The 5S rDNA and their chromosomal loci in autotriploid *Carassius auratus*

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Abstract

Background Polyploidization can induce a flurry of genetic events and plays an important role in speciation. In this study, we comparatively analyzed the molecular organization and chromosomal locus of 5S rDNA in natural autotriploid (3nCC, 3n=150) and artificial autotriploid gynogenetic (3nG, 3n=150) *Carassius auratus* as well as the related diploid ancestors, diploid *Carassius auratus* (2nCC, 2n=100) and *Carassius auratus* red var. (RCC, 2n=100), which provide an excellent model to ascertain the genomic changes in autotriploidization process. **Results** The similarities of the 5S rDNA coding region (class I, class II and class III) between RCC and 3nG were 98.3%, 100% and 99.1%, respectively, and the similarities of the 5S rDNA coding region (class IV, class V and class VI) between 2nCC and 3nCC were 97.5%, 100% and 100%, respectively. Fluorescence in situ hybridization (FISH) with 5S rDNA as probe indicated that the expected numbers of maternal chromosomal loci were found in 3nG and 3nCC, respectively. **Conclusions** These observations indicate that autotriploidization has little influence on the divergence of 5S rDNA family in *Carassius auratus*.

Background

Polyploidy is viewed as an important factor in eukaryote evolution [1-3]. Distant hybridization is a useful tool to produce hybrid offspring with altered genotypes and phenotypes or with different ploidy levels [4-6]. Fertile triploidy, as a special polyploidy population and a bridge to the formation of tetraploid populations, might play an important role in speciation [7, 8]. In previous studies, artificial autotriploid gynogenetic offspring (3n=150, AAA; abbreviated as 3nG) derived from *Carassius auratus* red var. (2n=100, AA; abbreviated as RCC) (♂) × *Megalobrama amblycephala* (2n=48, BB; abbreviated as BSB) (♀) were obtained [9]. The 3nG are fertile and can produce unreduced eggs (AAA) at an age of 1 year [10]. In the Dongting water system, crucian carp (*Carassius auratus* L., abbreviated as CC) complexes, including diploid (2n=100; 2nCC) [11], triploid (3n=150; 3nCC) and tetraploid (4n=200; 4nCC) forms exist [12]. Triploid *C. auratus* are considered of autotriploid origin [13] and have bisexual fertile and gynogenesis reproduction models [12]. These fertile autotriploids provide an attractive model for the study of the genomic changes.

The 5S rDNAs in higher eukaryotes are tandemly arrayed and involve a coding sequence (120bp) and a nontranscribed spacer (NTS) region containing some regulatory elements for coding sequence transcription [14, 15]. Variation within the NTS region is mainly due to its length and molecular composition [16]. Because of its special structure, the 5S rDNA multigene, is an important marker to analyze and trace rapid evolutionary events. Many 5S rDNA studies have demonstrated the influence of polyploidy on intragenomic variation and gene expression [17, 18]. The evolutionary dynamics of the 5S rDNA family likely reflect processes of chromosome repatterning that took place during speciation [19, 20]. Therefore, the inheritance pattern and chromosome constitution of 5S rDNAs among polyploid hybrids and their parents can be used to explore the associated hereditary relationships [21]. This study performed a comparative analysis of the 5S rDNA sequence and chromosomal locus in 3nG and 3nCC as well as the related diploid ancestors, RCC and 2nCC.

