VARIABILITY OF MITOCHONDRIAL DNA OF TRIPLOID HYBRIDS OF CRUCIAN CARP AND COMMON CARP

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Abstract: The article presented the results of the first regular population study of triploid hybrids of crucian carp and common carp by sequencing the tRNA-Thr gene, tRNA-Pro gene, and partial control region of mitochondrial DNA (mtDNA). The 26 sequenced fragments ranged from 837 to 839 bp in length. The termination associated sequence (TAS) motifs, central conserved sequence blocks (CSB-F, CSB-E, CSB-D), and conserved sequence block (CSB1) were identified in the mtDNA control region. Most heteroplasmic fish had 3—5 TAS repeat units. The above sequences from 26 triploid hybrids were detected 65 polymorphic sites, yielding 8 haplotypes. Sequence divergence among different haplotypes ranged from 0.1% to 6.3%. This study provided some invaluable information for the reproducibility and genetic improvement of triploid hybrids.

Key words: Triploid hybrids; Mitochondrial DNA; Control region; Sequence variation
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Red crucian carp (*Carassius auratus* red var., ♀, 2n = 100) and common carp (*Cyprinus carpio* L., ♂, 2n = 100) belong to different genera. The crossing between them is considered distal. The F_2 hybrids were derived from crossing males with females of F_1, and F_3 hybrids were developed by crossing males with females of F_2. The cytological analysis revealed that the females and males of F_2 hybrids were able to produce diploid eggs and diploid sperms that fertilized each other to form tetraploid fish in F_3. Thus, the hybrids of F_3 to F_{11} of red crucian carp and common carp were proved to be allotetraploids (4n = 200) with two chromosome sets of red crucian carp and two chromosome sets of common carp[1,2]. In the allotetraploid population, both females and males are fertile. Similar to F_3-F_{11}, F_{12}-F_{15} hybrids were also confirmed as allotetraploids (data not published). The shape of the F_1-F_2 was intermediate to red crucian carp and common carp. The tetraploids of F_3 to F_{15} generations displayed similar morphological phenotype (shape and color) also intermediate to red crucian carp and common carp, which can be stably inherited from generation to generation. The phenotype of F_3 to F_{15} hybrids is a little different from that of F_1-F_2. It is the first example of creation of an allotetraploid population in a vertebrate by successive generations of hybridization. Our successful establishment of the allotetraploid population in vertebrate provided important diploid-gamete resources to create triploid hybrids through mating F_3-F_{15} hybrids (♂) with Japanese crucian carps (*Carassius auratu* cuvieri) (♀). Triploid hybrids are sterile, grow faster and have high survival rate, and thus greatly enhance their annual production[3]. It is necessary to reveal the genetic diversity of triploid hybrids since they are so excellent fresh fish with high quality.

The mtDNA of most animals ranges in size from 16 kbp to 20 kbp and encodes 13 protein subunits, 22 tRNAs, and two rRNAs. The only substantial noncoding segment, the so-called control region or displacement loop (D-loop), encompasses the sites of initiation of H-strand replication and both H- and L-strand transcription. The

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mammalian control region is organized into three major regions, or domains, including the extended terminal associated sequences (ETAS), central, and conserved sequence block (CSB) domains[4]. The extended terminal associated sequences (ETAS), central conserved sequence blocks (CSB-F, CSB-E, CSB-D) and conserved sequence blocks (CSB-1, CSB-2, CSB-3) of the mitochondrial DNA control region in cyprinids with the different ploidy level were identified[5]. Because of the short conserved elements and the propensity for rapid change, the control region is one of the most interesting parts of the vertebrate mitochondrial genome. Substantial length variation has been found in the control region of many mammalian and fish mtDNA[6—8]. In addition to length variation, substantial nucleotide sequence variability in the control region has been recorded both within and between species[9,10]. Furthermore, the degree of genetic variability characteristic of the control region has been widely exploited in studies of population structure[11] and can be useful in identifying meaningful population subdivisions[12]. It is an important prerequisite to effective conservation, management and monitoring. Such analyses of mitochondrial sequence data have already proved invaluable in examining population genetic diversity and constructing phylogeny in a number of fish species[8,13,14]. In this study, we compared sequence information from the mitochondrial tRNA-Thr gene, tRNA-Pro gene, and part control region of triploid hybrids to assess the degree of population genetic diversity. At present, the population structure of triploid hybrids has attracted our considerable interests, not only because of its importance for the management of freshwater fisheries, but also because of fundamental interest in application for its popularization in national and international market.

