Molecular Phylogenetics and Evolution 66 (2013) 350–359

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

High male incidence and evolutionary implications of triploid form in northeast Asia *Carassius auratus* complex

Fang-Fang Jiang [a,b], Zhong-Wei Wang [a,b], Li Zhou [a,b], Long Jiang [a], Xiao-Juan Zhang [a], Olga V. Apalikova [c], Vladimir A. Brykov [c,d], Jian-Fang Gui [a,b,*]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100039, China
[c] A.V. Zhirmunsky Institute of Marine Biology, Far East Branch, Russian Academy of Science, Vladivostok 690059, Russia
[d] Far Eastern Federal University, School of Natural Science, Vladivostok, Russia

ARTICLE INFO

Article history:
Received 24 July 2012
Revised 1 October 2012
Accepted 8 October 2012
Available online 23 October 2012

Keywords:
Gynogenesis
Unisexual animals
Carassius auratus gibelio
Triploid
Reproduction mode
Evolutionary implication

ABSTRACT

Carassius auratus complex is believed to originate from East Eurasia and consist of diploid and triploid forms. Diploid form reproduces sexually, whereas triploid form possesses mixture modes of unisexual gynogenesis and sexual reproduction, which makes it a unique case to study evolutionary issues among vertebrates. In this study, we identified 337 triploid individuals from 386 specimens of *Carassius auratus* complex sampled from 4 different sites of Xingkai Lake and Suifen River on the northeast Asia transboundary areas of Russia and China, and found that triploids were ubiquitous, whereas diploids existed only in SII site of Suifen River. Triploid males were detected in all surveyed sites, and an unusually high triploid male incidence (23%) was found in the Chinese reach of Suifen River. Then, nuclear and cytoplasmic markers were used to analyze their genetic diversity and phylogenetic relationship. A total of 61 distinct *tf* alleles and 35 mtDNA CR haplotypes were revealed. Higher genetic diversity and divergence were confirmed in triploids than in diploids, and identical genetic background between triploid males and females was demonstrated. Moreover, evolutionary implications and roles of triploid males were suggested in population proliferation and diversity creation of the triploid form.

1. Introduction

It is theoretically known that in the absence of segregation and recombination, unisexual species cannot generate enough genetic variations to cope with changing environments and to purge deleterious mutations, and therefore generally be considered to be evolutionarily 'short-lived' in spite of their ecological advantage on fast colonizing a new habitat in a short term (Shcherbakov, 2010). However, most of unisexual vertebrates, such as kleptogenetic *Ambystoma* salamanders (Bi and Bogart, 2010; Spolsky et al., 1992), hybridogenetic *Poeciliopsis* fish (Quattro et al., 1992), gynogenetic Amazon molly (Lampert and Schartl, 2008; Schartl et al., 1995), *Phoxinus eosneogaeus* hybrids (Angers and Schlosser, 2007), and gynogenetic *Cobitis* (Janko et al., 2003), have been revealed to have long history and large ranges of geographical distribution (Avise, 2008). And, high genetic diversity has been extensively observed in gynogenetic or hybridogenetic fish (Angers and Schlosser, 2007; Cunha et al., 2011; Schmidt et al., 2011; Stöck et al., 2012), kleptogenetic amphibians (Bi and Bogart, 2010) and parthenogenetic reptiles (Fujita et al., 2007; Kupriyanova, 2009). Significantly, these rare unisexual animals are largely associated with polyploidy origin (Neaves and Baumann, 2011; Otto et al., 2007). In plants, polyploidy has been confirmed to be ubiquitous (Jiao et al., 2011), and polyploidy roles in increasing allelic diversity, altering genomic complexity, introducing novel traits, driving ecological transfiguration, and especially facilitating plant invasions have been suggested extensively (Doyle et al., 2008; Parisod et al., 2010; te Beest et al., 2012). Owing to the rarity of polyploid forms, the evolutionary consequence of polyploidy *per se* and the evolutionary potential of unisexual reproduction maintenance remain unknown in vertebrates.

