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Cytogenetic Mapping of the Retroelements *Rex1*, *Rex3* and *Rex6* among Cichlid Fish: New Insights on the Chromosomal Distribution of Transposable Elements

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Key Words
Chromosome evolution · Genome evolution · Transposable elements

Abstract
To enhance our understanding of the organization of the genome and chromosome evolution of cichlid fish species, we have isolated and physically mapped onto the chromosomes the transposable elements (TEs) *Rex1*, *Rex3* and *Rex6*, which are conserved in teleost fish, in the chromosomes of African and South American cichlid species. The physical mapping of different *Rex* elements showed that they are primarily compartmentalized in the pericentromeric heterochromatic regions, although dispersed or clustered signals in euchromatic regions were also observed. The presence of TEs in heterochromatin can be correlated with their role in the structure and organization of heterochromatic areas (such as centromeres) or with the lower selective pressure that act on these gene-poor regions. The *Rex* elements were also concentrated in the largest chromosome pair of the Nile tilapia, *Oreochromis niloticus*. This chromosome pair is supposed to have originated by fusions, demonstrating the possible involvement of TEs with chromosome rearrangements. Besides general patterns of chromosomal distribution, comparative analysis suggests that *Rex* elements could differ in their chromosomal distribution among different fish groups or species and that intrinsic aspects of the genomes could influence the spread, accumulation or elimination of TEs.

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One of the features of eukaryotic genomes is the presence of a great diversity of repetitive DNA sequences that can constitute a huge fraction of the genome. The portion of the genome composed of repetitive DNAs can represent 95% of the onion's genome, 50% or more of the human genome, 10% of the genome of the pufferfish, *Tetraodon nigroviridis* (which is among the smallest known vertebrate genomes), and about 14% of the genome of the flowering plant *Arabidopsis thaliana* [Flavell et al., 1974; The Arabidopsis Genome Initiative, 2000; International Human Genome Sequencing Consortium, 2001; Dasilva et al., 2002]. The two major classes of repetitive DNAs are: (i) tandem repeats, including the satellite, minisatellite and microsatellite DNAs, and (ii) transposable elements (TEs), including DNA transposons, the elements that transpose directly through DNA copies, and retrotransposons, which transpose through an intermediate RNA molecule that is reverse transcribed [Charlesworth et al., 1994; Böhne et al., 2008]. Transposons and retrotransposons may be arranged in clusters, thus being easily visual-

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ized in the chromosomes by cytogenetic methods [Ferreira and Martins, 2008; Gross et al., 2009; Mazzuchelli and Martins, 2009].

Although repetitive DNA sequences have been studied in almost all vertebrate groups, most of the information available is related to mammals; however, it is known that fish, the most diverse group of living vertebrates, contain all types of known repetitive DNAs. These sequences are spread over fish chromosomes, with enrichment in centromeric, pericentromeric, telomeric and subtelomeric regions [Lanfredi et al., 2001; Ferreira and Martins, 2008], and in the sex [Devlin et al., 1998; Stein et al., 2001; Cioffi et al., 2010] and supernumerary chromosomes [Mestriner et al., 2000; Poletto et al., 2010a]. However, the compact genomes of both pufferfish, *Takifugu rubripes* and *Tetraodon nigroviridis,* contain a smaller quantity of repeated sequences but a greater diversity of TE families than the much larger human and mouse genomes [Volff et al., 2003]. Almost every class of TEs known in eukaryotes is represented in the pufferfish genomes [Aparicio et al., 2002].

TEs were long considered to be junk DNA because they had no clearly recognized function, and they were believed not to be transcribed in eukaryotes [Doolittle and Sapienza, 1980]. Data accumulated from recent studies, however, have challenged this view, and it is becoming clear that these elements have had a significant influence on the evolution of genomes, particularly by controlling gene activity and by their involvement in chromosome rearrangements [Syvanen, 1994; Biémont and Viera, 2006; Raskina et al., 2008]. The role of TEs in generating genomic variation has been very important for the evolution of genome structure and gene function in vertebrates and other organisms. TEs have generated at least half of the human and mouse genome variation [Feschotte and Pritham, 2007].

