RESEARCH ARTICLE

Open Access

Chromosome spreading of associated transposable elements and ribosomal DNA in the fish *Erythrinus erythrinus*. Implications for genome change and karyoevolution in fish

Marcelo B Cioffi^{1*}, Cesar Martins², Luiz AC Bertollo¹

Abstract

Background: The fish, *Erythrinus erythrinus*, shows an interpopulation diversity, with four karyomorphs differing by chromosomal number, chromosomal morphology and heteromorphic sex chromosomes. Karyomorph A has a diploid number of $2n = 54$ and does not have differentiated sex chromosomes. Karyomorph D has $2n = 52$ chromosomes in females and $2n = 51$ in males, and it is most likely derived from karyomorph A by the differentiation of a multiple X_1X_2Y sex chromosome system. In this study, we analyzed karyomorphs A and D by means of cytogenetic approaches to evaluate their evolutionary relationship.

Results: Conspicuous differences in the distribution of the 5S rDNA and *Rex3* non-LTR retrotransposon were found between the two karyomorphs, while no changes in the heterochromatin and 18S rDNA patterns were found between them. *Rex3* was interstitially dispersed in most chromosomes. It had a compartmentalized distribution in the centromeric regions of only two acrocentric chromosomes in karyomorph A. In comparison, in karyomorph D, *Rex3* was found in 22 acrocentric chromosomes in females and 21 in males. All 5S rDNA sites co-localized with *Rex3*, suggesting that these are associated in the genome. In addition, the origin of the large metacentric Y chromosome in karyomorph D by centric fusion was highlighted by the presence of internal telomeric sites and 5S rDNA/*Rex3* sites on this chromosome.

Conclusion: We demonstrated that some repetitive DNAs (5S rDNA, *Rex3* retroelement and $(TTAGGG)_n$ telomeric repeats) were crucial for the evolutionary divergence inside *E. erythrinus*. These elements were strongly associated with the karyomorphic evolution of this species. Our results indicate that chromosomal rearrangements and genomic modifications were significant events during the course of evolution of this fish. We detected centric fusions that were associated with the differentiation of the multiple sex chromosomes in karyomorph D, as well as a surprising increase of associated 5S rDNA/*Rex3* loci, in contrast to karyomorph A. In this sense, *E. erythrinus* emerges as an excellent model system for better understanding the evolutionary mechanisms underlying the huge genome diversity in fish. This organism can also contribute to understanding vertebrate genome evolution as a whole.

Background

Repetitive DNA sequences include tandemly-arrayed satellites, as well as minisatellites, microsatellites and dispersed repeats such as transposable elements (TEs) [1]. Satellite DNAs are organized as long arrays of head-

to-tail linked repeats. TEs are DNA segments capable of integrating into new locations in the genome, and they also mobilize non-autonomous sequences [2,3]. TEs and satellite DNAs are some of the most important components of the genome that contribute to genetic variations within and between species [4]. The possible functions of these repetitive DNAs have been the focus of several studies, and there are indications that they

* Correspondence: mbello.ufscar@gmail.com

¹Universidade Federal de São Carlos, Departamento de Genética e Evolução, São Carlos, SP, Brazil

Full list of author information is available at the end of the article

could play important roles at both the chromosomal and nuclear levels [5-8].

Fish genomes contain all known types of transposable elements: classical DNA transposons, miniature inverted-repeat transposable elements and retroelements, which include long terminal repeat (LTR) retrotransposons and non-LTR retrotransposons [9]. While DNA transposons move directly as DNA molecules from one genomic site to another, retroelements transpose via an RNA intermediate. Among retrotransposable elements, *Rex* is comprised of various families of transposable elements that are abundant in teleosts. *Rex3*, the first reverse transcriptase (RT)-encoding retrotransposon isolated from the melanoma fish model, *Xiphophorus*, is a non-LTR element related to the RTE family that shows wide distribution and different patterns of organization in the genomes of several fish species [10,11].

The molecular organization and cytogenetic locations of repetitive DNAs, including rDNA repeats [12-18], satellite DNAs [19,20], telomeric sequences [18,21,22] and several classes of TEs [2,3,23,24], have been analyzed in a large number of fish species. These studies have demonstrated the enormous potential that the investigation of repetitive DNAs offers toward extending our knowledge of karyotype differentiation and sex chromosome evolution in fish [16,18,25-29]. These genomic components are able to change the molecular composition of sex chromosomes and reduce the rate of recombination between them, which are crucial steps in the differentiation of sex chromosomes [30-33].