1 Materials and methods

1.1 Fish samples The 28 triploid hybrids were captured from Chinese National Tetraploid Fish Protection Station located in Hunan Normal University.

1.2 Total DNA extraction The total DNA was isolated from blood samples collected from the triploid hybrid caudal veins by using DNA Extraction Kit from Shanghai Sangon, Inc. The extraction method was performed according to the manufacture's instructions. The concentration and quality of DNA were assessed by the agarose gel electrophoresis, and the final extracts were dissolved in TE buffer and kept in the 4 ℃ for later use.

1.3 PCR amplification The polymerase chain reaction (PCR) amplifications were carried out in a 50 μL final volume containing 100—200 ng DNA, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 1 μmol of each primer, 1 × of amplification buffer and 1 unit of Taq-polymerase (Promega). The following primers were used to amplify fragments of the entire mtDNA tRNA-Thr gene, tRNA-Pro gene, and partial control region sequences: forward primer L (+) (5′-GGACAAATTGCATCCGTCCT-3′); backward primer H (-) (5′-GTTTCGGGGTTTGACAAGGATA-3′). The amplification conditions (30 cycles) were the following: 94 ℃ for 45s, 55 ℃ for 45s, 72 ℃ for 1min, followed by final 5min extension at 72 ℃. PCR amplification was carried out on a programmable thermal controller (GeneAmp® PCR System 2700). To detect PCR products, 5 μL of the reaction mixture was applied to a gel of 1.5 % agarose and electrophoresed in the standard 1 × TAE buffer. The gel was stained with 0.1 μg/mL ethidium bromide for 20min.

1.4 mtDNA sequencing The final PCR products were run on a 1.5 % agarose gel at 120 V for 1.5h, and purified by QIAquick Gel Extraction Kit (Qiagen). Direct sequencing of the PCR product was performed with the above same PCR primers using an automated DNA sequencer (ABI PRISM 377, Pekin-Elmer) following the protocol for cycle sequencing in Shanghai Sangon, Inc.