Methods

Source of samples

All experiments were approved by the Animal Care Committee of Hunan Normal University and followed the guidelines statement of the Administration of Affairs Concerning Animal Experimentation of China. Allotetraploid hybrids ($4n=148$, AABB; abbreviated as $4nF_1$) derived from *Carassius auratus* red var. ($2n=100$, AA; abbreviated as RCC) ($\otimes$) $\times$ *Megalobrama amblycephala* ($2n=48$, BB; abbreviated as BSB) ($\otimes$) produced autotriploid gametes ($3n=150$, AAA) via abnormal chromosome behavior during meiosis [9]. Autotriploid gametes, activated by UV-irradiated BSB sperm, developed into gynogenetic offspring ($3n=150$, AAA; abbreviated as 3nG) [10]. Natural autotriploid *Carassius auratus* ($3nCC$, $3n=150$) were collected from the Yuanjiang River and Xiangjiang River in the Dongting water system, Hunan Province. The next generation of 3nCC were produced by artificial gynogenesis from 3nCC eggs. Autotriploid *Carassius auratus* are not endangered or protected species, thus no special permit was necessary for the collection. After blood and the fin tissue were gathered, all samples were kept alive. They were reared and bred at the Reproduction of State Education Ministry, China, located at Hunan Normal University.

PCR amplification and sequencing of the 5S rDNA sequences

A pair of primers (5SP1, 5'-GCTATGCCCGATCTCGTCTGA-3': 5SP2R,

5'-CAGGTTGGTATGGCCGTAAGC-3') were applied to amplify 5S rDNA products by PCR [18]. Sequences were analyzed with Clustal W [38]. To further evaluate differences in 5S rDNA patterns, 210 clones were sequenced, containing 30 clones from RCC (10 clones for each PCR fragment), 60 clones from 3nG (20 clones for each PCR fragment), 60 clones from the 2nCC (20 clones for each PCR fragment) and 60 clones from the 3nCC (20 clones for each PCR fragment).

Preparation of chromosome spreads

Chromosome preparation was carried out on the fin cell of RCC, 3nG, 2nCC and 3nCC. The fin cell culture and chromosome preparation was performed according to the method reported by Zhu et al. [39] and Earley [40] with minor modifications. Three hours prior to harvest the cells was added concanavalin ($0.1\mu\text{g/mL}$). The collected cells were ground in 0.0375% NaCl, followed by hypotonic treatment with 0.075 M KCl at 28°C for 15–20 min and then fixed in 3:1 methanol–acetic acid for 20–30 min with two changes. Chromosome preparations were examined under an oil lens at a magnification of $3330\times$. For each fish sample, 200 metaphase spreads (20 metaphase spreads in RCC, 3nG, 2nCC and 3nCC) were examined.

Fluorescence *in situ* Hybridization

The 5S gene probes for fluorescence *in situ* hybridization (FISH) were amplified from RCC (203bp, 340bp and 477bp) and 2nCC (203bp, 340bp and 490bp) by PCR using 5SP1 and 5SP2R primers labeled with Dig-11-dUTP (using a nick translation kit; Roche, Germany). FISH was performed according to the method

described by Qin et al. [41]. For each fish sample, 200 metaphase spreads (20 metaphase spreads in each sample) of the chromosomes were analyzed. Preparations were examined under an inverted microscope (CW4000, Leica, Germany) with a confocal imaging system (LCS SP2, Leica). Captured images were colored and superimposed in Adobe Photoshop CS6.

Results

Examination of chromosome number

In RCC individuals, 87.5% of the chromosomal metaphases had 100 chromosomes (Figure. 1a). In 3nG individuals, 90% of the chromosomal metaphases had 150 chromosomes (Figure. 1b). In 2nCC individuals, 89.5% of the chromosomal metaphases had 100 chromosomes (Figure. 1c). In 3nCC individuals, 94.5% of the chromosomal metaphases had 150 chromosomes (Figure. 1d).