Erythrinus is a cytogenetically poorly studied genus inside the Erythrinidae family. Until now, classical cytogenetic analyses have only been conducted with the species, *E. erythrinus*. These have shown a karyotypic diversity among populations, with four currently identified karyomorphs (A to D) [34]. Karyomorph A is comprised of populations with $2n = 54$ chromosomes, which have very similar karyotypic structures ($6m + 2st + 46a$) and an absence of differentiated sex chromosomes. Karyomorphs B, C and D share an $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system, but they differ in their diploid number and chromosomal morphology. It has been proposed that a centric fusion between two non-homologous acrocentric chromosomes may have created the specific Y chromosome and, consequently, the unpaired X_1 and X_2 chromosomes in the male karyotypes. Karyomorph B has $2n = 54$ ($6m + 2st + 46a$) chromosomes in females and $2n = 53$ ($7m + 2st + 44a$) in males. Both karyomorphs C and D show $2n = 52/51$ chromosomes but differ in their karyotypic formula, i.e., $6m + 2sm + 6st + 38a$ in females and $7m + 2sm + 6st + 36a$ in males of karyomorph C and $4m + 2sm + 2st + 44a$ in females and $5m + 2sm + 2st + 42a$ in males of karyomorph D. The distinct chromosomal features found

among isolated populations suggest the occurrence of several unnamed new species within this fish group [34].

In this report, new samples from allopatric populations of karyomorphs A and D were analyzed using new methodological approaches and molecular cytogenetic analyses to find useful new characteristics for comparative genomics at the chromosomal level and to provide insights into the karyoevolutionary pathways in this fish group. The results show that chromosomal rearrangements and genomic modifications were significant events during the course of evolution of this fish. Centric fusions were found to be clearly associated with the differentiation of the multiple sex chromosomes in karyomorph D. In addition, a surprising increase in the number of associated 5S rDNA/*Rex3* loci was found in karyomorph D, in contrast to karyomorph A.

Results

Karyotyping and C-banding

The two populations showed evidence of the general karyotypic structures of the *Erythrinus* species, with few biarmed chromosomes and a large number of acrocentric ones (Fig. 1). The sample from Penápolis-SP showed $2n = 54$ chromosomes ($6m + 2st + 46a$) and lacked morphologically differentiated sex chromosomes, which is characteristic of karyomorph A. The samples from Natal-RN showed $2n = 52$ chromosomes ($4m + 2sm + 2st + 42a$) in females and $2n = 51$ chromosomes ($5m + 2sm + 2st + 42a$) in males, with a multiple $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system, which is characteristic of karyomorph D (Fig. 1). Conspicuous C-positive bands were observed in the centromeric/pericentromeric region of several chromosomes, as well as in the telomeric region of some pairs, in both karyomorphs. A small but significant heterochromatic block was found in the interstitial region of the long arms of the Y and X_1 chromosomes of karyomorph D (Fig. 1).

Nucleotide sequences

Nucleotide sequences were determined for the *Rex3* clones to confirm that the PCR-isolated DNA fragments corresponded to copies of the retrotransposable element, *Rex3*. One of these sequences was deposited in GenBank under the accession number, GU989321. NCBI BlastN searches identified a similarity between the *E. erythrinus* *Rex3* sequence and sequences found in fish from other distinct orders, such as Anguilliformes, Perciformes, Beloniformes, Cyprinodontiformes and Tetraodontiformes.

Cytogenetic mapping of 18S and 5S rDNAs, *Rex3* and (TTAGGG)_n telomeric repeats

Double-FISH with 5S and 18S rDNAs showed a similar distribution pattern for the 18S rDNA sites in both the

Figure 1 Karyotypes of males and females of *Erythrurus erythrurus* (karyomorphs A and D) under different cytogenetic analyses. The karyotypes, arranged by sequentially Giemsa-stained and C-banded chromosomes, were probed with 5S rDNA and 18S rDNA after a double-FISH analysis. Note the significant increase of 5S rDNA sites in karyomorph D. m, metacentric chromosomes; sm, submetacentric chromosomes; st, subtelocentric chromosomes; a, acrocentric chromosomes. Bar = 5 μ m.

A and D karyomorphs. Five acrocentric pairs with telomeric sites on the long or short arms were found. In addition, bitelomeric sites were found on pair no. 14. In contrast, a large difference was seen in 5S rDNA distribution. Karyomorph A showed only two 5S rDNA sites in the centromeric region of acrocentric pair no. 8, which also bears a telomeric 18S rDNA site on its long arm. Although karyomorph D shared the syntenic condition seen in karyomorph A, it showed a surprising increase in the number of 5S rDNA sites, with 22 in females and 21 in males. These sites were all found in the centromeric region of acrocentric chromosomes except for a site in the metacentric Y chromosome in males (Fig. 1).

Double-FISH with 5S rDNA and *Rex3* probes showed that *Rex3* has an interstitial and dispersed distribution pattern along most chromosomes in both karyomorphs. In addition, *Rex3* clusters were predominantly located in the centromeric regions and co-localized with heterochromatic blocks in both karyomorphs, which matches the 5S rDNA distribution pattern (Fig. 2).

Mapping of the (TTAGGG)_n telomeric repeats in males of karyomorph D showed the typically expected telomeric signals on both telomeres of all chromosomes. Interstitial telomeric sites (ITS) were located in the centromeric regions of the only submetacentric pair and on the largest metacentric Y chromosome (Fig. 3).