Among the TEs, the elements *Rex1, Rex3* and *Rex6* are non-long terminal repeat (non-LTR) retrotransposons, firstly isolated from the melanoma fish model *Xiphophorus* and are widely distributed within fish genomes. The *Rex1* TE seems to be related to the *CR1* clade of long interspersed elements (LINEs) and encodes a reverse transcriptase and an apurinic/apyrimidinic endonuclease, which is frequently removed by incomplete reverse transcription [Volff et al., 2000]. *Rex3* is related to the RTE family and the essential features of the element are (i) coding regions for an endonuclease and a reverse transcriptase, (ii) truncations of most of the copies, (iii) a tail consisting of tandem repeats of the sequence GATG, and (iv) short target site sequence duplications of variable length [Volff et al., 1999]. *Rex6* encodes a reverse transcriptase and a putative restriction enzyme-like endonuclease and is a member of the *R4* family of non-LTR retrotransposons. *Rex6* was identified in many species of teleost and underwent several bursts of retrotransposition leading to a high copy number in the genome of numerous fish. Extremely truncated *Rex6*-related sequences were detected by database screening in reptiles, but not in sequences from the human genome, suggesting that this element might have been lost from certain vertebrate lineages [Volff et al., 2001].

Several classes of repetitive DNAs have been described among cichlid fish mostly in the Nile tilapia, *Oreochromis niloticus*, but few of them have been cytogenetically mapped onto chromosomes. They include satellite DNAs [Oliveira et al., 1998; Mazzuchelli and Martins, 2009; Mota-Velasco et al., 2010], transposable elements [Oliveira et al., 1999, 2003; Ferreira and Martins, 2008; Gross et al., 2009; Mazzuchelli and Martins, 2009; Teixeira et al., 2009; Ferreira et al., 2010], telomeric $(TTAGGG)_n$ repeats [Chew et al., 2002; Mota-Velasco et al., 2010; Poletto et al., 2010a], rDNA repeats [Martins et al., 2002; Vicari et al., 2006; Gross et al., 2010; Poletto et al., 2010b], and other repetitive sequences [Ferreira and Martins, 2008; Mazzuchelli and Martins, 2009; Valente et al., 2009]. The cichlids have attracted the attention of biologists due to the rapid radiation of some groups in the Great Lakes of East Africa, in which almost 2,000 species arose in the last 10 million years [Kocher, 2004]. In addition, some species of Cichlidae, principally the tilapiines, are very important for aquaculture and fisheries, and the Nile tilapia represents one of the most widely farmed freshwater fish in the world [FAO, 2008]. Because of this, the cichlids have become an important model for genome studies.

To extend our understanding of the genome organization and chromosome evolution in the cichlid group, we have cytogenetically mapped the transposable elements *Rex1, Rex3*, and *Rex6* among African and South American cichlid species by fluorescent in situ hybridization (FISH). The results are discussed in the context of understanding the organization of transposable elements and their role in the diversification of fish genomes.

Material and Methods

Fish Material and Chromosome Preparation

South American fish species were collected from the Araguaia River (São Félix do Araguaia and Barra do Garças, Mato Grosso State, Brazil) and the Tietê River (Botucatu, São Paulo State, Brazil), according to Brazilian laws for environmental protection

Table 1. Analyzed cichlid species and their origin

Origin of cichlids	Species	Number of analyzed animals	Origin of specimens
Africa	*Oreochromis niloticus*	4	Tietê River, Botucatu, SP, Brazil
	Haplochromis obliquidens	3	Aquarium, Botucatu, SP, Brazil
	Hemichromis bimaculatus	1	Aquarium, Botucatu, SP, Brazil
	Melanochromis auratus	3	Aquarium, Botucatu, SP, Brazil
South America	*Astronotus ocellatus*	2	Tietê River, Botucatu, SP, Brazil
	Chaetobranchus flavescens	2	Araguaia River, São Félix do Araguaia, MT, Brazil
	Satanoperca jurupari	2	Araguaia River, Barra do Garças, MT, Brazil
	Heros efasciatus	2	Araguaia River, São Félix do Araguaia, MT, Brazil

(wild collection permit, SISBIO/15729-1). African species were obtained from aquarium pet shops in Botucatu, SP, Brazil (table 1). Metaphase chromosome spreads were prepared from anterior kidney cells following the protocols of Bertollo et al. [1978]. Tissue samples were stored in 100% ethanol, and the genomic DNA was extracted using standard phenol-chloroform procedures [Sambrook and Russel, 2001].