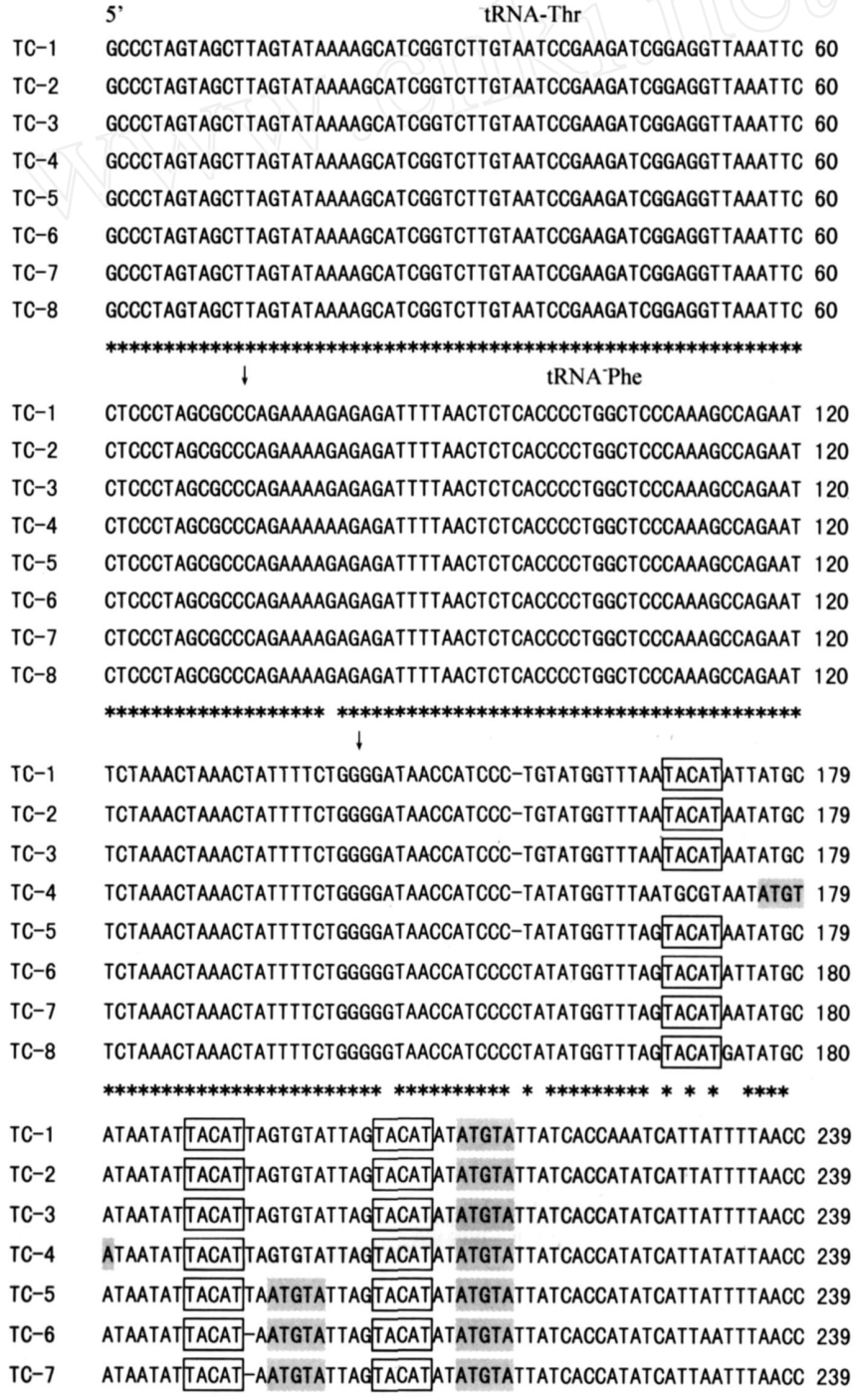
1.5 Sequence analyses Sequence analyses were performed using the GCG package of computer programs (Version 7.0; Genetics Computer Group Inc., Madison). Database searches were carried out using the Jellyfish program. The initial points of the CSB-F and CSB-1 were used as the demarcations of the extended terminal associated sequences, central and conserved sequence block domains, respectively. Localization of conserved sequences was done using BESTFIT. The mtDNA nucleotide sequences in the triploid hybrid population were aligned using CLUSTAL W package.

2 Results

2.1 Direct sequencing

Almost for all samples, PCR products generated clear sequencing results, though a few samples showed "messy" sequences at the beginning. This messy start was likely due

to incomplete removal of primer dimmer during the purification of PCR products or the presence of nonspecific PCR products, resulting in mixed and confusing base callings. All sequences were therefore double checked on electrophoregrams. Among 28 triploid hybrids, the PCR products of 26 fish were successfully sequenced except an unexpected "accident" for two individuals. Obviously, BLAST searches showed that the sequenced fragments of 26 fish included mtDNA tRNA-Thr gene (1—72bp), tRNA-Pro gene (71—142bp), and partial control region (143—837bp, 143—838bp, 143—839bp) after removing of areas of ambiguity as well as missing data (Fig. 1). The control region of the above 26 fish included the extended terminal associated sequence (ETAS) domain, central conserved sequence block domain, partial conserved sequence block domain. In the triploid hybrids, the central conserved sequence block domain contained three central conserved sequence blocks (CSB-F, CSB-E, CSB-D) identified in the 5′ end of the control regions. The CSB-1 conserved sequence block was identified in the 3′ end of the control regions (Fig. 1).



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TC-8    ATAATATTACATTACTGTATTACTACATATATGTATTATCACCATATCATTAATTTAACC 240
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TC-1    CCAAAGCAAGTACATATAAACTAAGGTATGCATAAAGCATAATCTTAAGACTCACAAGTT 299
TC-2    CCAAAGCAAGTACATATAAACTAAGGTATGCATAAAGCATAATCTTAAGACTCACAAGTT 299
TC-3    CCAAAGCAAGTACATATATTAAGGTATGCATAAAGCATAATCTTAAGACTCACAAATT 299
TC-4    CCAAAGCAAGTACATATAAACTAAGGTATGCATAAAGCATAATCTTAAGACTCACAAGTT 299
TC-5    CCAAAGCAAGTACATATGAACTAAGGTATGCATAAAGCATAATCTTAAGACTCACAAGTT 299
TC-6    ATAAAGCAGGGACATATATGTGAAGGTATACATAAAGCATAACATTAAGACTCACAAATT 299
TC-7    ATAAAGCAGGGACATATATGTGAAGGTATACATAAAGCATAACATTAAGACTCACAAATT 299
TC-8    ATAAAGCAAGTACATATATGTGAAGGTATACATAAAGCATAATCTTAAGACTCACAAGTT 300
          ****** * ******     ******* ***********  ************* **