Discussion

Among Characiformes fish, which include the Erythrinidae family, the most frequent chromosomal number is $2n = 54$, and this number may represent the basal diploid number of this order [35]. In this context, karyomorph A of *E. erythrurus*, which has a diploid

number of $2n = 54$, may have the most primitive karyotype found in the *Erythrurus* genus. This finding also takes into account the fact that differentiated sex chromosomes are absent in this karyomorph. Similarly, karyomorph D, which is most likely derived from karyomorph A, shows a smaller diploid number due to chromosomal rearrangements and a well-differentiated multiple $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system. However, despite differences in diploid numbers and the occurrence of differentiated sex chromosomes, karyomorphs A and D share a relatively similar karyotypic structure formed by several acrocentric and a few biarmed chromosomes with similarly distributed C-bands and 18S rDNA sites. However, the repetitive 5S rDNA and *Rex3* sequences have quite distinct distributions in the two karyomorphs.

A reduction in chromosome numbers often results from centric fusion rearrangements from acrocentric chromosomes. Based only on classical cytogenetic data, it was previously proposed that an initial centric fusion gave rise to the $X_1X_1X_2X_2/X_1X_2Y$ sex system found in karyomorphs B, C and D of *E. erythrurus*. Also, it was proposed that the differentiation of karyomorph D resulted from another centric fusion between two non-homologous acrocentric chromosomes, which was the origin of the only submetacentric pair found in the karyotype. In addition, pericentric inversions completed this karyotypic differentiation by decreasing the number of metacentric chromosomes and increasing the number of acrocentric chromosomes [34]. Indeed, this karyomorphic differentiation in *E. erythrurus* has now been corroborated by our FISH mapping of repetitive DNA sequences (Fig. 4).

Figure 2 Metaphase plates of karyomorphs A and D of *Erythrinus erythrinus* showing the locations of the 5S rDNA and the *Rex3* retroelement on the chromosomes using double-FISH analysis. Note the dispersed interstitial pattern of *Rex3* in both karyomorphs and its co-localization with the 5S rDNA on the centromeric region of the chromosomes. The box indicates the clear aggregation of some acrocentric chromosomes, which was seen in almost all chromosome preparations. The Y chromosome is indicated. Bar = 5 μ m.

The chromosomal location of 5S rDNA, *Rex3* and telomeric repeats clearly corroborates the centric fusions that occurred during the karyotypic differentiation of karyomorph D. The mapping of ITS in the centromeric region of the submetacentric pair highlights the centric fusion that was involved in the origin of this chromosome pair, which is not found in karyomorph A. Similarly, our results support the proposed origin for the largest metacentric Y chromosome from another centric fusion. As expected, (TTAGGG)_n repeats were also found in the centromeric region of this chromosome (Fig. 3). ITS have been found in the centromeric region of a large number of vertebrate species, suggesting that chromosomal rearrangements can occur without the loss of these telomeric sequences [36]. The general hypothesis that ITS may be remnants of chromosome rearrangements that occurred during genome evolution is supported by several investigations [37].

The location of 5SrDNA/*Rex3* sequences at the centromeric position of the Y chromosome is of particular relevance. These sequences were found in the centromeric region of several acrocentric chromosomes, including the ones proposed as X₁ (no. 5) and X₂ (no. 12) in the karyotype. The mapping of 5S rDNA/*Rex3* sites in the centromeric region of the Y chromosome suggests that this chromosome was created from a centric fusion of acrocentric pairs (nos. 5 and 12), which gave rise to the unpaired X₁ and X₂ chromosomes in the male karyotype (Figs. 1 and 2). Although the

identification of the X₂ chromosome remains unclear, the X₁ chromosome appears to be the first acrocentric pair (no. 5) in this karyotype. This result is supported by the C-banding pattern in which a faint but informative C-positive band occurs interstitially in corresponding regions of the X₁ chromosome and the long arm of the Y chromosome. It is probable that the same centric fusion also gave rise to the X₁X₂Y sex system in karyomorphs B and C, since it appears to have originated before the divergence of these three karyomorphs [34] (Fig. 4).

The most remarkable difference between karyomorphs A and D was the distribution of 5S rDNA/*Rex3* sites over the chromosomes. While only a single chromosome pair was found to bear these sites in karyomorph A, a surprisingly large number of these sites were found in karyomorph D, with 22 sites in females and 21 in males. (Figs 2 and 4). *Rex3*, a non-LTR retrotransposon first isolated from the platyfish, *Xiphophorus maculatus*, shows a wide distribution among teleost fishes, where it appears to be associated with the evolution of different lineages [10,11]. Although *Rex3* shows a preferential localization in the centromeric region of chromosomes in some fishes [23,24,38-40], it is widely scattered over all chromosomes in several Antarctic ice-fish species, with intense hybridization signals in some specific chromosomal regions [3]. Since a large majority of fish species studied until now harbor a low number of 5S rDNA sites, with a few exceptions [41,42], the high

Figure 3 Male metaphase plate of *Erythrinus erythrinus* karyomorph D showing the location of the telomeric hybridization signals on both telomeres of all chromosomes. In addition, ITS are found in the centromeric region of the only submetacentric pair in the karyotype (arrows) and on the large metacentric Y chromosome (arrowhead). Bar = 5 μ m.

number of 5S rDNA sites found in karyomorph D was an intriguing feature. According to the most probable hypothesis that karyomorph D represents a derivative form compared to karyomorph A, our results clearly show a huge dispersal of 5S rDNA/*Rex3* elements throughout the centromeric regions of the acrocentric chromosomes. We hypothesize that *Rex3* may have inserted into 5S rDNA sequences and that the 5S rDNA-*Rex3* complex moved and dispersed in the karyotype, although this hypothesis deserves further investigation and a molecular characterization. The clear association among the centromeric regions of the acrocentric chromosomes appears to be a favorable condition for this spreading (see details in Fig. 2).

