Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture 231 (2004) 37–49
Aquaculture
www.elsevier.com/locate/aqua-online

Physical mapping of the Nile tilapia (*Oreochromis niloticus*) genome by fluorescent in situ hybridization of repetitive DNAs to metaphase chromosomes—a review

Cesar Martins[a,*], Claudio Oliveira[a], Adriane P. Wasko[a], Jonathan M. Wright[b]

[a] *Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, CEP 18618-000, Botucatu, SP, Brazil*
[b] *Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1*

Received 30 May 2003; received in revised form 14 July 2003; accepted 6 August 2003

Abstract

The Nile tilapia (*Oreochromis niloticus*) has received increasing scientific interest over the past few decades for two reasons: first, tilapia is an enormously important species in aquaculture worldwide, especially in regions where there is a chronic shortage of animal protein; and second, this teleost fish belongs to the fascinating group of cichlid fishes that have undergone a rapid and extensive radiation of much interest to evolutionary biologists. Currently, studies based on physical and genetic mapping of the Nile tilapia genome offer the best opportunities for applying genomics to such diverse questions and issues as phylogeography, isolation of quantitative trait loci involved in behaviour, morphology, and disease, and overall improvement of aquacultural stocks. In this review, we have integrated molecular cytogenetic data for the Nile tilapia describing the chromosomal location of the repetitive DNA sequences, satellite DNAs, telomeres, 45S and 5S rDNAs, and the short and long interspersed nucleotide elements [short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs)], and provide the beginnings of a physical genome map for this important teleost fish.

Keywords: Physical mapping; Chromosomes; Repetitive sequences; Nile tilapia; Genome; *Oreochromis niloticus*

* Corresponding author. Tel.: +55-14-68026322; fax: +55-14-68213744.
E-mail address: cmartins@ibb.unesp.br (C. Martins).

0044-8486/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2003.08.017

1. Introduction

Cichlids are the predominant fishes of the Africa's Great Lakes that are now distributed worldwide in tropical and subtropical regions (Trewavas, 1983). Owing to their rapid adaptive radiation, which has led to an extensive ecological diversity, and the great importance of some species to tropical and subtropical aquaculture, the cichlid fishes have received increasing scientific interest (Pullin, 1991). The diversity of the species—more than 1500 species just in Africa—is of primary scientific interest. The African cichlids can be divided into three major groups: (1) Pelmatochromine cichlids, (2) Haplochromine cichlids and (3) Tilapiine cichlids (Lowe-McConnell, 1991). The Haplochromine cichlids of East and Central Africa, especially those of the Rift Valley Lakes, have undergone a remarkable speciation event and are now considered as a classic example of adaptive radiation (Liem, 1991). It is believed that there are 500–1000 different cichlid species adapted to specific niches solely in Lake Malawi (Stiassny, 1991). The Tilapiine tribe, commonly named tilapia, includes the genera *Sarotherodon*, *Oreochromis* and *Tilapia*, and the fourth genus, *Danakilia*, which comprises a single species.

Although approximately 70 species of cichlids are referred to as "tilapia", only *Oreochromis niloticus*, *Oreochromis mossambicus* and *Oreochromis aureus*, and their hybrids, have great importance in world fisheries. Nowadays, the Nile tilapia, *O. niloticus*, represents one of the most widely farmed freshwater fish in the world. Aquaculture practices may inadvertently decrease the genetic variability present in farmed stocks by the selection and breeding of related individuals, or by the use of a small number of parents as broodstock. Unless careful genetic records are maintained, there is a likelihood for increased inbreeding. Many of the domesticated strains of the Nile tilapia suffer from inbreeding depression and the best-performing strains are those most recently isolated from nature. In genetic terms, knowledge of the Nile tilapia genome is rather preliminary, and far behind pufferfish and the zebrafish. Owing to its scientific importance to fundamental and applied biology, genomic studies and genome maps for the Nile tilapia are timely and surely warranted.

2. Genome mapping

In the past decade, DNA-based genetic markers have been developed for use in aquaculture primarily with the goal of improving fish stocks and strains for important traits such as growth enhancement and resistance to viral and bacterial disease. Various DNA markers have been used for the construction of genetic maps that will also be of particular benefit in aquaculture, specifically for stock identification, selective breeding analysis of quantitative traits, and assessing the genetic variability of populations. Genome maps will also find applications in behavioural, morphological, phylogeographic and other evolutionary studies.

Currently, genome maps are constructed in one of three ways; (1) physical maps that localize DNA segments onto the karyotype of a species by cytogenetic methodologies; (2) genetic linkage maps that assign the linear order of DNA markers along a chromosome based on recombination frequencies between loci; and (3) the ultimate map, the complete

nucleotide sequence of a species' genome. The construction and integration of physical and genetic maps for the Nile tilapia currently represents the best strategy for the understanding of the structure and evolution of the cichlid genome.

