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Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*

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Abstract To contribute to the knowledge of fish genomes, we identified and characterized by means of nucleotide sequencing and physical chromosome mapping, three classes of repetitive DNAs in the genome of the South American cichlid fish *Astronotus ocellatus*. The first class corresponds to a satellite DNA family (*AoSat*) that shares similarity with a centromeric satellite DNA of the pufferfish *Tetraodon nigroviridis*. The second repetitive DNA class (*AoRex3*) is related to the retrotransposon *Rex3*, which is widely distributed among teleost fishes. The last repetitive element (*AoLINE*) shows a high similarity to the *CR1*-like LINE element of other teleosts. The three isolated repetitive elements are clustered in the centromeric heterochromatin of all chromosomes of the complement. The repetitive sequences are not randomly distributed in the genome, suggesting a pattern of compartmentalization on chromosomes.

Keywords Cytogenetics · Genome evolution · Chromosome · Heterochromatin · Transposon · Satellite DNA

Introduction

A substantial portion of eukaryotic genomes is composed of multiple DNA copies, known as "repetitive DNAs" (Jurka et al. 2005), which can account for more than 50% of the genome in some mammalian species (The genome international sequencing consortium 2001). These repetitive DNA sequences are generally classified into two main classes: the tandem repeats, such as the long tandem arrays termed satellite DNA; and the dispersed elements, such as transposons and retrotransposons (Jurka et al. 2005). Satellite DNAs are non-coding DNA sequences organized as long arrays of head-to-tail linked repeats (Plohl et al. 2008), and include satellite DNAs, minisatellites and microsatellites (Charlesworth et al. 1994). Transposable elements (TEs) represent a major fraction of vertebrate genomes; for instance, over 40% of the human genome is constituted of TEs (Böhne et al. 2008). The two known major classes of TEs, retrotransposable elements and DNA transposons, are both represented in vertebrate genomes.

Satellite DNAs and TEs were long considered to be junk DNA because they had no clearly identified function; the belief that they were not transcribed in eukaryotes seemed to confirm this (Doolitlle and Sapienza 1980; Orgel and Crick 1980). However, accumulated data from eukaryotic species of diverse taxonomic origins have challenged this view over the past few years (Bonaccorsi and Lohe 1991), supporting a major role of repetitive DNA in the structural and functional evolution of genes and genomes in a variety of organisms (Biémont and Vieira 2006). In addition, repetitive sequences can be involved with chromosome evolution by causing chromosome breakage, deletions, inversions and amplifications (Lim and Simmons 1994; Dimitri et al. 1997). The repeated DNA copies are closely associated in heterochromatic regions of the genomes of many distant eukaryotes such as *Drosophila* (Pimpinelli et al. 1995) and plants (Presting et al. 1998). This situation supports the structural role of these repeats in genome evolution (Dimitri and Junakovic 1999). Repetitive sequences such as transposons may be responsible for modifying the expression of flanking genes. It is believed

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that this has played a very important role in the evolution of genome structure and gene function in vertebrates and other organisms, and has generated at least half of the human and mouse genomes (Feschotte and Pritham 2007). The molecular characterization of repetitive elements is necessary to elucidate the structure and function of the genomes. Even among entire sequenced genomes, the repetitive areas remain as gaps because of the difficulty in determining their correct positioning and array in the genome. A complete understanding of the relationship between chromosome structure and function requires the repetitive segments to be fully resolved.

Teleost fishes are an outstanding model for the investigation of molecular processes driving diversity and speciation in living organisms. They have significantly contributed to a better understanding of the functioning, structure and evolution of vertebrate genomes. Fishes of the family Cichlidae are an interesting group to study because of their rapid speciation, species richness, and high levels of endemism, particularly in the East African lakes Victoria, Malawi, and Tanganyika (Koblmüller et al. 2007). The great majority of studies on cichlid fishes were conducted on African species. Some of their genomes are being completely sequenced (The international cichlid genome consortium 2006). It is therefore of particular interest to investigate the genome structure of South American cichlid species for purposes of comparative analysis. *Astronotus ocellatus* (Astronotinae, Cichlidae) is one of the most common cichlids in South America and is popularly known as "Oscar" or "Apaiari". This species is native to rivers of the Amazon basin (Pavanelli 2000) and it is an animal of great economic interest, mainly for aquarium hobbyists, sport fishing, and also as an important food item. The nucleotide sequence and chromosomal distribution of repetitive DNAs were investigated in *A. ocellatus*, allowing the discovery of new repetitive elements and also contributing to the knowledge of compartmentalization of fish genomes.

Materials and methods

Animals, DNA samples and chromosome preparation

Genomic DNA of 14 individuals of *A. ocellatus,* six males and eight females, from the Tietê River (Botucatu, state of São Paulo, Brazil) was extracted according to standard phenol–chloroform procedures (Sambrook and Russel 2001). Mitotic chromosomes were prepared from anterior kidney cells with in vivo colchicine treatment (Bertollo et al. 1978) and were submitted to the C-banding (Sumner 1972), Ag-NOR staining (Howell and Black 1980) and Fluorescence in situ hybridization (FISH).