Previous reports have suggested that the rDNA locus can serve as an ideal niche for the long-term survival of TEs [43], as seen in several organisms [44-46]. *In situ* hybridization revealed permanent clustering of different TEs in the NOR regions, as well as near or within clusters of 5S rDNA [45,47]. Hybridization on extended DNA fibers identified insertions of TEs inside the rDNA region, the overlap of rDNA and TE-enriched regions, and small fragments of rDNA inside TE-enriched regions. The presence of TEs in or around rDNA sites increases the possibility for recombination, which appears to be a common event in plant karyotype evolution [48]. In flowering plants, the distribution of 5S rDNA genes is highly variable and may be partially explained by the activity of small non-autonomous retrotransposons named Cassandra [49]. It is currently

believed that TEs tend to accumulate in heterochromatic regions because there are fewer genes and a weaker selection in the heterochromatin than in the euchromatin [50]. Recent studies have proposed that the activity of TEs is one possible source for rDNA movement [43,48]. Studies have also documented the ability of some classes of transposons to capture entire genes and move them to different parts of the genome [51,52].

Thus, considering the correlation between karyotype rearrangement and retrotransposon activity [3], and that rapid chromosomal evolution in some vertebrate lineages may be driven by the activity of repetitive sequences [53], we propose two probable alternatives during the karyotypic diversification of *E. erythrinus*, i.e., (i) the chromosomes of this species have undergone rearrangements during an evolutionary process mediated by retrotransposon activity or (ii) rearrangement events, including posterior mobilization of TEs, promoted the karyotypic differentiation among populations. However, it is difficult to state if a change in TE content or activity is the cause or the consequence of a speciation process because the true role of transposable elements in speciation is still a subject of large debate [6].

The frequent switching between different sex determination systems and the rapid evolution of sex chromosomes in fishes may also be linked to the formation of new species [54]. Recent comparative studies have revealed that teleost genomes have experienced a higher rate of gene-linkage disruption and chromosomal rearrangements compared to mammals, which may be linked to the apparent plasticity of their genomes [55,56]. Studies in fish models would therefore help in better understanding the molecular and evolutionary mechanisms underlying the huge genome diversity in this group, and these studies would also contribute to our understanding of vertebrate genome evolution.

Conclusion

Our *in situ* investigation of repetitive DNA sequences resulted in useful new characteristics for comparative genomics at the chromosomal level and provided insights into the karyoevolutionary pathways in *E. erythrinus* fish. Chromosomal rearrangements and genomic modifications were significant events during the course of karyoevolution of this fish. The spreading of associated transposable elements and ribosomal DNA in the genome and the differentiation of a multiple sex chromosome system was strongly associated with the evolution of karyomorph D. Considering the facts that fish occupy the basal position in the phylogeny of vertebrates, that they have a diversity of sex determining mechanisms and that many fish species lack heteromorphic sex chromosomes, *E. erythrinus* emerges as an excellent model system for better understanding the

Figure 4 Overview of the evolutionary karyotypic pathways proposed for karyomorphs A-D of *Erythrurus erythrurus* on the basis of their karyotypic features and FISH mapping results for karyomorphs A and D. The main chromosomal changes from the probable basal karyotype ($2n = 54$; $6m + 2st + 46a$) are underlined. The upward schematic figure illustrates the chromosomal distribution of the 18S rDNA and 5S rDNA/Rex3 sequences in karyomorph A and the expanded distribution of the latter sites in karyomorph D, including the Y, X_1 and the probable X_2 chromosome. The different colors represent the probes used for FISH.

evolutionary mechanisms underlying the huge genome diversity found among vertebrates.

Methods

Specimens, mitotic chromosome preparation, chromosome staining and karyotyping

In this study, we analyzed new samples from populations of karyomorphs A and D of the fish, *E. erythrurus*. We studied a total of 28 specimens (16 males and 12 females). Overall, 13 specimens (8 males and 5 females) of karyomorph A were obtained from Penápolis - São Paulo State, and 15 specimens (8 males and 7 females) of karyomorph D were obtained from Natal - Rio Grande do Norte State. These samples belong to distinct Brazilian hydrographic basins, which are isolated by thousands of kilometers.