TC-1    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAGAAATTGTCCTCACATTTTTCCTT 359
TC-2    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAGAAATTGTCCTCACATTTTTCCTT 359
TC-3    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAGAAATTGTCCTCACATTTTTCCTT 359
TC-4    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAGAAATTGTCCCCACATTTTTCCTT 359
TC-5    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAGAAATTGTCCCCACATTTTTCCTT 359
TC-6    AAATTATTTCGACCCGGGTAATATATTATTCCCCAAAAAATTGTCCTCACATTTTTCCTT 359
TC-7    AAATTATTTCGACCCGGGTAATATATTATTCCCCAAAAAATTGTCCTCACATTTTTCCTT 359
TC-8    AAATTATTTTAACCCGGGTAATATATTATTCCCCAAAAAATTGTCCTCACATTTTTCCTT 360
        *********  ************************* ********* *************
                                                  CSB-F
TC-1    GAATGATTCAGCTAAGGTTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 419
TC-2    GAATGATTCAGCTAAGGTTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 419
TC-3    GAATGATTCAGCTAAGGTTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 419
TC-4    GAATGACCCAACTAAGGTTTTATTCAAACATATTAATGTAGTAAGAGACCACCAACCA-T 418
TC-5    GAATGACTCAACTAAGGTTTTATTCAAACATATTAATGTAGTAAGAAACCACCAACCA-T 418
TC-6    GAATGGATCAACTAAGATTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 419
TC-7    GAATGGATCAACTAAGATTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 419
TC-8    GAATGGATCAGCTAAGGTTTTATTCGAACATATTAATGTAGTAAGAAACCACCAACTAAT 420