Carassius auratus complex has been generally thought to consist of both diploid form and triploid form that cannot be discriminated morphologically from each other (Apalikova et al., 2011; Gui and Zhou, 2010; Jakovlić and Gui, 2011; Takada et al., 2010), even though tetraploidization has been demonstrated to occur in the diploid form with 100 chromosomes (Ohno et al., 1967), and evolutionary hexaploid has been also suggested for the triploid form with more than 150 chromosomes (Zhou and Gui, 2002; Zhu et al., 2006). According to the current taxonomic category, the triploid form, also commonly known as gibel carp, silver crucian carp

* Corresponding author at: State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.
E-mail address: jfgui@ihb.ac.cn (J.-F. Gui).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.10.006

or Prussian carp, has been recognized as a subspecies gibel carp *Carassius auratus gibelio* (Cherfas, 1981; Jiang et al., 1983) of *Carassius auratus*, including the domestic goldfish, or even a separate species *Carassius gibelio* (Kalous et al., 2007; Kalous and Martin, 2011; Rylková et al., 2010). Previously, it was recorded only in Japan (Kobayasi, 1971), Russia (Cherfas, 1981), and China (Jiang et al., 1983; Zhou and Gui, 2002). Recently, along with cytogenetic and molecular marker development, it has been extensively observed in many countries of the Eurasian continent, such as in Britain, Italy, Germany (Hänfling et al., 2005), Hungary (Tóth et al., 2005), Greece (Liousia et al., 2008), Czech Republic (Kalous et al., 2007; Veteŝník et al., 2007), Kazakhstan (Sakai et al., 2009), and Croatia (Jakovlić and Gui, 2011). In the past decade, a lot of genetic knowledge including molecular basis of reproduction trait and numerous polymorphic DNA markers has been investigated and characterized from the triploid form (Gui and Zhou, 2010; Gui and Zhu, 2012). Similar to other polyploid salamanders, frogs and fish (Lampert and Schartl, 2010; Schlupp, 2005; Stöck et al., 2012), the triploid form can also reproduce by sperm-dependent gynogenesis, and many diverse gynogenetic clones have been discriminated by different genetic markers, such as transferrin (Yang and Gui, 2004; Yang et al., 2001, 2004), RAPD and SCAR markers (Zhou et al., 2001, 2000b), microsatellite (Guo and Gui, 2008) and mtDNA sequence (Apalikova et al., 2008; Brykov et al., 2005; Li and Gui, 2008). Interestingly, a minor but significant portion (approx 1–10%) of triploid males were found in the triploid form, and normal sperm production and their sexual reproduction ability have been demonstrated by experimental propagation and genetic analysis in the triploid form (Gui and Zhou, 2010; Peng et al., 2009; Sun et al., 2010; Zhou et al., 2000a). Moreover, a novel nucleo-cytoplasmic hybrid clone was synthesized from the sexual reproduction mating between clone D female and clone A male, and it was suggested through genetic analysis to arise via androgenesis by a mechanism of ploidy doubling of clone A sperm in clone D ooplasm through inhibiting the first mitotic division (Wang et al., 2011). Even though significant advances have been obtained in genetic identification and breeding application, we still do not know the evolutionary implications of male incidence and multiple reproduction mode maintenance in the triploid gibel carp.

All-triploid gibel carp and triploid–diploid *Carassius auratus* complex were initially recorded in Heilongjiang Province, northeast China (Jiang et al., 1983; Li and Gui, 2008), and recently, they were also reported in Far East of Russia (Apalikova et al., 2008, 2011). This study attempted to use nuclear and cytoplasmic markers including transferrin alleles and mitochondrial control region (CR) sequences to analyze genetic diversity and phylogenetic relationship of triploid form and diploid form in the northeast Asia *Carassius auratus* complex, and thereby to investigate the evolutionary implications of high male incidence and multiple reproduction mode maintenance in the triploid gibel carp.