Mitotic chromosomes were obtained from cell suspensions of the anterior kidney using the conventional air-drying method [57]. The experiments followed ethical conducts, and anesthesia was used prior to sacrificing the animals. The process was approved by the FAPESP committee under no. 2009/14881-3. Chromosomes were sequentially Giemsa-stained and C-banded using barium hydroxide to detect the C-positive heterochromatin [58].

Approximately 30 metaphase spreads were analyzed per specimen to determine the diploid chromosome number and karyotype structure. Images were captured by the CoolSNAP system software, Image Pro Plus, 4.1 (Media Cybernetics, Silver Spring, MD, USA), coupled to an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan). The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) or acrocentric (a) according to the arm ratios [59].

Chromosome probes

Two tandemly-arrayed DNA sequences isolated from the genome of another Erythruridae species, *Hoplias malabaricus*, were used. The first probe contained a 5S rDNA repeat copy and included 120 base pairs (bp) of the 5S rRNA transcribing gene and 200 bp of the non-transcribed spacer (NTS) [12]. The second probe corresponded to a 1,400-bp segment of the 18S rRNA gene obtained via PCR from nuclear DNA [16]. The 5S and 18S rDNA probes were cloned into plasmid vectors and propagated in DH5α *E. coli* competent cells (Invitrogen, San Diego, CA, USA).

The retrotransposable element, *Rex3*, was obtained by PCR directly from the genome of *E. erythrurus* using the

primers *Rex3f* (5'-CGG TGA YAA AGG GCA GCC CTG) and *Rex3r* (5'-TGG CAG ACN GGG GTG GTG GT- 3'), as previously described [2,3]. The obtained nucleotide segment of the *Rex3* transposon corresponds to the encoding domains 1, 2, 2A, A and B of the RT gene [2]. A PCR-generated amplicon (~500 bp) was isolated from a gel, purified with the Sepaglas Band Prep Kit (Pharmacia Biotech, Orsay, France) and ligated into the pGEM-T plasmid (Promega, Heidelberg, Germany). This plasmid was used to transform DH5α *E. coli* competent cells (Invitrogen, San Diego, CA, USA). The positive clones were sequenced on an ABI Prism 377 DNA sequencer (Perkin Elmer, Branchburg, NJ, USA) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Branchburg, NJ, USA). The nucleotide sequence was subjected to Blastn [60] searches at the National Center for Biotechnology Information (NCBI) website <http://www.ncbi.nlm.nih.gov/blast> for the identification of any similarity of the isolated sequences to any known sequences from the nucleotide collection (nt/nr), whole-genome shotgun reads (WGS), genomic survey sequences (GSS) and high-throughput genomic sequences (HTGS) in GenBank.

The 5S rDNA probe was labeled with biotin-14-dATP by nick translation according to the manufacturer's recommendations (BioNick™ Labeling System; Invitrogen, San Diego, CA, USA). The 18S rDNA and *Rex3* probes were labeled by nick translation with DIG-11-dUTP according to the manufacturer's instructions (Roche, Mannheim, Germany).

A probe from the telomeric DNA sequence (TTAGGG)_n was generated by PCR (PCR DIG-Probe Synthesis Kit, Roche) in the absence of a template using (TTAGGG)₅ and (CCCTAA)₅ as primers [37].

Fluorescent *in situ* hybridization (FISH) was performed under high stringency conditions on mitotic chromosome spreads [61]. The metaphase chromosome slides were incubated with RNase (40 µg/ml) for 1.5 h at 37°C. After denaturation of chromosomal DNA in 70% formamide/2× SSC at 70°C, spreads were incubated in 2× SSC for 4 min at 70°C. The hybridization mixture (2.5 ng/µl probes, 2 µg/µl salmon sperm DNA, 50% deionized formamide, 10% dextran sulphate) was dropped on the slides, and the hybridization was performed overnight at 37°C in a moist chamber containing 2× SSC. Two post-hybridization washes were carried out on a shaker (150 rpm) at 37°C. The first wash was in 2× SSC, 50% formamide for 15 min, followed by a second wash in 2× SSC for 15 min. A final wash was performed at room temperature in 4× SSC for 15 min. Avidin-FITC (Sigma, St. Louis, MO, USA) was used for signal detection of the 5S rDNA probe and anti-digoxigenin-rhodamine (Roche, Mannheim, Germany) for 18S rDNA, *Rex3* and

(TTAGGG)_n probes. One-color FISH was performed to detect (TTAGGG)_n repeats, while 5S/18S rDNA and 5S rDNA/*Rex3* were detected by double-FISH. The chromosomes were counterstained with DAPI (1.2 µg/ml), mounted in Antifade solution (Vector, Burlingame, CA, USA) and analyzed in an epifluorescence microscope Olympus BX50 (Olympus Corporation, Ishikawa, Japan)

Abbreviations

2n: diploid number; DAPI: 4'-6-Diamidino-2-phenylindole; FITC: fluorescein isothiocyanate; FISH: fluorescent in-situ hybridization; ITS: interstitial telomeric sites; LTR: long terminal repeats; NCBI: National Center for Biotechnology Information; NOR: nucleolar organizer region; NTS: non-transcribed spacer; PCR: polymerase chain reaction; RT: reverse transcriptase; rDNA: ribosomal DNA; SSC: sodium chloride-sodium citrate buffer; TEs: transposable elements.