        *****   ** ***** ******** ******************** ********* * *
                                          CSB-E
TC-1    TTACATAAAGGAATATCATGCATGATGGAATCAGGGACACCAACTGTGGGGGTTGCACAA 479
TC-2    TTACATAAAGGAATATCATGCATGATGGAATCAGGGACACCAACTGTGGGGGTTGCACAA 479
TC-3    TTACATAAAGGAATATCATGCATGATGGAATCAGGGACACCAACTGTGGGGGTTGCACAA 479
TC-4    TTATATAAAGGAATATCATGCATGATAGAATCAGGGACATCAATTGTGGGGGTTGCACAA 478
TC-5    TTACATAAAGGAATATCATGCATGATGGAATCAGGGACACCAACTGTGGGGGTCGCACAA 478
TC-6    TTACATAAAGGAATATCATGCATGATGGAATCAAGGACACTAATCGTGGGGGTTGCACAA 479
TC-7    TTACATAAAGGAATATCATGCATGATGGAATCAAGGACACTAATCGTGGGGGTTGCACAA 479
TC-8    TTACATAAAGGAATATCATGCATGATGGAATCAAGGACACTAATCGTGGGGGTTGCACAA 480
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TC-1    TGTGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCT 539
TC-2    TGTGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCT 539
TC-3    TGTGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCT 539
TC-4    TATGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCC 538
TC-5    TATGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCT 538
TC-6    TATGAACTATTACTGGCATCTGGTTCCTATTTCAGGGACATAACTGTAATATCCCACCCT 539
TC-7    TATGAACTATTACTGGCATCTGGTTCCTATTTCAGGGACATAACTGTAATATCCCACCCT 539
TC-8    TGTGAACTATTACTGGCATCTGGTTCCTATTTCAGGTACATAACTGTAATACTCCACCCT 540
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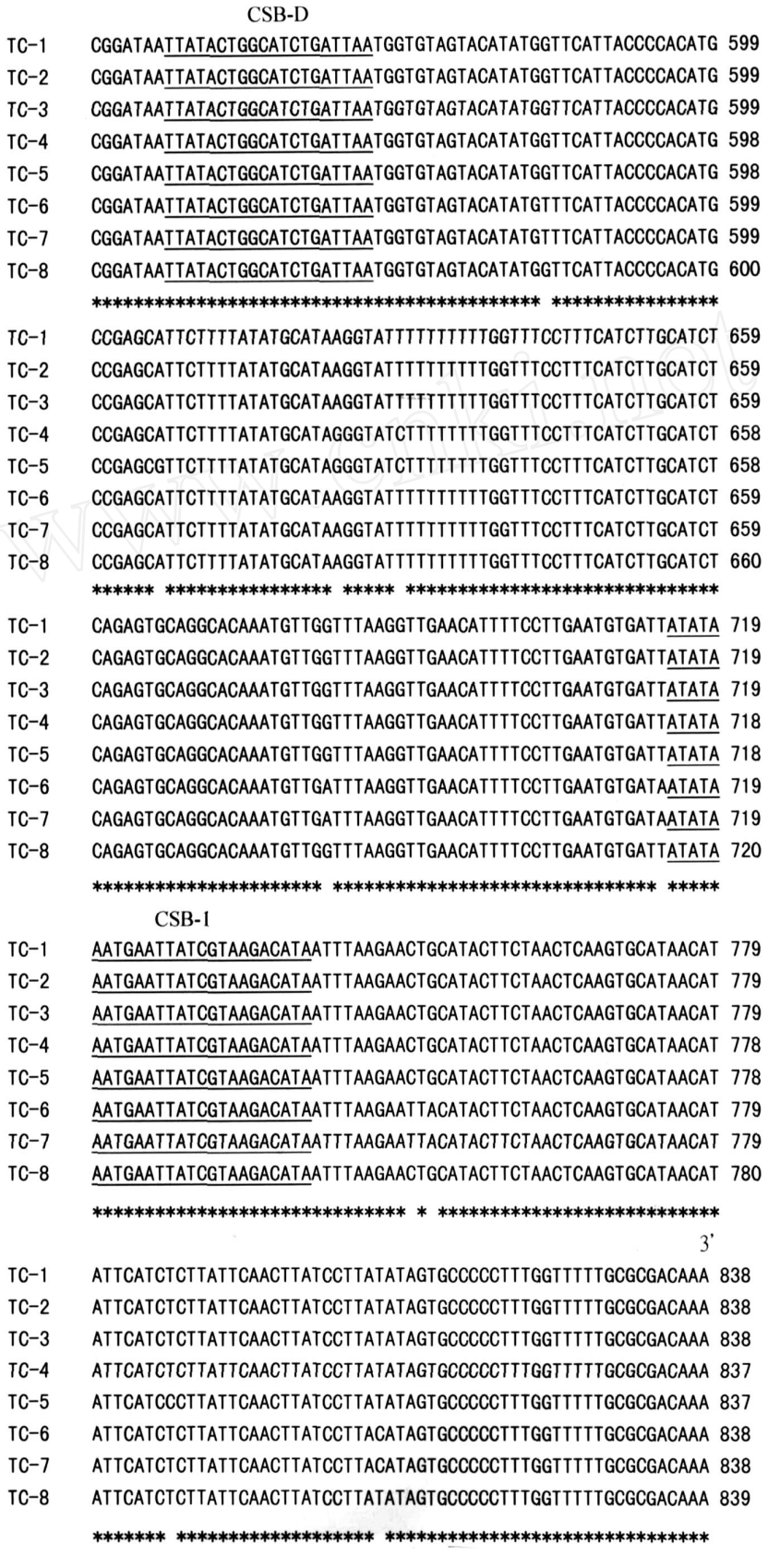