Cytogenetic studies have shown that the haploid genome of the Nile tilapia consists of 22 chromosomes (Kornfield et al., 1979; Majumdar and McAndrew, 1986) with a genome size of 1.2 pg, or about 10^9 bp (Hinegardner and Rosen, 1972). To date, polymorphic DNA markers have been applied in the construction of a genetic linkage map for the Nile tilapia (Kocher et al., 1998) with the goal of identifying single genes or quantitative trait loci which might be useful for the improvement of Nile tilapia production and other applications. Physical mapping of the chromosomes of *O. niloticus* also represents a powerful way to trace useful genetic markers for the improvement of the Nile tilapia production. Molecular cytogenetic information described for the Nile tilapia is related to the structure and the distribution of repetitive DNA sequences in the chromosomes of this species. Except for the first chromosome pair, the remaining chromosomes in the Nile tilapia karyotype are nearly identical in morphology and size, which makes identification of particular karyotypic elements difficult. The integration of a physical and a genetic map has been hindered due to the absence of specific chromosome markers for this species and, until recently, limited information on the structure of the Nile tilapia chromosomes and their identification was available.

3. Chromosome markers in the Nile tilapia

A substantial fraction of the eukaryotic genome, in some instances greater than 90%, consists of repetitive DNA sequences. These include satellite, minisatellite and microsatellite sequences, transposable elements, and multigene families such as the ribosomal RNA gene clusters. Although studied extensively for the past four decades, the molecular forces that propagate and maintain repetitive DNAs in the genome are still not well understood. However, the role of these DNAs in genome organization and evolution, and their likely impact on speciation is increasingly appreciated (Charlesworth et al., 1994).

In the course of identification of chromosome markers for the Nile tilapia, several repetitive DNA families have been isolated and their organization investigated in the genome of this species. Here we review the molecular cytogenetic data concerning satellite DNAs, telomeric sequences, 45S rDNA, 5S rDNA, SINEs and LINEs in the Nile tilapia.

3.1. Satellite DNAs

C-banding of metaphase chromosomes of *Oreochromis* provided evidence that heterochromatin is localized along and around the centromeres of the chromosomes of all species of the *Oreochromis* genus so far studied (Majumdar and McAndrew, 1986; Oliveira and Wright, 1998). As C-band positive segments are mostly composed of satellite DNAs, investigations have been directed towards the isolation and characterization of such sequences in the genome of the Nile tilapia. Two highly repeated tandemly arrayed sequences have been cloned, sequenced and their genomic organization determined. The first isolated satellite, SATA (Table 1), is approximately 237-bp

Table 1
Characteristics of repetitive sequences mapped in the chromosomes of the Nile tilapia (*O. niloticus*)

Repetitive sequence	Repeat type	Monomer size (bp)	Chromosome location
SATA	Satellite	237	Centromeric region of all chromosomes
SATB	Satellite	1900	Short arm of chromosome 4
$(TTAGGG)_n$	Telomeric		All telomeres and two interstitial loci in the long arm of chromosome 1
CiLINE2	LINE	1165[a]	Dispersed over all chromosomes and enriched in the long arm of chromosome 1
ROn-1	SINE	345	Small clusters dispersed in all chromosomes and a large cluster in the long arm of chromosome 1
ROn-2	SINE	359	Small clusters dispersed in all chromosomes
18S rDNA	45S rDNA		Chromosomes 8, 10 and 15
5S rDNA type I	5S rDNA	1417	Chromosome 3
5S rDNA type II	5S rDNA	475	Chromosomes 9 and 13

[a] Length of cloned element. Other CiLine2 elements in the Nile tilapia genome may be considerably larger.

length (size and sequence variation has been observed in related tilapiine species) (Wright, 1989; Franck et al., 1992) and is present in the genomes of other African cichlids with copy numbers ranging from 0.1 to 5.41×10^5 depending on the species examined (Franck et al., 1994). The second satellite sequence, SATB (Table 1), although not as abundant as SATA, is a highly reiterated sequence ($1-10 \times 10^3$ copies) of 1900 bp present in the Nile tilapia genome and also in the closely related cichlid tribe, Haplochrominae, but not present in the genomes of Asian or New World cichlids (Franck and Wright, 1993).

Fluorescent in situ hybridization (FISH) of SATA (Oliveira and Wright, 1998) showed that this abundant satellite DNA is localized primarily, if not exclusively, in the centromeric region of all the Nile tilapia chromosomes, and is also present in the short arms of two chromosome pairs (Fig. 1).

The presence of abundant copies of the SATA satellite at the centromeres of all chromosomes of the Nile tilapia, as detected under high stringency conditions, suggests that some form of amplification and intragenomic turnover must occur in this species. In situ hybridization data from mouse (Hamilton et al., 1990), in which satellite DNAs are distributed in all chromosomes, suggest that in some species, there is a tremendous intragenomic movement of these repetitive elements among non-homologous chromosomes and that mechanisms of genomic turnover are capable of distributing and homogenizing repeat units of a given satellite DNA family throughout the genome. Considering that SATA is also present in the genome of other tilapiine and haplochromine species (Franck et al., 1994), a possible alternative hypothesis is that this satellite sequence originated and spread in an ancestor of this group, and has been maintained in the centromeres of all chromosomes owing to the functionality of this repetitive sequence.