Acknowledgements

The authors are grateful to Dr. Christian Biémont for his helpful suggestions and critical review of the manuscript and to Dr. Wagner Franco Molina for supplying fish specimens. This work was supported by the Brazilian agencies FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Author details

¹Universidade Federal de São Carlos, Departamento de Genética e Evolução, São Carlos, SP, Brazil. ²UNESP- Universidade Estadual Paulista, Instituto de Biotecnologia, Departamento de Morfologia, Botucatu, SP, Brazil.

Authors' contributions

MBC performed the experiments and drafted the manuscript. CM helped in analysis and drafted the manuscript. LACB designed and coordinated the study, and drafted and revised the manuscript. All authors read and approved the final manuscript.

Received: 10 May 2010 Accepted: 6 September 2010

Published: 6 September 2010

References

1. Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J: **Repbase update, a database of eukaryotic repetitive elements.** *Cytogenet Genome Res* 2005, **110**:462-467.
2. Volff JN, Bouneau L, Ozouf-Costaz C, Fischer C: **Diversity of retrotransposable elements in compact pufferfish genomes.** *Trends Genet* 2003, **19**:674-678.
3. Ozouf-Costaz C, Brandt J, Körting C, Pisavo E, Bonillo C, Contanceau JP, Volff JN: **Genome dynamics and chromosomal localization of the non-LTR retrotransposons *Rex1* and *Rex3* in Antarctic fish.** *Antarct Sci* 2004, **16**:51-57.
4. Biémont C: **Within-species variation in genome size.** *Heredity* 2008, **101**:297-298.
5. Larin Z, Fricker MD, Tyler-Smith C: **De novo formation of several features of a centromere following introduction of an Y alphoid YAC into mammalian cells.** *Hum Mol Genet* 1994, **3**:689-695.
6. Biémont C, Vieira C: **Junk DNA as an evolutionary force.** *Nature* 2006, **443**:521-524.
7. Feschotte C, Pritham EJ: **DNA Transposons and the evolution of eukaryotic genomes.** *Annu Rev Genet* 2007, **41**:331-368.
8. Longo MS, Carone DM, Marzelli M, NISC Comparative Sequencing Program, Green ED, O'Neill MJ, O'Neill RJ: **Distinct retroelement classes define evolutionary breakpoints demarcating sites of evolutionary novelty.** *BMC Genomics* 2009, **10**:334.
9. Volff JN: **Genome evolution and biodiversity in teleost fish.** *Heredity* 2005, **94**:280-294.
10. Volff JN, Körting C, Sweeney K, Scharl M: **The non-LTR retrotransposon *Rex3* from the fish *Xiphophorus* is widespread among teleosts.** *Mol Biol Evol* 1999, **16**:1427-1438.