Isolation of Repeated DNA Elements

The retroelements *Rex1*, *Rex3* and *Rex6* were isolated by PCR (Polymerase Chain Reaction) from the African and South American species with the primer sets as follows: the set of primers, Rex1f (5′-TTC TCC AGT GCC TTC AAC ACC-3′) and Rex1r (5′-TCC CTC AGC AGA AAG AGT CTG CTC-3′), was designed to amplify *Rex1* segments corresponding to the coding domains 3–7 of the reverse transcriptase (RT) gene [Volff et al., 2000]; the primers Rex3f (5′-CGG TGA YAA AGG GCA GCC CTG-3′) and Rex3r (5′-TGG CAG ACN GGG GTG GTG GT-3′) were designed to amplify the coding domains 1, 2, 2A, A and B of the RT gene [Volff et al., 1999]; the primers Rex6f (5′-TAA AGC ATA CAT GGA GCG CCAC-3′) and Rex6r (5′-GGT CCT CTA CCA GAG GCC TGGG-3′) were used to amplify the C-terminal part of the restriction enzyme-like endonuclease of the retrotransposon element [Volff et al., 2001].

Standard PCR reactions were performed using 100–200 ng of total DNA, 0.2 μM of each primer, 0.04 mM of each nucleotide, 1.5 mM of magnesium chloride, 0.5 U of *Taq* DNA polymerase, 1× reaction buffer and ultrapure-water to a final volume of 25 μl. The PCR cycling conditions were as follows: 95°C for 5 min, 35 cycles of 95°C for 40 s, 55°C for 40 s and 72°C for 2 min, with a post-cycling extension at 72°C for 5 min. The PCR amplicons were analyzed by electrophoresis in 1-% agarose gels, quantified in a spectrophotometer Nanodrop ND-2000 and used as probes to perform FISH.

Sequencing and Sequence Analysis

The PCR products of *Astronotus ocellatus* were sequenced on an ABI Prism 3100 DNA sequencer (Applied Biosystems) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). 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 to the sequences deposited in the GenBank databases.

Chromosome in situ Hybridization

Mitotic chromosomes were labeled by FISH [Pinkel et al., 1986] using the PCR products from the repetitive elements *Rex1*, *Rex3* and *Rex6* as probes in independent assays. The probe labeling, hybridization and detection were performed as described in Teixeira et al. [2009]. Ten to 15 metaphases of each species were photographed and analyzed for the 3 TEs investigated.

Results

Isolation of Rex1, Rex3 *and* Rex6 *Elements*

The retrotransposons *Rex1, Rex3* and *Rex6* generated electrophoretic DNA bands of 560, 400 and 500 base pairs (bp), respectively, and no variation was found between the different species. The primer sets employed were very efficient in the amplification of these DNA fragments by PCR and have been employed in the isolation of these repeated elements in different fish groups [Capriglione et al., 2002; Ozouf-Costaz et al., 2004], including the South American cichlids [Gross et al., 2009; Teixeira et al., 2009]. The nucleotide sequence was determined for the PCR products of *Astronotus ocellatus*, confirming that the generated fragments correspond to the retrotransposons *Rex1, Rex3* and *Rex6* (GenBank accession numbers HM535301–HM535309).

Cytogenetic Mapping of the Rex1 *Element*

FISH, using PCR fragments of the *Rex1* element as probes, showed signals in the pericentromeric regions of almost all the chromosomes of all the cichlid species analyzed here. The 2 African cichlids, *H. obliquidens* and *O. niloticus*, showed dispersed signals along the q arm of the