Most copies of the satellite SATB sequence are located in the short arm of only one chromosome pair (chromosome 4—Fig. 1) of the Nile tilapia, as demonstrated by FISH experiments. However, copies of this repetitive DNA are probably also present in other chromosomes of the species (Oliveira and Wright, 1998) as weak fluorescent signals were

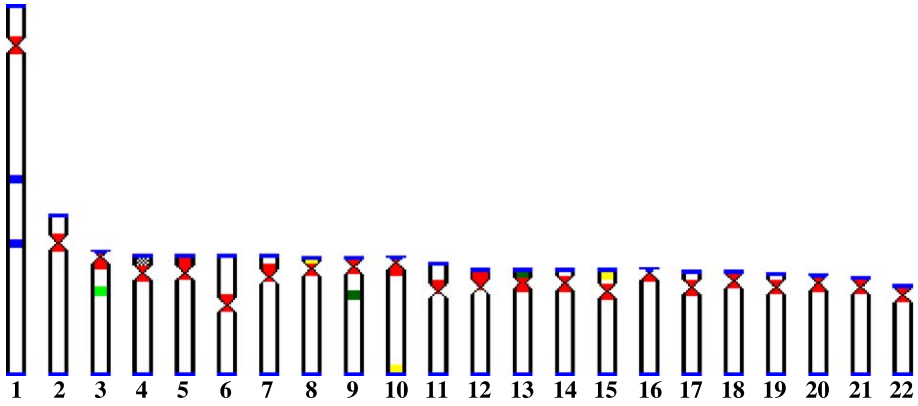

Fig. 1. Ideogram representing the physical mapping of repetitive sequences in the chromosomes of the Nile tilapia (*O. niloticus*). The chromosome position of the different sequences is distinguished by colour: red, SATA; speckled in black, SATB; yellow, 45S rDNA; blue, telomere; faint green, 5S rDNA type I; dark green, 5S rDNA type II.

observed in many chromosomes. Alternatively, these weak signals may be related to the presence of sequences exhibiting partial nucleotide identity to SATB sequences. The distribution of satellite DNA sequences in just one or in few chromosomes, as observed for SATB, seems to be a common pattern found in several fish species (Garrido-Ramos et al., 1994; Canapa et al., 2002).

Restriction digestion experiments with *Eco*RI and *Hae*III, enzymes that have recognition sites within the SATA and SATB sequences (Franck et al., 1992; Franck and Wright, 1993), were also performed on the chromosomes of the Nile tilapia (Oliveira and Wright, 1998) and supported the chromosomal location of SATA and SATB sequences determined by FISH. Most C-bands of the chromosomes of the Nile tilapia seem to be composed of the satellite DNA sequences, SATA and SATB. However, since some heterochromatin segments do not correspond to SATA or SATB, as for example the short arm of chromosome pair 1 (Oliveira and Wright, 1998), it is more likely that other repetitive sequences exist in the chromosomes of *O. niloticus* and their further isolation and characterization could contribute to the identification of other chromosome markers for the Nile tilapia.

3.2. Telomeres

Telomeres are composed of DNA and proteins found at the ends of linear eukaryotic chromosomes (Shippen, 1993). The basic structure and function of the telomere have been conserved throughout evolution, reflecting its important role in stabilizing chromosomes and blocking chromosome end-to-end fusion and degradation (Blackburn and Szostak, 1984). Telomeric DNA is composed of tandem arrays of a species-specific 5–8-bp GT-rich sequence, termed telomeric repeat (Shippen, 1993). Vertebrates from fish to humans share a common telomeric repeat sequence, $(TTAGGG)_n$. The length of these arrays varies from species to species.

Cytogenetic studies conducted in 115 species of the family Cichlidae showed that 51% of the species have a karyotype of $2n=48$ chromosomes (Klinkhardt et al., 1995). Moreover, the Neotropical cichlids *Cichlasoma* and *Geophagus*, which are the primitive sister group of the African cichlids, also have a karyotype of $2n=48$ consisting of acrocentric chromosomes (Thompson, 1979). In the Tilapiine tribe, most species have a karyotpye of $2n=44$. The presence of $2n=48$ chromosomes in *Oreochromis alcalicus* seems to be a plesiomorphic character and possibly predates the overall reduction to $2n=44$ in all other species of the group (Majumdar and McAndrew, 1986). It is likely, therefore, that three chromosomes may have undergone Robertsonian fusion and pericentric inversions to generate the large acrocentric chromosome 1 observed in Tilapiine species with $2n=44$. The detection of two interstitial $(TTAGGG)_n$ signals within chromosome 1 supports the hypothesis of chromosome number reduction in tilapia (Chew et al., 2002; Table 1). Oliveira and Wright (1998), however, did not detect SATA satellite DNA in interstitial regions of chromosome 1 of the Nile tilapia. This suggests that, if chromosome fusion has occurred, the satellite sequences, and possibly the inactive centromeres, have been eliminated from interstitial regions of chromosome 1 by deletion or mutation.