5S rDNA sequences

Three 5S PCR fragments (approximately 200, 340, and 500 bp) were amplified from the genomes of RCC, 3nG, 2nCC and 3nCC (Figure. 2). Based on the BLASTn analysis, all fragments from RCC, 3nG, 2nCC and 3nCC were confirmed as 5S rDNA repeat units, comprising a complete 5S rDNA region and a whole NTS region (Figure. 3). In RCC, three monomeric 5S rDNA (designated class I, class II and class III) were characterized by distinct NTS types (designated NTS-I: 83-bp, NTS-II: 220-bp and NTS-III: 357-bp). 3nG had three monomeric 5S rDNAs inherited from RCC (Figure. 4a and Figure. 5a-c). The coding region similarities (class I, class II and class III) between RCC and 3nG were 98.3%, 100% and 99.1%, respectively. In 2nCC, three monomeric 5S rDNAs (designated class IV, class V and class VI) possessed three NTS types (designated NTS-IV: 83-bp, NTS-V: 220-bp and NTS-VI: 370-bp). 3nCC had three monomeric 5S rDNAs, which were completely from 2nCC (Figure. 4b) and Figure. 5d-f). The coding region similarities (class IV, class V and class VI) between 2nCC and 3nCC were 97.5%, 100% and 100%, respectively. The accession numbers of 5S rDNA sequences in GenBank are listed in Table 1.

Fluorescence *in situ* Hybridization

The 5S rDNA probe (class I: 203 bp) hybridized with the metaphase chromosomes of RCC and 3nG. The 5S rDNA probe (class IV: 203 bp) hybridized with the metaphase chromosomes of 2nCC and 3nCC. The FISH results showed that eight 5S rDNA gene loci were present in 2nCC and RCC (Figure. 6a, c, respectively; Table 2). Twelve 5S rDNA gene loci were present in 3nG and 3nCC (Figure. 6b, d, respectively; Table 2).

The 5S rDNA probe (class II: 340 bp) hybridized with the metaphase chromosomes of RCC and 3nG. The 5S rDNA probe (class V: 340 bp) hybridized with the metaphase chromosomes of 2nCC and 3nCC. The FISH results showed that four 5S rDNA loci were present in 2nCC and RCC (Figure. 7a, c, respectively; Table 2). Six 5S rDNA loci were found in 3nG and 3nCC (Figure. 7b, d, respectively; Table 2).

The 5S rDNA probe (class III: 477 bp) hybridized with the metaphase chromosomes of RCC and 3nG. The 5S rDNA probe (class VI: 490 bp) hybridized with the metaphase chromosomes of 2nCC and 3nCC. The FISH results showed that eight 5S rDNA gene loci were identified in 2nCC and RCC (Figure. 8a, c, respectively; Table 2). Twelve 5S rDNA gene loci were identified in 3nG and 3nCC (Figure. 8b, d, respectively; Table 2).

Discussion

Birth-and-death and concerted evolution are important mechanisms in driving the long-term evolution of 5S rDNA [22]. Generally, fish genome has two 5S rDNA arrays [23, 24]. However, three 5S rDNA arrays were identified in RCC and 2nCC. In teleosts, fish-specific genome duplication has been revealed [25-27]. All *C. auratus* (Cypriniformes, Cyprinidae) karyotypes and genome sizes are approximately twice as large as those of standard diploid cyprinid fish [11, 28-30], which are viewed as undergoing an initial polyploidization process [31]. Reorganizations and variations of genomes in the polyploidization process probably lead to new 5S rDNA-arrays [18].

In this paper, three 5S rDNA classes of 3nG and 3nCC were completely inherited from RCC and 2nCC, respectively. The mapping of tandemly repeated sequences by means of FISH has proven to be very useful in analyzing fish genome architectures and large amounts of evolutionary, ecological and taxonomic questions [32]. The results of FISH showed that the 5S rDNA clusters (class I, class II and class III) were localized in the chromosomes of 3nG and the 5S rDNA clusters (class IV, class V and class VI) were localized in the chromosomes of 3nCC. Recurrent autopolyploidization has been reported to occur in the *C. auratus* complex [33]. The extant triploid form of the *C. auratus* complex with more than 150 chromosomes has undergone more than one genome polyploidy event and occurred on the basis of previous polyploidization [34]. Interestingly, it is difficult to distinguish 2nCC and 3nCC by appearance, and their mitochondrial DNA show high similarity [12]. Therefore, it is reasonable to infer that autotriploidization has little influence on the divergence of the 5S rDNA family in *C. auratus*.