11. Malik HS, Eickbush TH: The RTE class of non-LTR retrotransposons is widely distributed in animals and is the origin of many SINEs. *Mol Biol Evol* 1998, **15**:1123-1134.
12. Martins C, Ferreira IA, Oliveira C, Foresti F, Galetti PM Jr: A tandemly repetitive centromeric DNA sequence of the fish *Hoplias malabaricus* (Characiformes: Erythrinidae) is derived from 5 S rDNA. *Genetica* 2006, **127**:133-141.
13. Vicari MR, Artoni RF, Bertollo LAC: Comparative cytogenetics of *Hoplias malabaricus* (Pisces, Erythrinidae): A population analysis in adjacent hydrographic basins. *Genet Mol Biol* 2005, **28**:103-110.
14. Vicari MR, Artoni RF, Moreira-Filho O, Bertollo LAC: Co localization of repetitive DNAs and silencing of major rRNA genes. A case report of the fish *Astyanax jareiroensis*. *Cytogenet Genome Res* 2008, **122**:67-72.
15. Cioffi MB, Martins C, Bertollo LAC: Comparative chromosome mapping of repetitive sequences. Implications for genomic evolution in the fish, *Hoplias malabaricus*. *BMC Genetics* 2009, **10**:34.
16. Cioffi MB, Martins C, Centofante L, Jacobina U, Bertollo LAC: Chromosomal variability among allopatric populations of Erythrinidae Fish *Hoplias malabaricus*: mapping of three classes of repetitive DNAs. *Cytogenet Genome Res* 2009, **125**:132-141.
17. Blanco DR, Lui RL, Bertollo LAC, Diniz D, Moreira-Filho O: Characterization of invasive fish species in a river transposition region: evolutionary chromosome studies in the genus *Hoplias* (Characiformes, Erythrinidae). *Rev Fish Biol Fisheries* 2009, **20**:1-8.
18. Cioffi MB, Bertollo LAC: Initial steps in XY chromosome differentiation in *Hoplias malabaricus* and the origin of an X1X2Y sex chromosome system in this fish group. *Heredity* 2010.
19. Ferreira IA, Bertollo LAC, Martins C: Comparative chromosome mapping of 5 S rDNA and 5S HindIII repetitive sequences in Erythrinidae fish (Characiformes) with emphasis on *Hoplias malabaricus* "species complex". *Cytogenet Genome Res* 2007, **118**:78-83.
20. Pazza R, Kavalco KF, Bertollo LAC: Chromosome polymorphism in *Astyanax fasciatus* (Teleostei, Characidae). 2 Chromosomal location of a satellite DNA. *Cytogenet Genome Res* 2008, **122**:61-66.
21. Chew JSK, Oliveira C, Wright JM, Dobson MJ: Molecular and cytogenetic analysis of the telomeric (TTAGGG) $_n$ repetitive sequences in the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). *Chromosoma* 2002, **111**:45-52.
22. Sola L, De Innocentiis S, Gornung E, Papalia S, Rossi AR, Marino G, De Marco P, Cataudella S: Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18 S and 5 S rRNA genes and of the (TTAGGG) $_n$ telomeric sequence. *Marine Biol* 2000, **137**:47-51.
23. Mazzuchelli J, Martins C: Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*. *Genetica* 2009, **136**:461-469.
24. Teixeira WG, Ferreira IA, Cabral-de-Mello DC, Mazzuchelli J, Valente GT, Pinhal D, Poletto AB, Venere PC, Martins C: Organization of repeated DNA elements in the genome of the cichlid fish *Cichla kelberi* and its contributions to the knowledge of fish genomes. *Cytogenet Genome Res* 2009, **125**:224-234.
25. Nanda I, Feichtinger W, Schmid M, Schroder JH, Zischler H, Epplen JC: Simple repetitive sequences are associated with differentiation of the sex chromosomes in the guppy fish. *J Mol Evol* 1990, **30**:456-462.
26. Nanda I, Volff JN, Weis S, Körtling C, Froschauer A, Schimid M, Scharl M: Amplification of a long terminal repeat-like element on the Y chromosome of the platyfish, *Xiphophorus maculatus*. *Chromosoma* 2000, **109**:173-180.
27. Nanda I, Kondo M, Hornung U, Asakawa S, Winkler C, Shimizu A, Shan Z, Haaf T, Shimizu N, Shima A, Schimid M, Scharl M: A duplicated copy of DMRT1 in the sex determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proc Natl Acad Sci USA* 2002, **99**:11778-11783.
28. Stein J, Phillips RB, Devlin RH: Identification of the Y chromosome in Chinook salmon (*Oncorhynchus tshawytscha*). *Cytogenet Cell Genet* 2001, **92**:108-110.
29. Parise-Maltempi PP, Martins C, Oliveira C, Foresti F: Identification of a new repetitive element in the sex chromosomes of *Leporinus elongatus* (Teleostei: Characiformes: Anostomidae): new insights into the sex chromosomes of *Leporinus*. *Cytogenet Genome Res* 2007, **116**:218-223.
30. Liu ZY, Moore PH, Ma H, Ackerman CM, Ragiba M, Yu Q, Pearl HM, Kim MS, Charlton JW, Stiles JI, Zee FT, Peterson AH, Ming R: A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature* 2004, **427**:348-352.
31. Charlesworth D, Charlesworth B, Marais G: Steps in the evolution of heteromorphic sex chromosomes. *Heredity* 2005, **95**:118-128.
32. Marchal JA, Acosta MJ, Bullesos M, Puerma E, Díaz de la Guardia R, Sánchez A: Distribution of L1-retroposons on the giant sex chromosomes of *Microtus cabreræ* (Arvicolidae, Rodentia): functional and evolutionary implications. *Chromosome Res* 2006, **14**:177-186.
33. Kejnovsky E, Hobza R, Cermák T, Kubát Z, Vyskot B: The role of repetitive DNA in structure and evolution of sex chromosomes in plants. *Heredity* 2009, **102**:533-541.
34. Bertollo LAC, Oliveira C, Molina WF, Margarido VP, Fontes MS, Pastori MC, Falcão JN, Fenocchio : Chromosome evolution in the erythrinid fish, *Erythrinus erythrinus* (Teleostei: Characiformes). *Heredity* 2004, **93**:228-233.
35. Oliveira C, Almeida-Toledo LF, Foresti F: Karyotypic evolution in Neotropical fishes. In *Fish cytogenetics*. Edited by: Pisano E, Ozouf-Costaz C, Foresti F, Kappor BG. Enfield NH, USA: Science Publisher; 2007:111-164.
36. Meyne J, Ratliff RL, Moyzis RK: Conservation of the human telomere sequence (TTAGGG) $_n$ among vertebrates. *Proc Natl Acad Sci USA* 1989, **86**:7049-7053.
37. Ijdo JW, Wells RA, Baldini A, Reenders ST: Improved telomere detection using a telomere repeat probe (TTAGGG) $_n$ generated by PCR. *Nucleic Acids Res* 1991, **19**:4780.
38. Dasilva C, Hadji H, Ozouf-Costaz C, Nicaud S, Jaillon O, Weissenbach J, Crollius HR: Remarkable compartmentalization of transposable elements and pseudogenes in the heterochromatin of the *Tetraodon nigroviridis* genome. *Proc Natl Acad Sci USA* 2002, **99**:1636-1641.
39. Bouneau L, Fisher C, Ozouf-Costaz C, Froschauer A, Jaillon O, Coutanceau JP, Korting C, Weissenbach J, Bernot A, Volff JN: An active Non-LTR retrotransposon with tandem structure in the compact genome of the pufferfish *Tetraodon nigroviridis*. *Genome Res* 2003, **13**:1686-1695.
40. Gross MC, Schneider CH, Valente GT, Porto JIR, Martins C, Feldberg E: Comparative cytogenetic analysis of the genus *Symphysodon* (Discus Fishes, Cichlidae): chromosomal characteristics of retrotransposons and minor ribosomal DNA. *Cytogenet Genome Res* 2009, **127**:43-53.
41. Affonso PRAM, Galetti PM Jr: Chromosomal diversification of reef fishes from genus *Centropyge* (Perciformes, Pomacanthidae). *Genetica* 2005, **123**:227-233.
42. Poletto AB, Ferreira IA, Martins C: The B chromosome of the cichlid fish *Haplochromis obliquens* harbors 18 S rRNA genes. *BMC Genetics* 2010, **11**:1-8.
43. Zhang X, Eickbush MT, Eickbush TH: Role of recombination in the long-term retention of transposable elements in rRNA gene loci. *Genetics* 2008, **180**:1617-1626.
44. Eickbush TH: R2 and related site-specific non-long terminal repeat retrotransposons. In *Mobile DNA II*. Edited by: Craig NL, Craigie R, Gellart M, Lambowitz AM. Washington DC: American Society of Microbiology; 2002:813-835.
45. Belyayev A, Raskina O, Nevo E: Variability of Ty3 gypsy retrotransposons chromosomal distribution in populations of two wild Triticeae species. *Cytogenet Genome Res* 2005, **109**:43-50.
46. Ye J, Eickbush TH: Chromatin structure and transcription of the R1- and R2-inserted rRNA genes of *Drosophila melanogaster*. *Mol Cell Biol* 2006, **26**:8781-8790.
47. Raskina O, Belyayev A, Nevo E: Quantum speciation in *Aegilops*: molecular cytogenetic evidence from rDNA clusters variability in natural populations. *Proc Natl Acad Sci USA* 2004, **101**:14818-14823.
48. Raskina O, Barber JC, Nevo E, Belyayev A: Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. *Cytogenet Genome Res* 2008, **120**:351-357.
49. Kalendar R, Tanskanen J, Chang W, Antonius K, Sela H, Peleg O, Schulman A: Cassandra retrotransposons carry independently transcribed 5 S RNA. *Proc Natl Acad Sci USA* 2008, **105**:5833-5838.
50. Venner S, Feschotte C, Biémont C: Dynamics of transposable elements: Towards a community ecology of the genome. *Trends Genet* 2009, **25**:317-323.
51. Jiang N, Bao Z, Zhang X, Eddy SR, Wessler SR: Pack-MULE transposable elements mediate gene evolution in plants. *Nature* 2004, **431**:569-573.