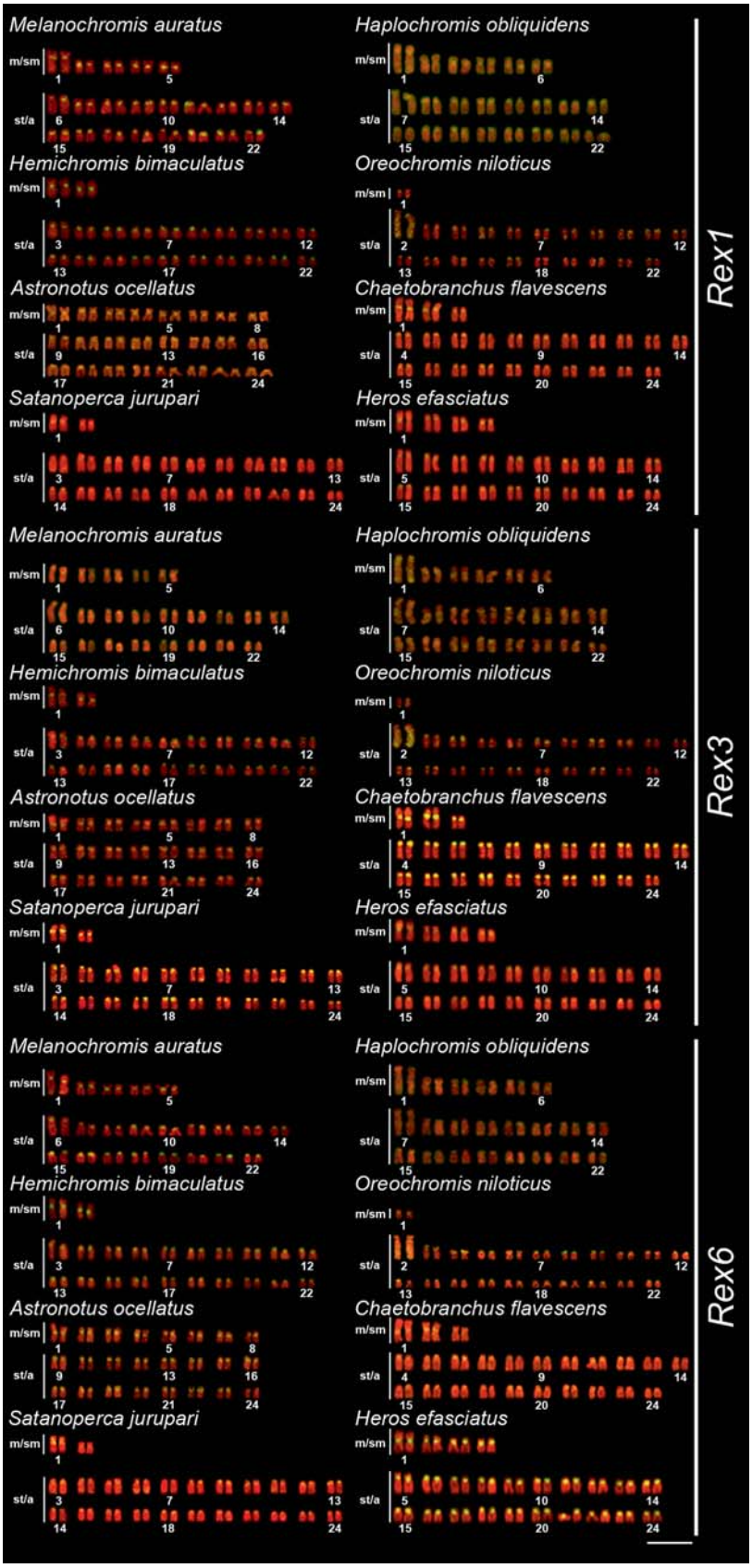

Fig. 1. Chromosome mapping of *Rex1*, *Rex3* and *Rex6* among cichlids. The chromosomal sites of the *Rex* retrotransposons were labeled with FITC (yellow), and the chromosomes were counterstained with propidium iodide (red). m/sm, meta/submetacentric chromosomes; st/a, subtelo/acrocentric chromosomes. Bar = 10 μm.

Table 2. Overview of hybridization patterns of *Rex1, Rex3* and *Rex6* elements in the chromosomes of several fish species investigated