Considering the general behavior of other polyploids, some changes following polyploidy were extremely probably to have occurred. Previous information has indicated that the uniparental loss of 5S rDNA classes and the variation of 5S rDNA chromosomal loci from progenitors have been detected in polyploid progeny [19, 35, 36]. These variations possibly reflect instability in polyploid genomes. However, maternal genome is stably inherited in progeny by the method of gynogenesis in autotriploid *C. auratus*. The conservation of 5S rDNA molecular organization and chromosomal loci was detected in 3nG and 3nCC. The high conservation in rDNA site number and chromosomal location, likely indicates conserved genome organization [37]. Thus, we speculate that the unique reproduction mode may allows them to reduce the pressure of evolution, resulting in the genetic conservation.

Because of the genetic conservation of 5S rDNA in 3nG and 3nCC, 5S rDNA is a useful genomic DNA maker. In our study, 3nG was derived from the hybridization of RCC (♂) × BSB (♀) which had a clear formation pathway. The 3nCC was derived from the crossing of diploid *C. auratus* (♂) with different

species (X) [12, 13]. However, it is still lack of clear molecular evidence to support this. The similar genetic characteristics of the 5S rDNA in 3nCC and 3nG revealed that 3nCC was of hybrid origin with the similar diploid ancestors as occurred in 3nG, which endorsed the viewpoint of the hybrid origin's natural autotriploid *C. auratus*.

Conclusions

The molecular organization and chromosomal locus of 5S rDNA showed conservation in 3nCC and 3nG. These data indicate that autotriploidization has little influence on the divergence of 5S rDNA family in *C. auratus*, which extend the knowledge of evolutionary dynamics of the 5S rDNA family in autotriploid fishes and have great significance in the study of *Carassius* taxa evolution.

Abbreviations

3nCC: Natural autotriploid *Carassius auratus*; 3nG: Artificial autotriploid gynogenetic offspring; 2nCC: Diploid *Carassius auratus*; RCC: *Carassius auratus* red var.; BSB: *Megalobrama amblycephala*; 4nF₁: Allotetraploid hybrids; NTS: Nontranscribed spacer; FISH: Fluorescence *in situ* Hybridization.

Declarations

Ethics approval and consent to participate

All experiments were approved by the Animal Care Committee of Hunan Normal University and followed the guidelines statement of the Administration of Affairs Concerning Animal Experimentation of China.

Consent to publish

Not applicable.

Authors' contributions

S.J.L. contributed to the conception and designed the study; L.C, C.Z., Q.B.Q. carried out the experimental work, participated in rafted the manuscript. Q.H.G., Y.W.Z., C.Q.W., Y.D.W., H.Q. analyzed sequences and M.T., C.Z., R.R.Z. participated in interpretation and discussion of the results. All authors read and approved the final manuscript.

Availability of data and materials

The complete clean reads for these libraries have been uploaded to the NCBI Sequence Read Archive site (<http://www.ncbi.nlm.nih.gov/sra/>; accession nos. GQ485555, GQ485556, GQ485557, MK073481, MK073482, MK073483, MK073487, MK073488, MK073489, MK073484, MK073485, MK073486).

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 GenBank accession numbers of the 5S rDNA sequences in RCC, 3nG, 2nCC and 3nCC

Fish type ^a	DNA fragments (bp)					
	203	340	477	490	492	495
RCC	GQ485555	GQ485556	GQ485557			
3nG	MK073481	MK073482				MK073483
2nCC	MK073487	MK073488		MK073489		
3nCC	MK073484	MK073485			MK073486	

^aRCC, *Carassius auratus* red var.; 3nG, Autotriploid gynogenetic offspring; 2nCC, Diploid *Carassius auratus*; 3nCC, Autotriploid *Carassius auratus*

Table 2 Examination of hybridising signals by FISH in RCC, 3nG, 2nCC and 3nCC

Fish type ^a	No. of Fish	No. of metaphases	203 bp	340 bp	477 bp	490 bp
			No. of loci	No. of loci	No. of loci	No. of loci
RCC	10	200	8	4	8	
3nG	10	200	12	6	12	
2nCC	10	200	8	4		8
3nCC	10	200	12	6		12