52. Lai Z, Nakazato T, Salmasso M, Burke JM, Tang S, Knapp SJ, Rieseberg LH: **Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species.** *Genetics* 2005, **171**:291-303.
53. Wichman HA, Payne CT, Ryder OA, Hamilton MJ, Maltbie M, Baker RJ: **Genomic distribution of heterochromatic sequences in equids: implications to rapid chromosomal evolution.** *J Hered* 1991, **82**:369-377.
54. Barske LA, Capel B: **Blurring the edges in vertebrate sex determination.** *Curr Opin Genet Dev* 2008, **18**:499-505.
55. Ravi V, Venkatesh B: **Rapidly evolving fish genomes and teleost diversity.** *Curr Opin Genet Dev* 2008, **18**:544-550.
56. Venkatesh B: **Evolution and diversity of fish genomes.** *Curr Opin Genet Dev* 2003, **13**:588-592.
57. Bertollo LAC, Takahashi CS, Moreira-Filho O: **Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae).** *Brazil J Genet* 1978, **1**:103-120.
58. Sumner AT: **A simple technique for demonstrating centromeric heterochromatin.** *Exp Cell Res* 1972, **75**:304-306.
59. Levan A, Fredga K, Sandberg AA: **Nomenclature for centromeric position on chromosomes.** *Hereditas* 1964, **52**:201-220.
60. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215**:403-410.
61. Pinkel D, Straume T, Gray J: **Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization.** *Proc Natl Acad Sci USA* 1986, **83**:2934-2938.

doi:10.1186/1471-2148-10-271

Cite this article as: Cioffi et al.: Chromosome spreading of associated transposable elements and ribosomal DNA in the fish *Erythrinus erythrinus*. Implications for genome change and karyoevolution in fish. *BMC Evolutionary Biology* 2010 **10**:271.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit