the sequences exactly are. Microsatellite sequences are widely distributed in the eukaryotic organisms and recent research found it play an important role on the regulation of gene expression (Lu *et al*., 2009; Storojeva *et al*., 2005). Our results demonstrated that changes in the location of the same microsatellite sequences resulted in the alteration of gene expression. Furthermore, the results obtained from carp No. 2 also indicated that the microsatellite sequences regulate gene expression by affecting the secondary structure of up- or downstream sequences.

In this study, we obtained a rearranged sequence related to body weight roughly. After the fragment was integrated into the genome and transferred into the F1 hybrid, the individuals which contain the sequence in the genome drift to the great direction in the distribution of the weight. Correlation analysis found that the relevance between the sequence and the weight is rough. Body weight is important quantitative trait and controlled by complex contributions of genetics and environment (Pomp *et al*., 2004). Therefore, the result still has a certain value. This fragment related to body weight has no coding function, but also may has no similar region to known regulatory function sequence. With earlier results obtained in this experiment, we found that the sequences related to body shape obtained in the study are all non-coding sequences.

The results of this study show that introducing homological rearranged DNA fragments into the host will have some impact on the phenotype and genotype of the host. But in this experiment, what we obtained is all rearranged DNA fragments with non-coding sequences and no rearranged DNA fragments found come from coding sequence. In 2009, we began to study what will happen after introducing the DNA fragment rearranged from the coding sequences or micro-satellite into the eggs of carp. The results show that these fragments can make significant changes in shape and lethal effects on the offspring. The results need further verification.

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