^aRCC, *Carassius auratus* red var.; 3nG, Autotriploid gynogenetic offspring; 2nCC, Diploid *Carassius auratus*; 3nCC, Autotriploid *Carassius auratus*

Figures

Figure 1

Chromosome spreads at metaphase in RCC, 3nG, 2nCC and 3nCC (a) The metaphase chromosome spreads of RCC individuals possess 100 chromosomes; (b) The metaphase chromosome spreads of 3nG individuals possess 150 chromosomes; (c) The metaphase chromosome spreads of 2nCC individuals possess 100 chromosomes; (d) The metaphase chromosome spreads of 3nCC individuals possess 150 chromosomes, in some samples uncounted micro-chromosomes were found (indicated by arrows).

Figure 2

Using the primer pair 5SP1-5SP2R, DNA fragments were amplified from RCC, 3nG, 2nCC and 3nCC. M, DNA ladder markers (100 bp increments); There were three DNA fragments (approximately 200, 340, and 500 bp) from RCC (lane 1), 3nG (lane 2), 2nCC (lane 3) and 3nCC (lane 4).

a

(Class I)	RCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class II)	RCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class III)	RCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class I)	3nG	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>GTCGGGCTG</u>	<u>GTTAGTACTT</u>
(Class II)	3nG	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class III)	3nG	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class I)	RCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class II)	RCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class III)	RCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	TTTGGAAATT	TTTCACCTAG	TATATAATAA	TTTTGCCAAA
(Class I)	3nG	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class II)	3nG	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class III)	3nG	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	TTTGGACATT	TTTCACCTAG	TATATAATAA	TTTTGCCAAA
(Class I)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class III)	RCC	AAATAGAGTC	AATGCCCGAT	CTCTGAATAT	TAGCAGGTTT	GGGCTGGTT	AGTACATGGA	TGGGAGACTG	CCTGGGAATA
(Class I)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class III)	3nG	AAATAGAGTC	AATGCCCGAT	CTCTGAATAT	TAGCAGGTTT	GGGCTGGTT	AGTACATGGA	TGGGAGATTG	CTTGGGAATA
(Class I)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class III)	RCC	CCAGGTGCTT	TAAGCTTTT	GGACATTTT	CACCTAGTAT	ATAATAATTT	TGCC-----	-----	-----
(Class I)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class III)	3nG	CCAGGTGCTT	TAATCTTTT	GGAAAAATT	CACGAATTAT	ATAATAATCT	TTCATTAAAA	AAAAAAAAAA	AAAAAAAAAA
(Class I)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	RCC	AGAGTCAATG	CCCGATCTCT	GAATCTTAGC	AGGTTTAGGT	CTGGTTAGAA	CTTTGATGAG	AGACTGCCTG	GGAATACCAAG
(Class III)	RCC	AGAGTCAATG	CCCGATCTCT	GAATCTTAGC	AGGTTTAGGT	CTGGTTAGTA	CTTTGATGAG	AGACTGCCTA	GGAATACCAAG
(Class I)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	3nG	AGAGTCAATG	CCCGATCTCT	GAATCTTAGA	AGGTTTAGGT	CTGGTTAGTA	CTTTGATGAG	AGACTTTCTA	GGAATACCAAG
(Class III)	3nG	AGAGTCAATG	CCCGATCTCT	GAATCTTAGC	AGGTTTAGGT	CTGGTTAGTA	CTTTGATGAG	AGACTGCCTA	GGAATACCAAG
(Class I)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	RCC	GTGCTTTAAG	CTTTTGGGTT	TTCTTTCTTA	CTTATATAAT	GTACTGGCGA	TAGATTGGC	TGGTCTTTAA	ATAGCCCTCT
(Class III)	RCC	GTGCTTTAAG	CTTTTGGGTT	TTCTTTCTTA	CTTATATAAT	GTACTGGCGA	TAGATTGGC	TGGTCTTTAA	ATAGCCCTCT
(Class I)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	3nG	-----	-----	-----	-----	-----	-----	-----	-----
(Class III)	3nG	GTGCTTTAAG	CTTTTGGGTT	TTCTTTCTTA	CTTATATAAT	GTACTGGCGA	TAGATTGGC	TGGTCTTTAA	ATAGCCCTCT
(Class I)	RCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class II)	RCC	CTTTGCAGCA	GTCTTC	-----	-----	-----	-----	-----	-----
(Class III)	RCC	CTTTGCAGCA	GTCTTC	-----	-----	-----	-----	-----	-----
(Class I)	3nG	CTTTGCAGCA	GTCTTC	-----	-----	-----	-----	-----	-----
(Class II)	3nG	CTTTGCAGCA	GTCTTC	-----	-----	-----	-----	-----	-----
(Class III)	3nG	CTTTGCAGCA	GTCTTC	-----	-----	-----	-----	-----	-----

b

(Class IV)	2nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class V)	2nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class VI)	2nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class IV)	3nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class V)	3nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class VI)	3nCC	<u>GCTTACGGCC</u>	<u>ATACCAACCT</u>	<u>GGCTATGCCC</u>	<u>GATCTCGTCT</u>	<u>GATCTCGGAA</u>	<u>GCTAAGCAGG</u>	<u>TTTGGGCTG</u>	<u>GTTAGTACTT</u>
(Class IV)	2nCC	<u>GGATGGGAGA</u>	<u>CCACCTAGGA</u>	<u>ATACCAGGTG</u>	<u>CTTTAAGCTT</u>	-----	-----	-----	-----
(Class V)	2nCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class VI)	2nCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	TTTGGACATT	TTTCACCTAG	TATATAATAA	TTTTGCCAAA
(Class IV)	3nCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTTTAAGCTT</u>	-----	-----	-----	-----
(Class V)	3nCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	-----	-----	-----	-----
(Class VI)	3nCC	<u>GGATGGGAGA</u>	<u>CCGCCTGGGA</u>	<u>ATACCAGGTG</u>	<u>CTGTAAGCTT</u>	TTTGGACATT	TTTCACCTAG	TATATAATAA	TTTTGCCAAA
(Class IV)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class VI)	2nCC	AAATAGAGTC	AATGCCCGAT	CTCTGAATAT	TAGCAGGTTT	GGGCTGGTT	AGTACATGGA	TGGGAGATTG	CTTGGGAATA
(Class IV)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class VI)	3nCC	AAATAGAGTC	AATGCCCGAT	CTCTGAATAT	TAGCAGGTTT	GGGCTGGTT	AGTACATGGA	TGGGAGATTG	CTTGGGAATA
(Class IV)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class VI)	2nCC	CCAGGTGCTT	TAATCTTTT	GGAAAAATT	ACGAATTATA	TAATAATCTT	TCATTAAAAA	AAAAAAAAAA	AAAAA--GAG
(Class IV)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class VI)	3nCC	CCAGGTGCTT	TAATCTTTT	GGAAAAATT	ACGAATTATA	TAATAATCTT	TCATTAAAAA	AAAAAAAAAA	AAAAAAGAG
(Class IV)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	2nCC	TCAATGCCCG	ATCTCTGAAT	CTTAGCAGGT	TTAGGTCTGG	TTAGTACTTT	GATGAGAGAC	TGCCTAGGAA	TACCAGGTGC
(Class VI)	2nCC	TCAATGCCCG	ATCTCTGAAT	CTTAGCAGGT	TTAGGTCTGT	TTAGTACTTT	GATGAGAGAC	TGCCTAGGAA	TACCAGGTGC
(Class IV)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	3nCC	TCAATGCCCG	ATCTCTGAAT	CTTAGCAGGT	TTAGGTCTGG	TTAGCACTTT	GATGAGAGAC	TGCCTAGGAT	TACCAGGTGC
(Class VI)	3nCC	TCAATGCCCG	ATCTCTGAAT	CTTAGCAGGT	TTAGGTCTGG	TTAGTACTTT	GATGAGAGAC	TGCCTAGGAA	TACCAGGTGC
(Class IV)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	2nCC	TTTAAGCTTT	TGGGTTTTCT	TTCTACTTAA	TATAATGTAC	CGGCGATTAG	ATTGGCTGAT	CTTTAAATAG	CCCTCTCTTT
(Class VI)	2nCC	TTTAAGCTTT	TGGGTTTTCT	TTCTACTTAA	TATAATGTAC	TGGCGATTAG	ATTGGCTGGT	CTTTAAATAG	CCCTCTCTTT
(Class IV)	3nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	3nCC	TTTAAGCTTT	TGGGTTTTCT	TTCTACTTAA	TATAATGTAC	CGGCGATTAG	ATTGGCTGAT	CTTTAAATAG	CCCTCTCTTT
(Class VI)	3nCC	TTTAAGCTTT	TGGGTTTTCT	TTCTACTTAA	TATAATGTAC	TGGCGATTAG	ATTGGCTGGT	CTGTAATAG	CCCTCTCTTT
(Class IV)	2nCC	-----	-----	-----	-----	-----	-----	-----	-----
(Class V)	2nCC	GCAGCAGTCT	TC	-----	-----	-----	-----	-----	-----
(Class VI)	2nCC	GCAGCAGTCT	TC	-----	-----	-----	-----	-----	-----
(Class IV)	3nCC	GCAGCAGTCT	TC	-----	-----	-----	-----	-----	-----
(Class V)	3nCC	GCAGCAGTCT	TC	-----	-----	-----	-----	-----	-----
(Class VI)	3nCC	GCAGCAGTCT	TC	-----	-----	-----	-----	-----	-----

Figure 3

Representative sequences of 5S rDNA-classes from RCC, 3nG, 2nCC and 3nCC (a) Representative sequences of 5S rDNA class I, II, and III from RCC and 3nG; (b) Representative sequences of 5S rDNA class IV, V, and VI from 2nCC and 3nCC. Complete 5S coding regions are shaded; the NTS upstream TATA elements are underlined.

Figure 5

Comparison of the NTS sequences from RCC, 3nG, 2nCC and 3nCC (a) NTS-I sequences from RCC and 3nG; (b) NTS-II from RCC and 3nG; (c) NTS-III from RCC and 3nG; (d) NTS-IV sequences from 2nCC and 3nCC; (e) NTS-V from 2nCC and 3nCC; (f) NTS-VI from 2nCC and 3nCC. The NTS upstream TATA elements are shaded; asterisks mark variable sites in the NTS.

Figure 6

Examination of hybridizing signals by FISH (probes with 203 bp) in RCC, 3nG, 2nCC and 3nCC (a) There were eight 5S gene loci (white arrows) in RCC; (b) There were twelve 5S gene loci (white arrows) in 3nG; (c) There were eight 5S gene loci (white arrows) in 2nCC; (d) There were twelve 5S gene loci (white arrows) in 3nCC. Bar = 3 μ m.

Figure 7

Examination of hybridizing signals by FISH (probes with 340 bp) in RCC, 3nG, 2nCC and 3nCC (a) There were two big (yellow arrows) and two small 5S gene loci (white arrows) in RCC; (b) There were three big (yellow arrows) and three small 5S gene loci (white arrows) in 3nG; (c) There were two big (yellow arrows) and two small 5S gene loci (white arrows) in 2nCC; (d) There were three big (yellow arrows) and three small 5S gene loci (white arrows) in 3nCC. Bar = 3 μ m.

Figure 8

Examination of hybridizing signals by FISH in RCC and 3nG with 477 bp probes, in 2nCC and 3nCC with 490 bp probes (a) There were eight 5S gene loci (white arrows) in RCC; (b) There were twelve 5S gene loci (white arrows) in 3nG; (c) There were eight 5S gene loci (white arrows) in 2nCC; (d) There were twelve 5S gene loci (white arrows) in 3nCC. Bar = 3 μ m.