Isolation of repetitive sequences

Repetitive DNA sequences were isolated by restriction endonuclease digestion and Degenerate Oligonucleotide Primer-PCR (DOP-PCR). Genomic DNA was digested with six restriction endonucleases, *Hae*III, *Hind*III, *Msp*I, *Pvu*I, *Xba*I and *Hinf*I, size-fractionated by electrophoresis on 1% agarose gel (Sambrook and Russel 2001), and stained with ethidium bromide. The endonucleases *Hinf*I and *Hae*III revealed conspicuous bands, and these prominent DNA bands, candidates to contain repetitive sequences, were isolated from the gel. The DNA fragments were purified using a GFX^{TM} PCR DNA and Gel Band Purification kit (GE Healthcare, Amersham Biosciences), cloned into *pMOS Blue* plasmid vector (GE Healthcare, Amersham Biosciences), and used for transformations in *E. coli* DH5α competent cells. Clones containing the digested DNA fragments were stored for nucleotide sequencing and to be used as probes in FISH.

The DOP-PCR amplification was performed using 2.25 μM of the DOP primer (5′CCG ACT CGA GNN NNN NAT GTG G3′), 0.5 mM deoxynucleotide triphosphates (dNTPs), 1× polymerase reaction buffer, and 10 U of *Taq* platinum DNA polymerase (Invitrogen). Cycling conditions were as follows: (1) 3 min at 95°C; (2) 1.5 min at 94°C; (3) 3 min at 30°C; (4) a ramp step to 72°C (0.2°C/s); (5) 0.5 min at 72°C; (6) 10 cycles of step 2–5; (7) 1.5 min at 94°C; (8) 1.5 min at 56°C; (9) 1.5 min to 72°C; (10) 35 cycles of steps 6–8. DOP-PCR could amplify template DNA from concentrations as low as 25 pg (Kukasjärvi et al. 1997). The principle of the DOP-PCR technology allows the amplification of sequences that cover the entire genome, with preferential amplification of repetitive DNAs (Telenius et al. 1992). This methodology makes possible the isolation and use of the repetitive fraction of the genome as a probe for chromosome mapping purposes.

Sequencing and sequence analysis

The positive clones obtained with the restriction endonuclease digestion were sequenced on an ABI Prism 3100 DNA sequencer (Perkin-Elmer) using the Kit BigDye Terminator Cycle Sequencing (Perkin-Elmer). The sequences were subjected to Blastn (Altschul et al. 1990) searches at the National Center for Biotechnology Information (NCBI), website (http://www.ncbi.nlm.nih.gov/blast) to check for any similarity of the isolated sequences to the sequences deposited in the GenBank databases. Sequences with high similarity to the repetitive DNAs isolated from *A. ocellatus* were retrieved from the NCBI database and used in comparative and evolutionary analyses. The sequences thus obtained were aligned online using the program Clustal W

(Thompson et al. 1994), website (http://www2.ebi.ac.uk/clustalw), and the alignment checked manually. Similar sequences were submitted to genetic distance analysis employing the Kimura-2-parameter genetic distance model (Kimura 1980) implemented in the program MEGA 3.1 (Kumar et al. 2004).

Fluorescence in situ hybridization

The probes were labeled by nick translation with biotin 14-dATP (BioNick™ Labeling System) (Invitrogen). The chromosomal DNA was denatured in 70% formamide/2× SSC for 28 s at 67°C, pH 7. Hybridization mixtures containing 100 ng of denatured probe, 10 µg/µl dextran sulfate, 2× SSC, and 50% formamide, in a total volume of 30 µl, were dropped on the slides, and the hybridization was performed overnight at 37°C in a 2× SSC moist chamber. Post-hybridization washes were carried out at 37°C in 2× SSC/50% formamide for 15 min, followed by a second wash in 2× SSC for 15 min, and a final wash at room temperature in 4× SSC for 15 min. Detection of hybridized probes was carried out with 0.07% avidin–FITC conjugate (Sigma) in C buffer (0.1 M $NaHCO_3$, 0.15 M NaCl) for 30 min followed by a signal amplification using 2.5% anti-avidin biotin conjugate (Sigma) in blocking buffer (4× SSC, 0.5% triton and 1% nonfat dried milk) for 10 min, and then followed again by a treatment with avidin–FITC. The treatments with avidin–FITC and anti-avidin–biotin were conducted in a 2× SSC moist chamber at 37°C. After each amplification step, the slides were washed three times for 5 min each in blocking buffer at 42°C. Chromosomes were counterstained with propidium iodide (0.2%) diluted in antifade (Vector).

Chromosomal analysis

Cytogenetic analyses were conducted with the use of an Zeiss Axiophot 2 microscope; the images were captured with an Axioplan 2 HRC digital camera with the program Axiovision 4 (Zeiss), and processed with the program Adobe Photoshop. The chromosomes were organized as meta-submetacentric (m/sm) and subtelo-acrocentric (st/a) in karyotypes.

Results and discussion

Isolation, nucleotide sequence and comparative analyses of repetitive DNAs

The digestion with *Hinf*I and *Hae*III produced bands around 270 bp, which were isolated from the gel and cloned. The recovered bacterial clones were stored in 25% glycerol at −80°C. The positive clones were denominated *AoHinfI* and *AoHaeIII* and were submitted to nucleotide sequencing. The sequences obtained were analyzed against the NCBI database through the program BLAST/N (Altschul et al. 1990) to search for similarities. In general, the sequences showed high similarity to several classes of repeated sequences of other organisms, mainly fish species. Several isolated clones showed similarity with retrotransposons and satellite sequences (Table 1).

The alignment of the clones *AoHae*III-2, *AoHae*III-3, *AoHae*III-5, *AoHae*III-6, *AoHae*III-16 and *AoHae*III-24, and *AoHinf*I-*8* and *AoHinf*I-10 showed that the isolated sequences belong to a satellite DNA family (named *AoSat*) composed of 265–268 bp repeat units (Fig. 1). The repeat units differed from each other by insertions/deletions and base substitutions, and had a mean Kimura-2-parameter genetic distance of 0.022. The *AoSat* units contained short internal motifs with similarities to several organisms, including fish species (Table 1). The most interesting characteristic of the *AoSat* family is its similarity to the 118-bp centromeric satellite DNA family of *Tetraodon nigroviridis* (Crollius et al. 2000). Although the *AoSat* sequence and the *T. nigroviridis* centromeric satellite have a high value of Kimura-2-parameter genetic distance (0.815), it was possible to detect a higher similarity in a 21-bp AT-rich motif (Fig. 2). Considering that the *AoSat* is also clustered in the centromeres of *A. ocellatus* chromosomes (see Results and discussion), it might be speculated that both satellite families could have arisen from an ancestor repetitive DNA, and the 21-bp AT-rich motif was inherited from the common ancestor.

The second class of repetitive DNAs identified (clones *AoHae*III-9 and *AoHae*III-15) contains sequences with a high similarity to the non-LTR retrotransposon *Rex3* of *Xiphophorus maculatus* and *T. nigroviridis*, and dispersed sequences in the genomes of several fish species including *Oryzias latipes*, *Takifugu rubripes* and *Gasterosteus aculeatus*, and a large number of sequences of the *Danio rerio* genome (Table 1). The isolated repetitive sequences contained in the clones *AoHae*III-9 and *AoHae*III-15 were named *AoRex3*, and contain two different segments of the Rex3 element previously characterized in the genome of *X. maculatus* (Volff et al. 1999). The clone *AoHae*III-9 contains a segment that corresponds to the nucleotide positions 463–733, and the clone *AoHae*III-15 contains a segment corresponding to the nucleotide position 800–1,065 of the Rex3b-XmJ copy (GenBank accession AF125982) of the *Rex3* element of *X. maculatus* (Volff et al. 1999). The isolated sequences correspond to the 5′ flanking region of the reverse transcriptase (RT) gene of the *Rex3* element. By searching sequence databases it was possible to identify high levels of similarity of the *AoRex3* element from *A. ocellatus* with fish species representative

Table 1 Characteristics of isolated repetitive DNAs from the genome of *Astronotus ocellatus*

Clones	GeneBank entries	Size of repeat (bp)	Similarity
AoHaeIII-2	FJ164033	266	Low similarity to repeats of centromeric satellite DNA of *Tetraodon nigroviridis* (AJ 270048*) and dispersed short sequences in the genome of *Populus trichocarpa* (AC213494*, AC216843*), *Danio rerio* (CR847973*, CR388171*, CT971502*), *Mus musculus* (AC147567*) and *Homo sapiens* (AC103770*, AC116096*)
AoHaeIII-3	FJ164034	266	
AoHaeIII-5	FJ164035	269	
AoHaeIII-6	FJ164036	266	
AoHaeIII-16	FJ164037	265	
AoHaeIII-24	FJ164038	265	
AoHinfI-8	FJ164039	265	
AoHinfI-10	FJ164040	265	
AoHaeIII-9	FJ164041	283	74–80% of similarity to *Rex3* non-LTR retrotransposons of *Xiphophorus maculatus* (AY298859*, AF125982*, AF125983*) and *T. nigroviridis* (AJ621035*); sequences of *Oryzias latipes* (AB111925*), *T. nigroviridis* (BX629355*, BX908814*), *T. rubripes* (AC091292*), *Gasterosteus aculeatus* (AC174771*, AC145725*) and a large number of sequences in the *D. rerio* genome (CR628327*, CR855302*, CR388175*)
AoHaeIII-15	FJ164042	276	
AoHinfI-4	FJ164043	231	75–95% of similarity to *LINE CR1*-like retrotransposons of *Paralichthys olivaceus* (AY136821*) and *T. rubripes* (AJ459419*); dispersed sequences in the genome of the cichlids *O. niloticus* (AB270897*) and *A. burtoni* (DQ386647*), and *D. rerio* (CR855317*) and *S. salar* (EU025708*)