Interstitial telomeric sequences have been found in the pericentric regions in a number of vertebrate species (Meyne et al., 1990), suggesting that Robertsonian fusions can occur without a resulting loss of telomeric sequences. These interstitial sites of telomeric DNA have been interpreted as "footprints" of previous chromosomal fusions and the presence of these sites especially in the pericentric region may promote the fission/fusion of chromosomes during karyotype evolution.

The distribution of $(TTAGGG)_n$ repeats among several fish species showed that these sequences were primarily localized in the telomeres although some non-telomeric sites have also been observed. Moreover, these sequences can be related to the nucleolus organizing regions (NORs), as seen in the rainbow trout (*Oncorhynchus mykiss*) (Abuín et al., 1996). Telomeric repeats were also visualized in the flanking regions of three NOR sites of *Salvelinus namaycush* (Reed and Phillips, 1995). In the Nile tilapia, NORs are found at a terminal location on three chromosome pairs (Foresti et al., 1993; Martins et al., 2000). Hybridization of a $(TTAGGG)_4$ probe to chromosomes of the Nile tilapia did not generate intense hybridization signals in any subset of chromosome ends suggesting that extensive tracts of telomeric repeats do not flank the NORs (Chew et al., 2002).

Telomere-associated sequences (TAS) are stretches of DNA adjacent to telomeres and comprised of middle repetitive DNA. The regions containing these TAS repeats vary in length from chromosome to chromosome and can be up to several hundred kb in length (reviewed in Biessmann and Mason, 1992). Although the function of TAS is not known, they may to be involved either in chromosome movement, telomere–telomere interactions and/or interactions between telomeres and the nuclear envelope (Blackburn and Szostak, 1984). Many TAS loci are polymorphic and as such have proven useful as markers in genome mapping and identification of specific sets of human chromosomes.

*Bal*31 exonuclease and restriction endonuclease digestion experiments followed by Southern blot-hybridization of a $(TTAGGG)_4$ probe to the Nile tilapia genomic DNA suggest that discrete *Eco*RI fragments of 160, 195 and 300 kb likely represent individual chromosome ends at which TAS repeats lack a recognition site for *Eco*RI (Chew et al.,

2002). These DNA fragments may well be ideal hybridization probes to distinguish specific chromosome ends.

The telomeric repeat in the Nile tilapia varies in size from 4–10 kb (Chew et al., 2002). The 100-kb *Bal*31-insensitive *Eco*RI fragments detected by Southern blot-hybridization probably represents the two interstitial $(TTAGGG)_n$ sites detected by FISH in chromosome 1. Analysis of the chromosomal distribution of telomeric sequences in New World cichlids like *Crenicara filamentosa* and *Apistogramma agassizi* ($2n=46$) and Old World cichlids like *Pelvicachromis pulcher* ($2n=48$) and *Etroplus maculatus* ($2n=46$) (Klinkhardt et al., 1995) would further elucidate the chromosomal evolution in this fascinating group of teleost fishes.

3.3. 45S and 5S rDNA

Studies on ribosomal RNA genes have gained prominence in a broad range of animals and plants, especially in relation to species or population characterization, evolutionary relationships and genome structuring. In higher eukaryotes, ribosomal RNA (rRNA) genes are organized as two distinct multigene families comprised of tandemly arrayed repeats composed of hundreds to thousands of copies. One class is represented by the 45S rDNA which consists of a transcriptional unit that codes for the 18S, 5.8S and 28S rRNAs, and an intergenic non-transcribed spacer (IGS). Multiple copies of this array correspond to the nucleolar organizer regions (NORs). The other class of rRNA genes code for the 5S rRNA and consists of a highly conserved coding sequence of 120 bp which is separated from each transcriptional unit by a variable non-transcribed spacer (NTS) (reviewed in Long and David, 1980). While the sequence of rRNA genes are conserved even among non-related taxa, the non-transcribed spacers show extensive length and sequence variation, which is thought to provide an accentuated dynamism to the rRNA genes.