Fig. 1　The nucleotide sequence comparison of the mtDNA tRNA-Thr genes, tRNA - Pro genes and partial control regions in the 8 triploid cruian carp haplotypes. The alphabets ahead every row are abbreviations of the triploid cruian carp haplotypes, dashes are gaps required for alignment, and " * " indicates the same base site. The borders of the tRNAs are indicated by arrows. The conserved sequence block (CSB) is underlined. The TAS motifs (TACAT) are framed, and the palindromic motifs (ATGTA) are indicated by shadows

2.2 Sequence diversity

For the 26 samples examined, the length of the mtDNA tRNA-Thr gene, tRNA-Pro gene, and partial control region sequences ranged from 837 to 839 bp (Fig. 1). On average, base composition was A 31.9 %, T 33.1 %, C 19.5 % and G 15.5 %. Among the 26 triploid hybrids, 65 polymorphic sites were detected, and yielded 8 haplotypes that were named TC-1, TC-2, TC-3, TC-4, TC-5, TC-6, TC-7, and TC-8, respectively (Fig. 1). Sequence divergence calculated by nucleotide diversity among different haplotypes ranged from 0.1 % to 6.3 % (Tab. 1). When all the DNA sequences were aligned together, variations can clearly be seen among different haplotypes (Fig. 1). As expected, individual variations were mainly observed in amplified control region fragments. We observed 64 polymorphic sites of sequence in mtDNA control region and 1 polymorphic sites of sequence in the mtDNA tRNA-Thr gene, tRNA-Pro gene. Thus, more than 98.5 % polymorphic sites occurred in mtDNA control region. Especially, about 60 % polymorphic sites were concentrated at position 143—395 of the 5' end sequence of mtDNA control region, which was an active hypervariable region. Therefore the terminal associated sequence domain showed a greater number of variable sites, while the less variability was found in conserved blocks between pair position 143 and 839. All these polymorphisms included 48 transitions, 14 transversions and 3 indels. So the main variation detected in the population was due to nucleotide substitutions, rather than insertion/deletion mutational events. The distribution showed a large bias towards transitional changes rather than transversional changes.

Tab. 1 The sequence variation rate of the mtDNA tRNA-Thr genes, tRNA-Pro genes and partial control regions in the 8 triploid hybrid haplotypes

Haplotypes	TC-1	TC-2	CTC-3	TC-4	TC-5	TC-6	TC-7
TC-1							
TC-2	0.2						
TC-3	0.6	0.4					
TC-4	2.8	2.6	3.0				
TC-5	2.1	1.8	2.2	2.2			
TC-6	4.7	4.7	4.3	6.3	5.2		
TC-7	4.8	4.6	4.2	6.2	5.1	0.1	
TC-8	2.7	2.4	2.3	4.5	3.6	2.8	2.7

2.3 Repeat sequences and length variations

The repeat region, responsible for length variations and heteroplasmy, was located near 5' end of the control region. In contrast to some other fish species[15], these repeat units contained the terminal associated sequence (TAS) motifs. TAS sequences were also present in the 8 triploid hybrid haplotypes (Fig. 1). There were three TAS motifs in the TC-4 haplotype, four TAS motifs in the TC-1, TC-2, TC-3, TC-5, TC-6 and AT-7 haplotypes, and five TAS motifs in the TC-8 haplotype. Moreover, TC-6 and TC-7 haplotypes had 1 bp deletion at position 193, and TC-4 and TC-5 haplotypes had 1 bp deletion at position 417 in the conserved sequence block domain, however, TC-6, TC-7 and TC-8 haplotypes had 1 bp insert at position 156 in the terminal associated sequence domain (Fig. 1).

3 Discussion

The control region is unique because of a faster rate of evolution as compared with the rRNA and protein-coding genes of mitochondrial genome. This region in the triploid hybrid follows the general structure previously described[4]. Concerning the selection of an appropriate section of the control region for maximum variability, the ETAS and central CSB domains provide the greatest variability for population analyses. The ETAS has been shown to be effective for population analysis[16—20]. If small indels are a concern, the CSB region should be avoided. However, if indels are of interest for the analysis, the CSB domain is an ideal region for examination. The mtDNA control region can provide an appropriate region for examination for a number of different types of studies. However, the use of a single domain may provide all of the resolution necessary for a specific analysis, thus reducing the cost of a research project by reducing the required amount of DNA sequencing.

In the present study, summarizing the distribution of repeat units in the 8 haplotypes of the 26 triploid hybrids, there had three TAS motifs in the TC-4, four TAS motifs in the TC-1, TC-2, TC-3, TC-5, TC-6 and TC-7, respectively, and five TAS motifs in the TC-8. This indicated a selective mechanism within the distribution of tandem arrays because without such a driving force a more or less even magnitude of distribution of tandem arrays would be expected or theoretically the number of repeat units should be unlimited. The TAS motif and its palindromic motif

(ATGTA) can form the stable hairpin-loop structure, which may be the main body of conserved extended termination-associated sequences (ETASs), suggesting it was a conserved secondary structure that may contribute to TAS function. Generally, in some TAS tandem arrays, only one TAS was the functional motif, and other TASes were the results of the intra-and intermolecular recombination or illegitimate elongation without performing function.