* GenBank accession numbers for the sequences at NCBI

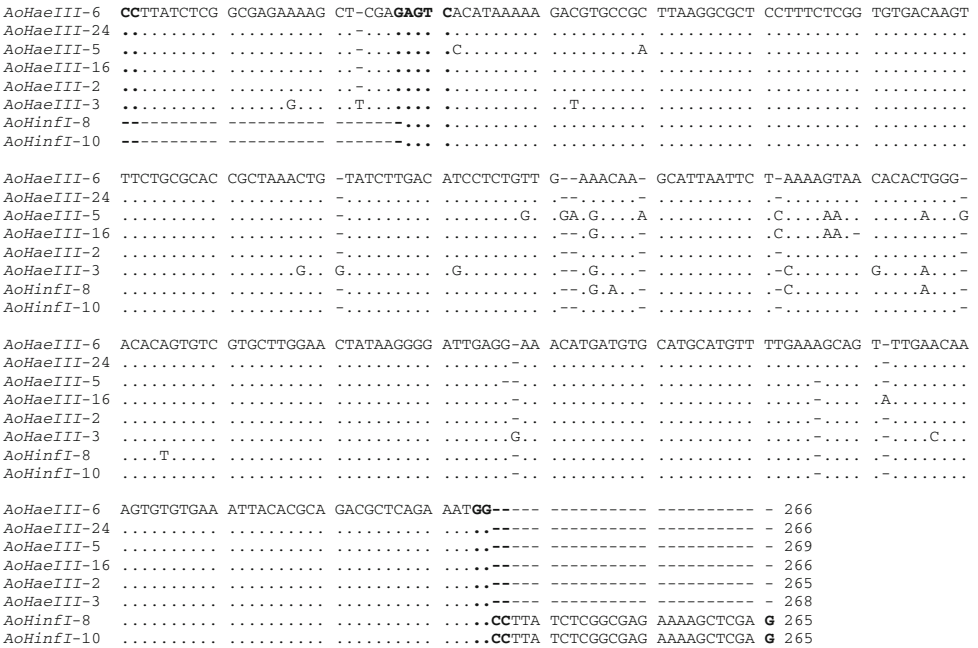

Fig. 1 Nucleotide alignments of sequences containing the *AoSat* repeat units isolated from the genome of *A. ocellatus* after digestion with the restriction enzymes *Hae*III and *Hinf*I. The restriction sites for *Hae*III (GGCC) and *Hinf*I (GANTC) are indicated in *boldface*. *Dashes* indicate insertion/deletion, *dots* similarity in sequence, and *N* non-identified nucleotides

of several orders, including the Gasterosteiformes, Cypriniformes, Cyprinodontiformes, Tetraodontiformes and Beloniformes, as also demonstrated in previous studies that showed that this element is widely distributed in fish genomes (Volff et al. 2001; Ozouf-Costaz et al. 2004). Although several *Rex3* sequences, related to the RT domain, are available for other fish species and orders, including cichlids, the nucleotide sequence for the 5′ flanking domain of the *Rex3* element is limited to one or two representative species per order, which makes more-detailed evolutionary analysis difficult.

The third repetitive DNA class identified was represented by only one clone (*AoHinf*I-4), and showed a similarity to *CR1-like* LINE retrotransposons of fish species and to dispersed sequences in the genome of cichlids and other fishes (Table 1). The isolated sequence was named *AoLINE* and contains a segment of the coding region of the reverse transcriptase-like protein. The comparative analysis of *AoLINE* to the nucleotide sequences of other fishes available at NCBI evidenced a close relationship among the *AoLINE* elements of cichlids (Fig. 3). Among cichlids, the African species *O. niloticus* and

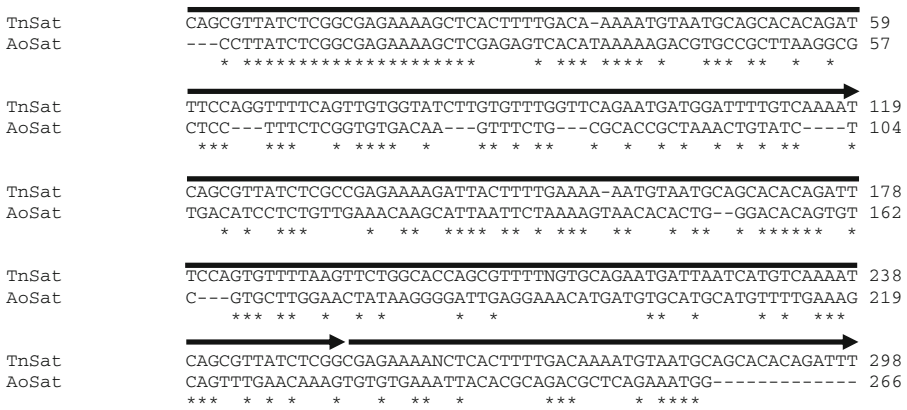

Fig. 2 Nucleotide alignment of the consensus sequence of the *AoSat* repeat unit and the 118-bp centromeric satellite of *T. nigroviridis* (TnSat). The repeat units of *TnSat* are indicated by an *arrow* above the sequence. *Dashes* indicate insertion/deletion, *asterisks* similarity in sequence, and *N* non-identified nucleotides

A. burtoni branch out together in the phylogenetic analysis, and are quite divergent (bootstrap value of 99) from the South American *A. ocellatus* (Fig. 2). Although there are only a few available sequences in the genomic databases of the *AoLINE* element, its presence in several different orders suggests that *AoLINE* is widespread in teleosts. More detailed analysis involving this element would be of great interest, to investigate its complete genomic structure and its distribution in other fish and vertebrate groups.

Chromosomal distribution of repetitive DNAs

The diploid chromosome number of *A. ocellatus* was 48 (16 m/sm and 32 st/a), that is in agreement with the modal chromosome formula for South American cichlids (Feldberg et al. 2003). Constitutive heterochromatin (Fig. 4) was present in the centromeric region of all the chromosomes. In addition to the centromeric location, C-banding-positive heterochromatin was present in a large interstitial region of the short arm of the chromosomes pair no. 1. A heteromorphic condition for this interstitial region in the first chromosome pair was frequently observed and seems to be associated with the nucleolus organizer regions (NORs) also present in this region (Fig. 4).