The chromosomal mapping of the 18S, 5.8S and 28S rRNAs (45S rDNA) genes has been done for many fish species, and these chromosomal markers have been applied to various phylogeographic questions. By contrast, few studies have explored the structure and chromosomal location of 45S rDNA sequence in the Cichlidae. Of the few studies that have been conducted in the Cichlidae, they describe the chromosomal mapping of the 18S, 5.8S and 28S rRNAs genes by FISH or by the indirect detection of the NORs using Ag-staining. FISH analysis using biotinylated 18S rDNA probes or Ag-staining identified three labelled-chromosome pairs (pairs 8, 10 and 15) in the Nile tilapia chromosomes (Fig. 1) (Foresti et al., 1993; Martins et al., 2000). Using sequential Ag-staining of 5S rDNA-FISH-metaphases, Martins et al. (2000) showed that 5S rDNA and 18S rDNA loci are distributed on different chromosomes. Different chromosomal sites for NOR and 5S rDNA loci have already been reported for *Anguilla anguilla* (Martínez et al., 1996), *Salmo trutta* (Móran et al., 1996), *Coregonus artedti*, *C. zenithicus* (Sajdak et al., 1998) and the genus *Leporinus* (Martins and Galetti, 2001a), an arrangement thus far most frequently observed in vertebrates. In contrast, in *Salmo salar* and *O. mykiss* (Pendás et al., 1994; Móran et al., 1996), the 5S and 45S rDNA loci are linked on the same chromosome.

5S rDNA sequences exist as two types in the genome of the Nile tilapia: tandemly arrayed, type I 5S rDNA composed of monomers of 1405 bp and the tandemly arrayed, type II 5S rDNA composed of monomers of 475 bp (Table 1). An inverted 5S rRNA

pseudogene and two putative 5S rRNA bona fide genes (one of them inverted) were also detected in the type I 5S rDNA (Martins et al., 2002). Both classes were clustered in distinct chromosomes (Table 1, Fig. 1). While the type I 5S rDNA was detected in an interstitial position in the long arm of a subtelo-acrocentric chromosome pair (chromosome 3), the type II 5S rDNA was identified interstitially in the long arm of a different subtelo-acrocentric pair and at the terminal region of the short arm of another subtelo-acrocentric chromosome pair (chromosomes 9 and 13) (Table 1, Fig. 1). The assignment of different classes of 5S rDNA to distinct chromosome loci was previously described for the fish *Leporinus* (Martins and Galetti, 2001a), reinforcing the idea that distinct 5S rDNA classes occupy different chromosome loci and seem to be evolving independently in their respective chromosomal domains. The detection of two different types of tandem repeats located in distinct chromosome loci in this species, which could even represent a general feature for 5S rDNA in fishes (Martins and Galetti, 2001b), and the detection of inverted 5S rRNA genes and pseudogenes reflect the intense dynamics of the evolution of such tandem repeat elements (Martins et al., 2002).

Several studies of 5S rDNA sequences among fish species have identified variant types of the 5S rDNA tandem repeats characterized by remarkable differences in the NTSs. The presence of two types of tandem repeats of this ribosomal DNA has been observed in Characiformes (Martins and Galetti, 2001a; Wasko et al., 2001), Perciformes (Martins et al., 2002) and Salmoniformes (Pendás et al., 1994; Móran et al., 1996; Sajdak et al., 1998). In the tilapiine cichlid fish *O. niloticus*, two distinct 5S rDNA units were also identified and seem to have a common origin, since they present some conserved sequences (Martins et al., 2002). Each 5S rDNA class is characterized by a distinct NTS that varied in nucleotide sequence and length between the loci. A dual 5S rRNA gene system, differently regulated in somatic and oocyte cells, has been described for a number of vertebrate species, including fish and amphibians (Komiya et al., 1986). The variant classes of the 5S rDNA observed in the Nile tilapia could also represent distinct oocyte and somatic 5S rRNA gene types.

The chromosomal locations of the 5S rRNA genes have been described for 48 fish species representing distinct groups, such as Acipenseriformes, Anguilliformes, Cypriniformes, Characiformes, Salmoniformes, Perciformes, and Tetraodontiformes. For most fish species, 5S rRNA genes have an interstitial position in the chromosomes, which suggests that such localization could represent some advantage related to the organization of these genes in the genome (Martins and Galetti, 2001b).

The distinct chromosome location of 45S and 5S rDNAs can demark six different chromosome pairs in the Nile tilapia karyotype (Fig. 1). The chromosomal mapping of such sequences in other Cichlidae may provide insight into the evolution of the Cichlidae karyotype.

3.4. SINEs and LINEs

The short interspersed nuclear elements (SINEs) and the long interspersed nuclear elements (LINEs) found in eukaryotic genomes of organisms as diverse as plants and mammals, are some of the most extensively studied dispersed repetitive elements (reviewed in Okada et al., 1997). All SINE elements described so far, except the primate

Alu and the rodent B1 families, are derived from tRNAs. Okada et al. (1997) have proposed that the 3′-end of tRNA-derived SINEs arose from the 3′ -end of LINE elements and that this organization may be related to the acquisition of retropositional activity of SINE elements.

Both in situ hybridization (Baker and Kass, 1994) and studies of chromosomal DNA replication (Holmquist, 1988) have shown that LINEs are concentrated in late-replicating DNA, or positive G-band segments, while SINEs occur predominantly in the early replicating, negative G-band segments of DNA. Since mammalian SINEs and LINEs are G/C- and A/T-rich, respectively, it was suggested that their base composition might be responsible for the transposition of these elements into DNA segments that show the same base composition (Bailey and Shen, 1993). In addition, biased base composition of SINEs and LINEs may also explain the base-specific fluorochrome banding of mammalian chromosomes (Korenberg and Rykowski, 1988).

Although studies of SINE and LINE distribution in fish chromosomes, to date, are modest compared to SINE and LINE cytogenetic studies in mammals, preliminary results suggest that the uniform distribution of these elements in chromosomes may account for the absence of base-specific fluorochrome bands in the karyptypes of fish species. The LINE element, CiLINE2, isolated from the Nile tilapia (Oliveira et al., 1999) detected by Southern blot-hybridization related sequences in the genomic DNA of all Tilapiini species tested of the genera *Oreochromis*, *Tilapia*, and *Sarotherodon*. It is interesting to note that DNA from *Oreochromis* and *Sarotherodon* species produce a hybridization pattern distinct of *Tilapia* species. Thus, if this pattern is confirmed for other species of the three tilapiine genera, this probe could be used to distinguish fish of the genera *Tilapia* from those of *Oreochromis* and *Sarotherodon*. Fluorescent in situ hybridization with the CiLINE2 element showed that all chromosomes exhibit very small hybridization signals distributed more or less randomly over the chromatids, but strikingly enriched along the terminal two-thirds of the long arm of chromosome 1 (Oliveira et al., 1999; Table 1).

The distribution of two SINE sequences in the Nile tilapia chromosomes, the ROn-1 and the ROn-2, was investigated by fluorescent in situ hybridization (Oliveira et al., 2003). FISH of ROn-1 and ROn-2 to denatured metaphase spreads of the Nile tilapia showed that both SINE sequences are organized in small clusters dispersed in all chromosomes. The only exception was a large cluster of ROn-1 found in the middle of the long arm of chromosome 1 (Table 1). A comparative analysis showed that while the ROn-1 elements are almost exclusively distributed in interstitial regions of chromosomes, copies of ROn-2 are localized near the telomeric region of several chromosomes. No similarity was observed in the distribution of SINEs and LINEs in the Nile tilapia chromosomes and the distribution of these elements in mammalian chromosomes.

Considering that the chromosomes of the Nile tilapia do not display longitudinal bands when stained with the fluorochromes chromomycin A_3 or DAPI (unpublished data), and the chromosomal distribution of SINE and LINE elements described above, Oliveira et al. (2002) suggested that the absence of longitudinal euchromatic bands in the Nile tilapia chromosomes is due to the absence of clusters of large numbers of A/T-rich LINE and G/C-rich SINE elements in this species. Since many SINE and LINE elements described for several cold-blooded vertebrates have similar characteristics to those found in the Nile tilapia (Okada et al., 1997), further analysis of the structure and distribution of these

elements in the chromosomes of other fish species, and also in the chromosomes of other cold-blooded vertebrates, may lead to a better understanding of the role of retroelements and other dispersed repetitive sequences in the origin of A/T- and G/C-rich euchromatic bands.

Whole chromosome probes DOP-PCR-amplified-microdissected from chromosome 1 of the Nile tilapia hybridized most intensely in the long arm of chromosome 1, suggesting the presence of large numbers of repetitive elements in this chromosomal region (Harvey et al., 2002). The presence of an enriched region with the CiLINE2 and ROn-1 elements in the long arm of chromosome 1 is in agreement with the studies of Harvey et al. (2002). Considering that the chromosome pair 1 of the Nile tilapia is the sex chromosomes and that the main differences detected between the X and Y resides in the long arm (Foresti et al., 1993), the development of genetic markers capable of distinguishing the X and Y chromosomes will be of considerable value for tilapiine aquaculture.

4. Integrating genetic mapping and physical mapping

Molecular cytogenetic studies have shown that repetitive DNA sequences, such as those reviewed here, represent useful tools for defining the structure, and revealing the evolution, of the tilapia genome. Recently, a major contribution to the genetics of the Nile tilapia has been the construction of a high-density linkage map based on polymorphic DNA markers (Kocher et al., 1998). The present paper represents the first initiative in the construction of a physical chromosome map for the Nile tilapia.

Centromere markers in *O. niloticus*, such as SATA and SATB, and other sequences, like the 45S and 5S rDNA, that function as markers for a specific subset of chromosomes, are powerful tools to detect chromosomal rearrangements in Nile tilapia and related cichlids. Also, new markers for the X and Y sex chromosomes (chromosome pair 1), as well as other sex markers that could be present in other chromosomes, will elucidate the origin and evolution of sex chromosomes in cichlids. We view the next phase in the physical mapping of the Nile tilapia genome will be the use of Bacteria Artificial Chromosomes (BAC) containing large inserts of its genome that can be readily localized to specific chromosomes by FISH. Many of the DNA sequences in these BAC clones to be used for physical mapping by FISH will contain polymorphic DNA markers already assigned to linkage groups in the current, and to-be-updated, genetic map of Kocher et al. (1998). As such, an integrated genome map of *O. niloticus*, based on both physical and genetical data, will be generated and will allow for development of tools and strategies to investigate a myriad of questions and issues, such as genome evolution, phylogeography, isolation of quantitative trait loci for important aquacultural traits (e.g., growth performance, disease resistance) and genes involved in behavioural and morphological diversity of this astonishing group of fishes.

Acknowledgements

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil

to APW, CM and CO and from Natural Sciences and Engineering Research Council of Canada to JMW.

References

Abuín, M., Martínez, P., Sánchez, L., 1996. Localization of the repetitive telomeric sequence $(TTAGGG)_n$ in four salmonid species. Genome 39, 1035–1038.

Bailey, A.D., Shen, C.K.J., 1993. Sequential insertion of *Alu* family repeats into specific genomic sites of higher primates. PNAS 90, 7205–7209.

Baker, R.J., Kass, D.H., 1994. Comparison of chromosomal distribution of a retroposon (LINE) and a retrovirus-like element *mys* in *Peromyscus maniculatus* and *P. leucopus*. Chomosom. Res. 2, 185–189.

Biessmann, H., Mason, J.M., 1992. Genetics and molecular biology of telomeres. Adv. Genet. 30, 185–249.

Blackburn, E.H., Szostak, J.W., 1984. The molecular structure of centromeres and telomeres. Ann. Rev. Biochem. 53, 163–194.

Canapa, A., Cerioni, P.N., Barucca, M., Olmo, E., Caputo, V., 2002. A centromeric satellite DNA may be involved in heterochromatin compactness in gobiid fishes. Chomosom. Res. 10, 297–304.

Charlesworth, B., Snlegowski, P., Stephan, W., 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371, 215–220.

Chew, J.S.K., Oliveira, C., Wright, J.M., Dobson, M.J., 2002. Molecular and cytogenetic analysis of the telomeric $(TTAGGG)_n$ repetitive sequences in the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Chromosoma 111, 45–52.

Foresti, F., Oliveira, C., Galetti Jr., P.M., Almeida-Toledo, L.F., 1993. Synaptonemal complex analysis in spermatocytes of tilapia, *Oreochromis niloticus* (Pisces, Cichlidae). Genome 36, 1124–1128.

Franck, J.P.C., Wright, J.M., 1993. Conservation of a satellite DNA sequence (SATB) in the tilapiine and haplochromine genome (Pisces: Cichlidae). Genome 36, 187–194.

Franck, J.P.C., Wright, J.M., McAndrew, B.J., 1992. Genetic variability in a family of satellite DNAs from tilapia (Pisces: Cichlidae). Genome 35, 719–725.

Franck, J.P.C., Kornfield, I., Wright, J.M., 1994. The utility of SATA satellite DNA sequences for inferring phylogenetic relationships among the three major genera of tilapiine cichlid fishes. Mol. Phylogenet. Evol. 3, 10–16.

Garrido-Ramos, M.A., Jamilena, M., Lozano, R., Rejon, C.R., Rejon, M.R., 1994. Cloning and characterization of a fish centromeric satellite DNA. Cytogenet. Cell Genet. 65, 233–237.

Hamilton, M.J., Honeycutt, R.L., Baker, R.J., 1990. Intragenomic movement, sequence amplification and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys*: evidence from *in situ* hybridization. Chromosoma 99, 321–329.

Harvey, S.C., Masabanda, J., Carrasco, L.A.P., Bromage, N.R., Penman, D.J., Griffin, D.K., 2002. Molecular cytogenetic analysis reveals sequence differences between the sex chromosomes of *Oreochromis niloticus*: evidence for an early stage of sex-chromosome differentiation. Cytogenet. Genome Res. 79, 76–80.

Hinegardner, R., Rosen, D.E., 1972. Cellular DNA content and evolution of teleostean fishes. Am. Nat. 106, 621–644.

Holmquist, G.P., 1988. DNA sequences in G-bands and R-bands. In: Adolph, K.W. (Ed.), Chromosomes and Chromatin. CRC Press, Boca Raton, pp. 76–121.

Klinkhardt, M., Tesche, M., Greeven, H., 1995. Database of Fish Chromosomes. Westarp-Wiss., Magdeburg, Germany. 237 pp.

Kocher, T.D., Lee, W.J., Sobolewsha, H., Penman, D., McAndrew, B., 1998. A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). Genetics 148, 1225–1232.

Komiya, H., Hasegawa, M., Takemura, S., 1986. Differentiation of oocyte- and somatic-type 5S rRNAs in animals. J. Biochem. 100, 369–374.

Korenberg, J.R., Rykowski, M.C., 1988. Human genome organization: Alu, Lines, and the molecular structure of metaphase chromosome bands. Cell 53, 391–400.

Kornfield, I.L., Rittle, U., Richler, C., Warhman, J., 1979. Biochemical and cytological differentiation among cichlid fishes of the Sea of Galilee. Evolution 33, 1–14.