Among the 26 triploid hybrids, 65 polymorphic sites were detected and 8 haplotypes (TC-1, TC-2, TC-3, TC-4, TC-5, TC-6, TC-7 and TC-8, respectively) (Fig. 1) were yielded. Sequence divergence among different haplotypes ranged from 0.1% to 6.3% (Tab.1). The results indicated that the triploid hybrid population had the relatively abundant genetic diversity. Triploid hybrids were produced through mating allotetraploid hybrids (♂) with Japanese crucian carps (♀). More generally, the mtDNA is inherited maternally. According to the mtDNA maternal heritance characteristic, in fact, the analysis of the mtDNA polymorphism of triploid hybrids is equivalent to study on the variability of mtDNA coming from female parents. Thus this research indicated that Japanese crucian carp population also had the relatively abundant genetic diversity. The presence of the genetic diversity of Japanese crucian carps indicated that this population from Chinese National Tetraploid Fish Protection Station located in Hunan Normal University could have the abundant genetic resources for their genetic breeding such as producing the high quality sterile triploids on a large scale. The maintenance of genetic variation is a major objective of most species conservation plans[21]. From the results obtained, it is possible to draw a number of inferences on the breeding, genetic conservation, and population rejuvenation of triploid hybrid.

The amount of genetic diversity is important because it represents the raw material for evolution and adaptation. More genetic diversity in a species or population means a greater ability for some of the individuals in it to adapt to changing environments, including new pests and diseases and new climatic conditions. Less diversity leads to uniformity, which is a problem in the long term, as it is unlikely that any individual in the population would be able to adapt to changing conditions. The analysis at the physiological, cytological and biochemical revealed that triploid hybrids possessed many advantages such as sterility, faster growth, high survival rate, facile fishing and delicious taste[1]. The present study showed the triploid hybrid population had the abundant genetic diversity by analysis of mtDNA tRNA-Thr genes, tRNA-Pro genes, and partial control regions from the molecular level, providing the supporting evidence that this population may have a greater ability to adapt to changing environments. In conclusion, the triploid hybrid is a species of excellent fresh fish with high quality. It is worth popularizing in the national and international markets. We thus are amplifying the breeding area of triploid hybrids to increase production of the fresh fish that not only supply the actual requirement in the national market, but also provide the wide foreground to make profit in foreign markets.

References:

[1] Liu S J, Liu Y, Zhou G J, *et al*. The formation of tetraploid stocks of red crucian carp ×common carp hybrids as an effect of interspecific hybridization [J]. *Aquaculture*, 2001, **192**: 171—186

[2] Sun Y D, Liu S J, Zhang C, *et al*. The chromosome number and gonadal structure of F_9-F_{11} allotetraploid crucian carp [J]. *Acta Genetica Sinica*, 2003, **30**(5): 37—41 [**孙远东, 刘少军, 张纯, 等. 异源四倍体鲤鲫 F9-F11 代的染色体和性腺观察. 遗传学报**, 2003, **30**(5): 37—41]

[3] Guo X H, Liu S J, Xiang B, Liu Y*et al*. Sequence analysis of three *sox* gene hmg box in the triploid crucian carp [J]. *Acta Hydrobiologica Sinica*, 2005, **29**(1): 102—105 [**郭新红, 刘少军, 向兵, 刘筠, 等. 三倍体湘云鲫 3 个 *Sox* 基因保守区的序列分析, 水生生物学报**, 2005, **29**(1): 102—105]

[4] Pesole G, Gissi C, Chirico D E, *et al*. Nucleotide substitution rate of mammalian mitochondrial genomes [J]. *J. Mol. Evol.*, 1999, **48**: 427—434

[5] Guo X H, Liu S J, Liu Y. Comparative analysis of the mitochondrial DNA control region in cyprinids with different ploidy level [J]. *Aquaculture*, 2003, **224**: 25—38

[6] Moritz C, Dowling T E, Brown W M. Evolution of animal mitochondrial DNA: relevance for population biology and systematics [J]. *Annu. Rev. Ecol. Syst.*, 1987, **18**: 269—292

[7] Rand D M. Endotherms, ectotherms, and mitochondrial genome-size variation [J]. *J. Mol. Evol.*, 1993, **37**: 281—295

[8] Sell J, Spirkovski Z. Mitochondrial DNA differentiation between two forms of trout Salmo letnica, endemic to the Balkan Lake Ohrid, reflects their reproductive isolation [J]. *Mol. Ecol.*, 2004, **13**(12): 3633—3644

[9] Foran D R, Hixson J E, Brown W M. Similarities of ape and human sequences that regulate mitochondrial DNA transcription and their DNA synthesis [J]. *Nucleic Acids Res.*, 1989, **17**: 5841—5861

[10] Shaffer H B, McKnight M L. The polytypic species revisited: Genetic

differentiation and molecular phylogenetic of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex [J]. *Evolution*, 1996, **50**: 417—433

[11] Avise J C. Molecular markers, natural history and evolution [M]. Chapman and Hall, New York. 1994

[12] Moritz C. Applications of mitochondrial DNA analysis in conservation: a critical review [J]. *Mol. Ecol.*, 1994, **3**: 401—411

[13] Tang Q Y, Liu H Z, Yang X P, *et al*. Studies on the structure of the mitochondrial dna control region and phylogenetic relationships of the subfamily botiinae [J]. *Acta Hydrobiologica Sinica*, 2005, **29**(6): 47—55 [唐琼英, 刘焕章, 杨秀平, 等. 沙鳅亚科鱼类线粒体 DNA 控制区结构分析及系统发育关系的研究. 水生生物学报, 2005, **29**(6): 47—55]

[14] Aboim M A, Menezes G M, MSchlitt T, *et al*. Genetic structure and history of populations of the deep-sea fish Helicolenus dactylopterus (Delaroche, 1809) inferred from mtDNA sequence analysis [J]. *Mol Ecol.*, 2005, **14**(5): 1343—1354

[15] Nesbø C L, Arab M O, Jakobsen K S. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*) [J]. *Genetics*, 1998, **148**: 1907—1919

[16] Rosel P E, France S C, Wang J Y, *et al*. Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers [J]. *Mol. Ecol.*, 1999, **8**: S41—S54

[17] Ehrich D, Federov V B, Stenseth N C, *et al*. Phylogeography and mitochondrial DNA (mtDNA) diversity in North American collared lemmings (*Dicrostonyx groenlandicus*) [J]. *Mol. Ecol.*, 2000, **9**: 329—337

[18] Matson C W, Rodgers R E, Chesser R K, *et al*. Genetic diversity of *Clethrionomys glareolus* populations from highly contaminated sites in the Chornobyl region [J]. *Environ. Toxicol. Chem.*, 2000, **19**: 2130—2135

[19] Zardoya R, Castilho R, Grande C, *et al*. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea [J]. *Mol. Ecol.*, 2004, **13**(7): 1785—1798

[20] Kochzius M, D. Blohm D. Genetic population structure of the lionfish Pterois miles (*Scorpaenidae*, *Pteroinae*) in the Gulf of Aqaba and northern Red Sea [J]. *Gene*, 2005, **347**: 295—301

[21] Avise J C, Hamrick J L, Conservation genetics: case histories from nature [M]. Chapman & Hall, NY, 1996

三倍体湘云鲫线粒体 DNA 序列变异性分析

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摘要: 对 26 尾三倍体湘云鲫的线粒体 tRNA-Thr 基因、tRNA-Pro 基因和部分控制区的核苷酸序列进行了测定, 获得 26 条长度为 837—839 bp 的同源基因序列, 共发现 65 个多态性核苷酸变异位点, 多态位点比例为 0.077, 定义了 8 种单元型。在湘云鲫 8 种单元型中确认了 DNA 复制终止相关的序列 TAS、中央保守区序列 (CSB-F、CSB-E 和 CSB-D) 和保守序列 CSB1, 8 种单元型含有 3—5 个 TAS 序列。在 65 个变异位点中, 大部分序列变异为转换, 8 种单元型之间的序列差异在 0.1%—6.3%之间。该研究为三倍体湘云鲫的繁殖和遗传改良提供了一些有价值的信息。

关键词: 三倍体湘云鲫; 线粒体 DNA; 控制区; 序列变异