The repetitive elements identified in this study (*AoSat*, *AoRex3* and *AoLINE*), were located preferentially in the centromeric regions of the chromosomes (Fig. 5), thus revealing a possible structural role as component of the

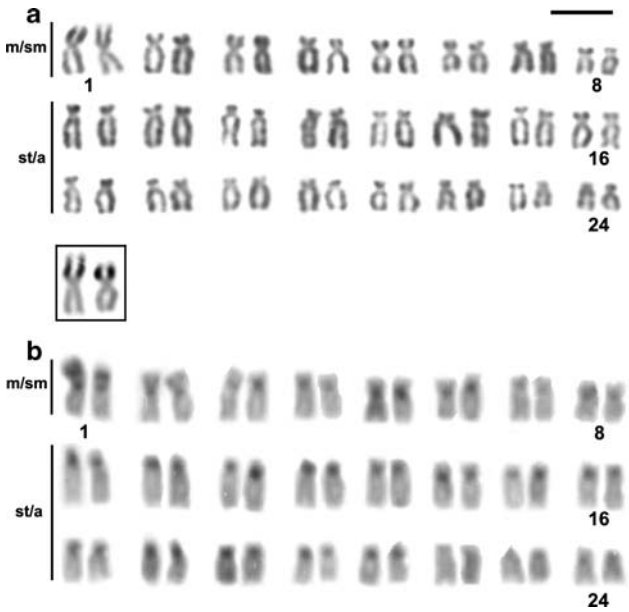

Fig. 4 Karyotypes of *A. ocellatus* after Giemsa staining (**a**) and C-banding (**b**). The NOR region in the first chromosome pair is showed in detail in the *box*. m/sm, metacentric and submetacentric chromosomes; st/a, subtelocentric and acrocentric chromosomes. *Scale bar*: 5 μm

constitutive centromeric heterochromatins. This organization pattern is apparently widespread among multicellular eukaryotes, and suggests the involvement of repetitive DNAs in centromeric functions (Dawe 2003). However,

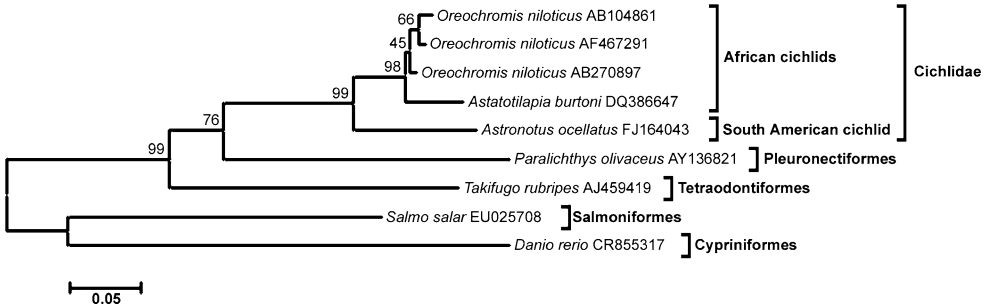

Fig. 3 Phylogenetic relationships of *AoLINE* sequences of several fish species, including *A. ocellatus*. Branch lengths are proportional to evolutionary distance (*scale bar*) and *bootstrap* values are indicated on the nodes. Fish orders and species, and accession numbers of the sequences are indicated

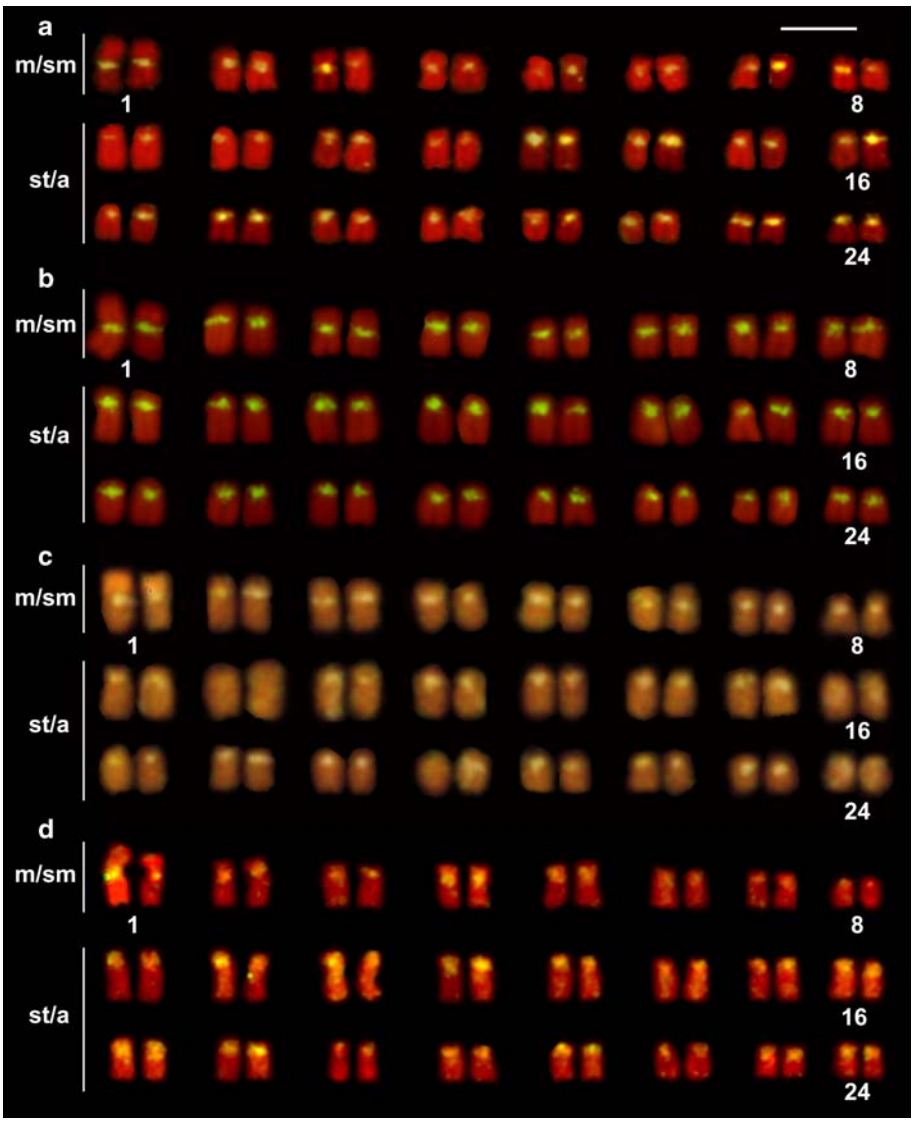

Fig. 5 Karyotypes of *A. ocellatus* after FISH with probes of the repeated elements *AoSat* (**a**), *AoRex3* (**b**), *AoLINE* (**c**), and DOP-PCR (**d**). m/sm, meta- and submetacentric chromosomes; st/a, subtelo- and acrocentric chromosomes. *Scale bar*: 5 μm

our probes did not hybridize with the interstitial region of the short arms of chromosome 1, thus indicating a different composition of the NORs associated heterochromatin.

The similarity among *AoSat* sequences and the *T. nigroviridis* centromeric satellite units suggests that these sequences were preserved during the divergence between Cichlidae and Tetraodontiformes, perhaps because of their centromeric function. On the other hand, the repetitive DNAs such as the transposons *AoRex3* and *AoLINE* might have accumulated in the centromeric heterochromatic regions as a consequence of the lower selective pressure that acts on these genomic regions.

The chromosomal hybridization of the DOP-PCR generated probe confirms that repetitive DNAs are accumulated in heterochromatic areas in this species, showing stronger signals in the centromeric areas and some weak signals spread out in the chromosomal arms (Fig. 5).

Repetitive DNA sequences have been extensively mapped in fish chromosomes by means of cytogenetic techniques (Martins 2007). The repeats are, in most cases, compartmentalized in the heterochromatins and are not randomly distributed in the genome. Analysis of the chromosomal location of various types of TEs in the compact genome of the pufferfish *T. nigroviridis* showed that these sequences are generally excluded from gene-rich regions (Dasilva et al. 2002; Bouneau et al. 2003; Fisher et al. 2004). They accumulate together with other categories of repeats (duplicated pseudogenes, minisatellites) in particular heterochromatic regions of the genome (DaSilva et al. 2002). Such a situation is not observed in humans, where repeated sequences constitute an important fraction of euchromatic DNA (Volff et al. 2003). Repetitive DNAs, particularly TEs, also have been mapped in the chromosomes of several species of Antarctic fishes of the suborder

Notothenioidei, and have a homogeneous distribution in some species and are accumulated in peri-centromeric regions and sex chromosomes in others (Ozouf-Costaz et al. 2004). Among cichlid fishes, the chromosome organization of repetitive DNAs has only been studied in *O. niloticus*, and several classes of repetitive elements including rDNA repeats (Martins et al. 2000, 2002), satellite DNAs (Oliveira and Wright 1998), telomeric sequences (Chew et al. 2002), SINES (Oliveira et al. 2003), LINES (Oliveira et al. 1999), and BACs (Bacteria Artificial Chromosomes) enriched from repetitive sequences (Ferreira and Martins 2008), have had their chromosomal distribution elucidated. The repetitive DNAs are accumulated in peri-centromeric regions and in the supposed sex chromosomes of *O. niloticus*. The organization of repetitive DNAs in the chromosomes of *A. ocellatus* is in agreement with the results observed in other fishes, including *O. niloticus*.

In a number of other genomes, DNA transposons and retrotransposons appear to be more abundant within the heterochromatin. In dipterans, TEs accumulate near centromeres and telomeres. This was observed in *Drosophila*, where TEs account for 8% of heterochromatin and 4–5% of euchromatin (Bartolomé et al. 2002). In plants such as *Oryza sativa* or *Arabidopsis thaliana*, centromeres and pericentromeric regions also contain high levels of TEs. In both these plants, the retroelements appeared to be centromeric and the DNA transposons more predominantly pericentromeric. Some classical DNA families and MITEs, as well as SINEs, are an exception to this clustering, since these TEs are distributed throughout the chromosomes in *A. thaliana* (The arabidopsis genome initiative 2000; Lenoir et al. 2001).

All classes of repetitive DNA seem to accumulate preferentially in the heterochromatin in fishes, and also in other eukaryotic groups, as can be observed in corn (Dimitri and Junakovic 1999; Bartolomé et al. 2002), reptiles (Yamada et al. 2005) and rodents (Yamada et al. 2006). Repetitive sequences have been isolated in some avian species (Psittaciformes, Passeriformes and Strigiformes), and were located on all or most centromeres (Madsen et al. 1992; Saifitdinova et al. 2001). In animals and plants, centromeres are rich regions of highly repetitive satellite DNAs and are vital for the correct sorting of chromosomes during cell division (Henikoff et al. 2001). The highly repeated DNA sequences of no obvious functional significance are associated with regions of restricted crossing over, such as the centromeric area (Charlesworth et al. 1986). Therefore, we can speculate that the centromeric heterochromatins are refuges that protect the repetitive DNAs against the selective pressure that acts on the gene-rich euchromatins.