Suborder/Family	Group/Tribe	Species	*Rex1*	*Rex3*	*Rex6*	Reference
Labroidei/Cichlidae	haplochromine[a]	*M. auratus*	CPR	CPR	CPR	Present study
	hemichromine[a]	*H. bimaculatus*	CPR	CPR	CPR	Present study
	haplochromine[a]	*H. obliquidens*	CPR; DS[c]	CPR; DS[c]	CPR; DS[c]	Present study
	tilapiine[a]	*O. niloticus*	CPR; DS	CPR; DS	CPR; DS	Present study
	Chaetobranchini[b]	*Chaetobranchus flavescens*	CPR	CPR, DS[c]	CPR; DS[c]	Present study
	Geophagini[b]	*Satanoperca jurupari*	CPR; DS[c]	CPR; DS[c]	CPR; DS[c]	Present study
	Astronotini[b]	*Astronotus ocellatus*	CPR	CPR	CPR	Present study; Mazzuchelli and Martins [2009]
	Cichlini[b]	*Cichla kelberi*	CPR; DS[c]	CPR; DS[c]	DS[c]	Teixeira et al. [2009]
	Heroini[b]	*Symphysodon* genus	n.d.	CPR	n.d.	Gross et al. [2009]
	Heroini[b]	*Heros efasciatus*	CPR	CPR	CPR	Present study
Notothenioidei/ Nototheniidae		*Notothenia coriiceps*	Spots	CPR	n.d.	Ozouf-Costaz et al. [2004]
		Trematomus newnesi	S	H	n.d.	Ozouf-Costaz et al. [2004]
		Dissostichus mawsoni	S	H	n.d.	Ozouf-Costaz et al. [2004]
Notothenioidei/ Bathydraconidae		*Gymnodraco acuticeps*	S	H	n.d.	Ozouf-Costaz et al. [2004]
Notothenioidei/ Channichthyidae		*Chionodraco hamatus*	S	H	n.d.	Ozouf-Costaz et al. [2004]
		Neopagetopsis ionah	S	H	n.d.	Ozouf-Costaz et al. [2004]
Tetraodontidae		*Tetraodon nigroviridis*	comp.	comp.	n.d.	Dasilva et al. [2002]; Bouneau et al. [2003]; Fisher et al. [2004]

CPR: Clustered over pericentromeric regions; comp.: compartmentalized; DS: dispersed signals; H: homogeneous distribution of spots; n.d.: not described; S: superimposed with *Rex3* signals.

[a] According to the classification of Lowe-McConnell [1999]. [b] According to the classification of Smith et al. [2008]. [c] Euchromatic areas.

first chromosome pair. The signals in this region were less evident in *H. obliquidens* than in *O. niloticus*. Dispersed weak signals were also detected in interstitial and terminal positions in 2 chromosomes (pairs 3 and 23) of the South American cichlid *S. jurupari* (fig. 1, table 2).

Cytogenetic Mapping of the Rex3 *Element*

In general, the FISH results obtained using a partial *Rex3* element as probe showed sites in pericentromeric regions in all the cichlid species analyzed here. Dispersed signals were also observed in *H. obliquidens* (along 8q and 17q, in segments of 1q and 12q, and in interstitial sites in 3q, 7q and 11q), *O. niloticus* (along the 1q arm), *C. flavescens* (along 2p and in the terminal region of 8q) and *S. jurupari* (part of 3q and in the terminal region of 8q and 11q) (fig. 1, table 2). The signals generated in the chromosomes of *A. ocellatus* are in agreement with previous data [Mazzuchelli and Martins, 2009].

Cytogenetic Mapping of the Rex6 *Element*

All the cichlid species analyzed here showed sites of *Rex6* in pericentromeric regions in almost all chromosomes. Only *H. obliquidens* showed a general pattern with dispersed signals (including part of 1q) and only 1 pericentromeric cluster restricted to chromosome pair no. 15. Dispersed weak signals were also observed in *O. niloticus* (along 1q), *C. flavescens* (terminal region of 12q) and *S. jurupari* (part of 6q and in the terminal region of 11q) (fig. 1, table 2).

Discussion

General Features of Rex *Elements in the Fish Chromosomes*

It is known that TEs may accumulate in regions far from genes (such as heterochromatin or intergenic regions) and into or near gene sequences [Kidwell, 2005]. Generally this distribution pattern is non-random and seems to have some relation to specific characteristics of subregions of the host genomes [Kidwell and Lisch, 2000].