2. Materials and methods

2.1. Specimen collection

A total of 386 individuals that belong to *Carassius auratus* complex were collected from four sampled sites in Xingkai Lake (Khanka Lake, Russian) and Suifen River (Razdolnaya, Russian) respectively. The sampled sites XI and SI located in China and XII and SII in Russia (Fig. 1).

For each specimen, caudal fin and blood were obtained. Caudal fin was fixed in 100% ethanol directly and used for DNA extraction. Blood was centrifuged at 1000 rpm for 5 min, and the supernatant sera were gently decanted and stored at 4 °C for transferrin preparation. The deposited blood cells were then washed 2–3 times with 1 × PBS solution and fixed in 75% ethanol. After being dissected to examine their gender, all the specimens were preserved in 100% ethanol and stored at the Institute of Hydrobiology in China.

2.2. Ploidy determination

Flow cytometry (Beckmen) was carried out to determine the sample ploidy by measuring the relative DNA content of their fixed blood cells following the documented instructions (Wei et al., 2003). Chicken blood cells with known DNA content of 2.5 pg/nucleus were used as an internally quantitative standard for each individual FCM profile.

2.3. Transferrin isolation and electrophoresis

Transferrins were isolated from each individual according to the rivanol-treatment procedure (Yang et al., 2001), and the isolated transferrins were subjected to 10% polyacrylamide gel electrophoresis (PAGE) followed the report by Li and Gui (2008).

2.4. DNA extraction and mtDNA sequencing

Total genomic DNA was extracted from a small piece of caudal fin for each sample by QIAGEN (Valencia CA) kit following the manufacturer's protocol. A fragment of mtDNA including partial tRNA-Pro gene, complete tRNA-Thr gene and the first third of control region was amplified by polymerase chain reaction (PCR) with the primer pair $L15923_c$ and $H16500_d$ (Takada et al., 2010) as described previously (Li and Gui, 2008). The amplified mtDNA fragments (described as CR for short) were purified by 1.0% low-melting agarose gel electrophoresis, and sequenced from both sides with the same primer pair through an ABI PRISM 3700 sequencing system.

2.5. Transferrin allele amplification and sequencing

A pair of primers *Tf-F* (CTCCTCAAAGAGCCTCGCCAT) and *Tf-R* (TACACCTGGCCACCATCAACTG) was designed to amplify 1100 bp fragment from the 7th exon to 10th exon according to 4 full length transferrin alleles (GenBank accession numbers: JQ822131–JQ822134) and 10 complete transferrin cDNA sequences (Electronic supplementary material, Table S1), and used to identify distinct transferrin alleles as described previously (Yang and Gui, 2004; Yang et al., 2004). PCR products were cloned into PMD-18T vector (Takara), and at least 12 positive clones were sequenced for each specimen in both directions to guarantee that the alleles were detected as many as possible.

2.6. Data analyses

Sequences were edited and assembled by Seqman software (SeqMan, 2011; Zhou et al., 2012) and aligned by Clustal package (Thompson et al., 1997). Haplotype files were generated by DnaSP version 4.10 (Rozas et al., 2003) program. For both CR and *tf* data sets, HKY+I+G was selected as the best fit model of sequence evolution by MODELTEST version 3.7 (Posada and Crandall, 1998) using the program PAUP4.0b8a (Swofford, 2003), and neighbor-joining (NJ) and maximum-parsimony (MP) phylogenetic trees were constructed with the same program. The branch confidence values were estimated by the bootstrap method with 1000 replications. For the CR tree, two corresponding *Cyprinus carpio* mtDNA CR sequences (GenBank accession numbers: X61010 and Ap009047.1) were used as outgroup. Same segment of transferrin gene in *Cyprinus carpio* (GenBank accession number: JQ822196) was also sequenced to root the *tf* tree. Moreover, a parsimony network was constructed to better resolve phylogenetic relationships among