The results presented herein will contribute to elucidate the genome organization of repetitive elements in cichlid genomes and also, the presence of these repeated elements preferably in the centromeric region, provides a good molecular marker to be used in evolutionary studies of chromosomal rearrangements.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bartolomé C, Maside X, Charlesworth B (2002) On the abundance and distribution of transposable elements in the genome of *Drosophila melanogaster*. Mol Biol Evol 19:926–937

Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Citotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). Braz J Genet 1:103–120

Biémont C, Vieira C (2006) Genetics: junk DNA as an evolutionary force. Nature 443:521–524. doi:10.1038/443521a

Böhne A, Brunet F, Galiana-Arnoux D, Schultheis C, Volff JN (2008) Transposable elements as drivers of genomic and biological diversity in vertebrates. Chromosome Res 16:203–215. doi:10.1007/s10577-007-1202-6

Bonaccorsi S, Lohe A (1991) Fine mapping of satellite DNA sequences along the Y chromosome of *Drosophila melanogaster*: relationships between satellite sequences and fertility factors. Genetics 129:177–189

Bouneau L, Fisher C, Ozouf-Costaz C, Froschauer A, Jaillon O, Coutanceau JP, Körting C, Weissenbach J, Bernot A, Volff JN (2003) An active Non-LTR retrotransposon with tandem structure in the compact genome of the pufferfish *Tetraodon nigroviridis*. Genome Res 13:1686–1695. doi:10.1101/gr.726003

Charlesworth B, Langley CH, Stephan W (1986) The evolution of restricted recombination and the accumulation of repeated DNA sequences. Genetics 112:947–962

Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371:215–220. doi:10.1038/371215a0

Chew JSK, Oliveira C, Wright JM, Dobson MJ (2002) Molecular and cytogenetic analysis of the telomeric (TTAGGG)n repetitive sequences in the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Chromosoma 111:45–52. doi:10.1007/s00412-002-0187-3

Crollius HR, Jaillon O, DaSilva C, Ozouf-Costaz C, Fizames C, Fischer C, Bouneau L, Billault A, Quetier F, Saurin W, Bernot A, Weissenbach J (2000) Characterization and repeat analysis of the compact genome of the freshwater pufferfish Tetraodon nigroviridis. Genome Res 10:939–949

Dasilva C, Hadji H, Ozouf-Costaz C, Nicaud S, Jaillon O, Weissenbach J, Crollius HR (2002) Remarkable compartmentalization of transposable elements and pseudogenes in the heterochromatin of the *Tetraodon nigroviridis* genome. Proc Natl Acad Sci USA 99:1636–1641. doi:10.1073/pnas.202284199

Dawe RK (2003) RNA interference, transposons, and the centromere. Plant Cell 15:297–302. doi:10.1105/tpc.150230

Dimitri P, Junakovic N (1999) Revising the selfish DNA hypothesis. New evidence on accumulation of transposable elements in heterochromatin. Trends Genet 15:123–124. doi:10.1016/S0168-9525(99)01711-4

Dimitri P, Arca B, Berghella L, Mei E (1997) High genetic instability of heterochromatin after transposition of the LINE-like I factor in *Drosophilamelanogaster*. Proc Natl Acad Sci USA 94:8052–8057. doi:10.1073/pnas.94.15.8052

Doolitlle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature 284:601–603. doi:10.1038/284601a0

Feldberg E, Porto JIR, Bertollo LAC (2003) Chromosomal changes and adaptation of cichlid fishes during evolution. In: Val AL, Kapoor BG (eds) Fish adaptations. Science Publishers, Inc., New Delhi, pp 285–308

Ferreira IA, Martins C (2008) Physical chromosome mapping of repetitive DNA sequences in Nile tilapia *Oreochromis niloticus*: evidences for a differential distribution of repetitive elements in the sex chromosomes. Micron 39:411–418. doi:10.1016/j.micron.2007.02.010

Feschotte C, Pritham EJ (2007) DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet 41:331–368. doi:10.1146/annurev.genet.40.110405.090448

Fisher C, Bouneau L, Coutanceau JP, Weissenbach J, Volff JN, Ozouf-Costaz C (2004) Global heterochromatic colocalization of transposable elements with minisatellites in the compact genome of the pufferfish *Tetraodon nigroviridis*. Gene 336:175–184. doi:10.1016/j.gene.2004.04.014

Henikoff S, Ahmad K, Malik HS (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293:1098–1102. doi:10.1126/science.1062939

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36:1014–1015. doi:10.1007/BF01953855

Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J (2005) Repbase update, a database of eukaryotic repetitive elements. Cytogenet Genome Res 110:462–467. doi:10.1159/000084979

Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. doi:10.1007/BF01731581

Koblmüller S, Egger B, Sturmbauer C, Sefc KM (2007) Evolutionary history of Lake Tanganyika's scale-eating cichlid fishes. Mol Phylogenet Evol 44:1295–1305. doi:10.1016/j.ympev.2007.02.010

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163. doi:10.1093/bib/5.2.150

Kuukasjärvi T, Tanner M, Pennanen S, Karhu R, Visakorpi T, Isola J (1997) Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridisation. Genes Chromosomes Cancer 18:94–101. doi:10.1002/(SICI)1098-2264(199702)18:2<94::AID-GCC3>3.0.CO;2-W

Lenoir A, Lavie L, Prieto JL, Goubely C, Cote JC, Pélissier T, Deragon JM (2001) The evolutionary origin and genomic organization of SINEs in *Arabidopsis thaliana*. Mol Biol Evol 18:2315–2322

Lim JK, Simmons MJ (1994) Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. Bioessays 16:269–275. doi:10.1002/bies.950160410

Madsen CS, de Kloet DH, Brooks JE, de Kloet SR (1992) Highly repeated DNA sequences in birds: the structure and evolution of an abundant, tandemly repeated 190-bp DNA fragment in parrots. Genomics 14:462–469. doi:10.1016/S0888-7543(05)80242-3