The physical mapping of different *Rex* elements showed that they are generally compartmentalized in the pericentromeric regions in the cichlid species analyzed here (some exceptions were also observed) and are coincident with heterochromatic regions in the cichlid species. The general compartmentalization of the *Rex1* element was also previously noticed in 2 other fish: the

South American cichlid *Cichla kelberi* (Perciformes) [Teixeira et al., 2009] and the pufferfish *Tetraodon nigroviridis* (Tetraodontiformes) [Dasilva et al., 2002; Fisher et al., 2004]. In addition, the *Rex3* element showed a general compartmentalization in the South American cichlids *Symphysodon aequifasciatus*, *S. discus*, *S. haraldi* [Gross et al., 2009] and in *C. kelberi* [Teixeira et al., 2009], in *T. nigroviridis* [Bouneau et al., 2003; Fisher et al., 2004], and in the Antarctic fish *Notothenia coriiceps* (Perciformes, Notothenioidei) [Ozouf-Costaz et al., 2004]. Among the notothenioids, both *Rex1* and *Rex3* showed accumulation in some regions (spots) but were not clearly compartmentalized [Ozouf-Costaz et al., 2004].

The physical mapping of these elements in all fish analyzed so far showed a similar general pattern of arrangement over the chromosomes of each group (cichlids, notothenioids and tetraodontiforms), with exceptions for some cichlids and Antarctic species (table 2). Thus, it is suggested that these elements are able to accumulate in specific genomic regions within each fish group. The tendency to accumulate seems to be shared by the 3 *Rex* elements within the same fish group, but differs among different fish groups. In fact, it is known that TEs may accumulate in particular regions in some fish species like *C. hamatus* and *T. nigroviridis* [Dasilva et al., 2002; Bouneau et al., 2003; Ozouf-Costaz et al., 2004]. Although there are no data for the cytogenetic mapping of *Rex6* elements in other non-cichlid fish, we propose extending this conclusion to the *Rex6* elements because they have a hybridization pattern similar to the other *Rex* elements in all cichlid species analyzed so far.

Furthermore, dispersed signals of the 3 *Rex* elements in the largest chromosome pair of *O. niloticus* and *H. obliquidens* were observed, being more clearly seen in the heterochromatic areas of *O. niloticus*. In *O. niloticus* the largest chromosome pair might have originated by chromosome fusions [Chew et al., 2002; Ferreira et al., 2010] and is recognized as the sex chromosome [Carrasco et al., 1999; Griffin et al., 2002]. It has been broadly reported that TEs have been important to genome evolution in a range of species, causing in some cases loss and gain of sequences and chromosomal rearrangements [Lyttle and Haymer, 1992; Cáceres et al., 1999, 2001; Zhang and Peterson, 1999; Evgen'ev et al., 2000; Biémont and Vieira, 2006]. Moreover, it has been predicted that the TEs are important in the sex chromosome evolution of eukaryotic genomes [Bachtrog, 2005; Charlesworth et al., 2005; Fraser and Heitman, 2005; Ming and Moore, 2007]. The enrichment of *Rex* elements in the largest chromosome pair of *O. niloticus* suggests their involvement in the proposed chromosome fusions and sex chromosome differentiation. As the largest chromosome pair of *O. niloticus* may reflect an ancient state for the sex chromosome in the tilapiine group [Cnaani et al., 2008], the accumulation of *Rex* elements along the length of the q arm, together with other repeated sequences [Harvey et al., 2003; Ferreira and Martins, 2008; Ferreira et al., 2010], indicates that the repetitive elements played important roles in the differentiation of sex chromosomes in the group. Similarly, in *C. hamatus*, the retrotransposon *Rex3* has also accumulated in the long arm of the male Y chromosome, indicating that the TE could have been involved in the fusion process and molecular differentiation that created the sex chromosomes in this species [Ozouf-Costaz et al., 2004].

Dispersed signals of *Rex* elements were also detected in some euchromatic areas, as observed in *H. obliquidens*, *S. jurupari* and *C. flavescens*; however, the signals are more obvious in *H. obliquidens*. Considering that this signal pattern was not seen in the haplochromine *M. auratus*, we speculate that the dispersed pattern of *Rex* elements in *H. obliquidens* may be related to some genetic traits of the *Haplochromis* genus. In fact, genomic in situ hybridization (GISH) analysis revealed divergence of the haplochromines *(H. obliquidens)* from other African cichlids in the distribution of repetitive DNAs [Valente et al., 2009]. Furthermore, there is evidence that *Haplochromis* is a highly diverse group of African cichlids [Kornfield et al., 1979; Liem, 1991; Turner, 2007].