Liem, K.F., 1991. Functional morphology. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes: Behavior, Ecology and Evolution. Chapman & Hall, London, pp. 129–150.

Long, E.O., David, I.D., 1980. Repeated genes in eukaryotes. Ann. Rev. Biochem. 49, 727–764.

Lowe-McConnell, R.H., 1991. Ecology of cichlids in South American and African waters, excluding the African Great Lakes. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman & Hall, London, pp. 60–85.

Majumdar, K.C., McAndrew, B.J., 1986. Relative DNA content of somatic nuclei and chromosomal studies in three genera, *Tilapia*, *Sarotherodon*, and *Oreochromis* of the tribe Tilapiini (Pisces, Cichlidae). Genetica 68, 175–188.

Martínez, J.L., Móran, P., Garcia-Vásquez, E., Pendás, A.M., 1996. Chromosomal localization of the major and minor 5S rRNA genes in the European eel (*Anguilla anguilla*). Cytogenet. Cell Genet. 73, 149–152.

Martins, C., Galetti Jr., P.M., 2001a. Organization of 5S rDNA in species of the fish *Leporinus*: two different genomic locations are characterized by distinct nontranscribed spacers. Genome 44, 903–910.

Martins, C., Galetti Jr., P.M., 2001b. Two 5S rDNA arrays in Neotropical fish species: is it a general rule for fishes? Genetica 111, 439–446.

Martins, C., Wasko, A.P., Oliveira, C., Wright, J.M., 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.

Martins, C., Wasko, A.P., Oliveira, C., Porto-Foresti, F., Parise-Maltempi, P.P., Wright, J.M., 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, 76–85.

Meyne, J., Baker, R.J., Hobart, H.H., Hsu, T.C., Ryder, O.A., Ward, O.G., Wiley, J.E., Wurster-Hill, D.H., Yates, T.L., Moyzis, R.K., 1990. Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99, 3–10.

Móran, P., Martínez, J.L., Garcia-Vásquez, E., Pendás, A.M., 1996. Sex linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenet. Cell Genet. 75, 145–150.

Okada, N., Hamada, M., Ogiwara, I., Ohshima, K., 1997. SINEs and LINEs share common 3′ sequences: a review. Gene 205, 229–243.

Oliveira, C., Wright, J.M., 1998. Molecular cytogenetic analysis of heterochromatin in the chromosomes of tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Chomosom. Res. 6, 205–211.

Oliveira, C., Chew, J.S.K., Porto-Foresti, F., Dobson, M.J., Wright, J.M., 1999. A LINE-like repetitive DNA sequence from the cichlid fish, *Oreochromis niloticus*: sequence analysis and chromosomal distribution. Chromosoma 108, 457–468.

Oliveira, C., Wright, J.M., Foresti, F., 2002. Chromosome structure in fishes. In: Sobti, R.C., Obe, G. (Eds.), Some Aspects of Chromosome Structure. Narosa Publishing, New Delhi, India, pp. 103–108.

Oliveira, C., Wang, Y., Bryden, L.J., Wright, J.M., 2003. Short interspersed repetitive elements (SINEs) from the cichlid fish, *Oreochromis niloticus*, and their chromosomal localization by fluorescent in situ hybridization. Caryologia 56, 177–185.

Pendás, A.M., Móran, P., Freije, J.P., Garcia-Vásquez, E., 1994. Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. Cytogenet. Cell Genet. 67, 31–36.

Pullin, R.S.V., 1991. Cichlids in aquaculture. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman & Hall, New York, pp. 280–309.

Reed, K.M., Phillips, R.B., 1995. Molecular characterization and cytogenetic analysis of highly repeated DNAs of lake trout, *Salvelinus namaycush*. Chromosoma 104, 242–251.

Sajdak, S.L., Reed, K.M., Phillips, R.B., 1998. Intraindividual and interspecies variation in the 5S rDNA of coregonid fish. J. Mol. Evol. 46, 680–688.

Shippen, D.E., 1993. Telomeres and telomerases. Curr. Opin. Genet. Dev. 3, 759–763.

Stiassny, M.L., 1991. Phylogenetic intrarelationships of the family Cichlidae: an overview. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman & Hall, London, pp. 1–35.

Thompson, K.W., 1979. Cytotaxonomy of 41 species of Neotropical Cichlidae. Copeia 1979, 679–691.

Trewavas, E., 1983. Tilapiine Fishes of the Genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum (Natural History), London.

Wasko, A.P., Martins, C., Wright, J.M., Galetti Jr., P.M., 2001. Molecular organization of 5S rDNA in fishes of the genus *Brycon*. Genome 44, 893–902.

Wright, J.M., 1989. Nucleotide sequence, genomic organization and the evolution of a major repetitive DNA family in tilapia (*Oreochromis mossambicus/hornorum*). Nucleic Acids Res. 17, 5071–5079.