Martins C (2007) Chromosomes and repetitive DNAs: a contribution to the knowledge of fish genome. In: Pisano E, Ozouf-Costaz C, Foresti F, Kapoor BG (eds) Fish Cytogenetics. Science Publisher, Inc., Enfield, pp 421–453

Martins C, Wasko AP, Oliveira C, Wright JM (2000) Nucleotide sequence of 5S rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the Tilapiine cichlid fish, *Oreochromis niloticus*. Hereditas 133:39–46. doi:10.1111/j.1601-5223.2000.00039.x

Martins C, Wasko AP, Oliveira C, Porto-Foresti F, Maltempi PPP, Wright JM, Foresti F (2002) Dynamics of 5S rDNA in the tilapia (*Oreochromis niloticus*) genome: repeat units, inverted sequences, pseudogenes and chromosome loci. Cytogenet Genome Res 98:78–85. doi:10.1159/000068542

Oliveira C, Wright JM (1998) Molecular cytogenetic analysis of heterochromatin in the chromosomes of tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Chromosome Res 6:205–211. doi:10.1023/A:1009211701829

Oliveira C, Chew JSK, Foresti FP, Dobson M, Wright JM (1999) A LINE2 repetitive DNA sequence from the cichlid fish, *Oreochromis niloticus*: sequence analysis and chromosomal distribution. Chromosoma 108:457–468. doi:10.1007/s004120050397

Oliveira C, Wang Y, Bryden LJ, Wright JM (2003) Short interspersed repetitive elements (SINEs) from the cichlid fish, *Oreochromis niloticus*, and their chromosomal localization by fluorescence in situ hybridization. Caryologia 56:181–189

Orgel LE, Crick FH (1980) Selfish DNA: the ultimate parasite. Nature 284:604–607. doi:10.1038/284604a0

Ozouf-Costaz C, Brandt J, Körting C, Pisano E, Bonillo C, Countaceau JP, Volff JN (2004) Genome dynamics and chromosomal localization of the non-LTR retrotransposons *Rex1* and *Rex3* in Antarctic fish. Antarct Sci 16:51–57. doi:10.1017/S0954102004001816

Pavanelli GC (2000) Sanidade de peixes, rãs, crustáceos e moluscos. In: Valenti WC, Poli CR, Pereira JA, Borghetti JR (eds) Aqüicultura no Brasil: bases para um desenvolvimento sustentável. CNPq, Brasília, pp 197–246

Pimpinelli S, Berloco M, Fanti L, Dimitri P, Bonaccorsi S, Marchetti E, Caizzi R (1995) Transposable elements are stable structural components of *Drosophila melanogaster* heterochromatin. Proc Natl Acad Sci USA 92:3804–3808. doi:10.1073/pnas.92.9.3804

Plohl M, Luchetti A, Meštrović N, Mantovani B (2008) Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. Gene 409:72–82. doi:10.1016/j.gene.2007.11.013

Presting GG, Malysheva L, Fuchs J, Schubert I (1998) A Ty3/gypsy retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes. Plant J 6:721–728. doi:10.1046/j.1365-313x.1998.00341.x

Saifitdinova AF, Derjusheva SE, Malykh AG, Zhurov VG, Andreeva TF, Gaginskaya ER (2001) Centromeric tandem repeat from the chaffinch genome: isolation and molecular characterization. Genome 44:96–103. doi:10.1139/gen-44-1-96

Sambrook J, Russel DW (2001) Molecular cloning. A laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York

Sumner AT (1972) A single technic for demonstrating centromere heterochromatin. Exp Cell Res 75:304–306. doi:10.1016/0014-4827(72)90558-7

Telenius H, Polmear AH, Tunnacliffe A, Carter NP, Behmel A, Ferguson-Smith MA, Nordenskjold M, Pfragner R, Ponder BAJ (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. Genes Chromosomes Cancer 4:257–263. doi:10.1002/gcc.2870040311

The arabidopsis genome initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

The genome international sequencing consortium (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921

The international cichlid genome consortium (2006) Genetic basis of vertebrate diversity: the cichlid fish model. Available at http://hcgs.unh.edu/cichlid/

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. doi:10.1093/nar/22.22.4673

Volff JN, Korting C, Sweeney K, Schartl M (1999) The non-LTR retrotransposon *Rex3* from the fish *Xiphophorus* is widespread among teleosts. Mol Biol Evol 16:1427–1438

Volff JN, Körting C, Meyer A, Schartl M (2001) Evolution and discontinuous distribution of *Rex3* retrotransposons in fish. Mol Biol Evol 18:427–431

Volff JN, Bouneau L, Ozouf-Costaz C, Fischer C (2003) Diversity of retrotransposable elements in compact pufferfish genomes. Trends Genet 19:674–678. doi:10.1016/j.tig.2003.10.006

Yamada K, Nishida-Umehara C, Matsuda Y (2005) Molecular and cytogenetic characterization of site-specific repetitive DNA sequences in the Chinese soft-shelled turtle (*Pelodiscus sinensis*, Trionychidae). Chromosome Res 13:33–46. doi:10.1007/s10577-005-2351-0

Yamada K, Kamimura E, Kondo M, Tsuchiya K, Nishida-Umehara C, Matsuda Y (2006) New families of site-specific repetitive DNA sequences that comprise constitutive heterochromatin of the Syrian hamster (*Mesocricetus auratus*, Cricetinae, Rodentia). Chromosoma 115:36–49. doi:10.1007/s00412-005-0012-x