The cichlid *Cichla kelberi* has an accumulation of *Rex1* and *Rex3* in euchromatic regions of 2 chromosome pairs [Teixeira et al., 2009], and this pattern is different from the dispersed signals in euchromatic regions found in the other cichlids analyzed here (such as *H. obliquidens*, *S. jurupari* and *C. flavescens*). *Cichla* is the sister-group of all Cichlinae (Neotropical cichlids) [Smith et al., 2008] and *C. kelberi* is more closely related to the possible ancestral karyotype of the cichlid groups. The accumulation of *Rex* in the interstitial euchromatic areas could be more closely related to a karyotype with few changes, such as observed in *C. kelberi* [Teixeira et al., 2009], whereas rearranged karyotypes could harbor dispersed clusters of *Rex* elements. Although TEs have been associated with karyotype rearrangements [Lim and Simmons, 1994; Dimitri et al., 1997], changes in the genome could eliminate the *Rex* elements in interstitial euchromatic areas and lead to their accumulation in heterochromatic regions. In *H. efasciatus* and *Symphysodon* species, which belong to groups with rearranged karyotypes, the *Rex* elements are clustered in heterochromatic areas [Gross et al., 2009; present study].

The *Rex3* element showed less intense signals than the other elements in *H. bimaculatus* and *M. auratus*. On the other hand, in *H. obliquidens*, *C. flavescens* and *S. jurupari Rex3* showed more intense signals than *Rex1* and *Rex6*. In addition, *Rex6* showed intense signals in *A. ocellatus* and *H. efasciatus*. The differences in signal intensity seem to be related to the copy number of these elements in the genome of the cichlid species analyzed. The differences in the copy number and chromosomal distribution of the different *Rex* elements suggested that these repetitive sequences were independently amplified or excluded after the split of the ancestral cichlid lineages. In fact, the results of physical mapping of *Rex1* and *Rex3* in Antarctic fish species revealed variations in the accumulation of these elements in different species of the same suborder [Ozouf-Costaz et al., 2004].

Special attention must be exercised concerning the relationship between the data obtained through the cytogenetic mapping of dispersed repeated DNAs and the data provided through the complete sequencing of genomes. The dispersed signals of transposable elements revealed through the molecular cytogenetic analysis does not represent dispersed single copies, but small clusters of at least few copies of the DNA element. The current FISH procedure conducted in most cytogenetic laboratories permits the visualization of segments of DNA that encompass at least 10 kb [Jiang et al., 1995]. In this way, several copies of the *Rex* transposons have to be closely organized in the genome to generate a single detectable signal onto chromosomes. At the same time, dispersed cytogenetic signals need careful analysis concerning several aspects of the FISH procedure employed as size, amount and nucleotide content of the probe, and stringency conditions of the hybridization procedure. Such FISH conditions can generate background easily misunderstood as real signals in the chromosomes.

The Relationship between Rex *Elements and Chromatin Traits*

It is believed that some factors such as gene density, chromatin structure and recombination rate may have a role in the maintenance of TEs at a specific genomic region [Hua-Van et al., 2005]. Generally, TEs seem to be more abundant in heterochromatin in several genomes, and their presence in these regions seems to be common among multicellular eukaryotes [Dawe, 2003; Hua-Van et al., 2005]. Despite some discordance [for more details see Dimitri and Junakovic, 1999], there are many possible explanations for the relationship between TEs and heterochromatin: (i) TEs tend to accumulate in regions with low recombination rates as a consequence of their removal from regions with high recombination rates, where ectopic recombination could have more deleterious effects; (ii) there is more elimination of TEs in gene-rich regions because of their potential deleterious effects when inserted within genes; (iii) the high expression of TE-encoded products could have negative consequences for the genome due to the cost for the cell; thus, these TEs would be eliminated from regions with high expression levels [Hua-Van et al., 2005]; (iv) TEs could accumulate in heterochromatin as a consequence of their functional involvement in the maintenance of specific genomic regions, such as the pericentromeric and telomeric regions [Dimitri and Junakovic, 1999; Dawe, 2003].

The absence of genomic data for the cichlids analyzed here, such as gene density, GC content, recombination rate, and epigenetic data, among others, make the elucidation of the mechanism(s) for *Rex* distribution in these species difficult. Considering that the genomes of several African cichlid species will soon be sequenced [proposal by Kocher, 2006], the knowledge of the chromosomal distribution of DNA sequences, as exemplified here by the TEs *Rex1*, *Rex3* and *Rex6*, will be of particular value for achieving an integrated view of the cichlid genomes.

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