Micron 45 (2013) 129–135

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Comparative chromosomal mapping in *Triportheus* fish species. Analysis of synteny between ribosomal genes

Vinicius Marquioni [a], Luiz Antonio Carlos Bertollo [a], Débora Diniz [b], Marcelo de Bello Cioffi [a,*]

[a] Universidade Federal de São Carlos, Departamento de Genética e Evolução, São Carlos, SP, Brazil
[b] Universidade Estadual do Sudoeste da Bahia, Departamento de Ciências Biológicas, Jequié, BA, Brazil

ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form
25 November 2012
Accepted 27 November 2012

Keywords:
Fishes
Molecular cytogenetics
Triportheus
Ribosomal DNA

ABSTRACT

All *Triportheus* species show the conserved diploid number of 52 chromosomes and a ZZ/ZW sex chromosome system. Previous studies conducted on *Triportheus nematurus* reported a syntenical location of 18S and 5S sites on this species, in addition to some indications that this condition could be shared by other *Triportheus* species, possibly constituting a synapomorphy for this genus. In the present study, fluorescence in situ hybridization (FISH) experiments were performed in seven *Triportheus* species in view of a comparative analysis of the distribution of the 18S and 5S ribosomal DNAs on the chromosomes. The double-FISH experiments have showed that the synteny of the 18S and 5S rDNA genes is not a synapomorphy for the genus, since it is not present in all the species investigated, although it is present in most of them. The findings suggest that the syntenical location of the ribosomal genes is an ancestral trait in *Triportheus*, which was changed during the course of evolution of this group.

1. Introduction

Triportheus (Characiformes, Characidae) is widely distributed throughout South America, being found from Colombia to Uruguay. Its species may reach 20–24 cm in length and are popularly known as "sardinha-facão", "sardela" and "sardinha de água doce". Sixteen nominal species have been described for this genus so far (Malabarba, 2004).

All *Triportheus* species analyzed have a similar karyotype macrostructure, presenting a diploid number of 52 chromosomes, with predominance of meta- and submetacentric chromosomes, as well as a conserved ZZ/ZW sex chromosome system, the Z chromosome being always the biggest of the karyotype (Artoni et al., 2001). The W chromosome is probably derived from an ancestral homologue of the Z chromosome through a degeneration process leading to its length reduction and large-scale heterochromatinization (Artoni et al., 2001; Artoni and Bertollo, 2002). In fact, the W chromosome of all cytogenetically described species varies in length from almost as long as the corresponding Z to much smaller than it, while the Z chromosome shows heterochromatin accumulation only at the centromeric and telomeric regions, the W chromosome is predominantly heterochromatic throughout its length. Thus, the sex chromosomes evolution in *Triportheus* is more similar to that of higher vertebrates, in contrast to some other fish groups, making *Triportheus* a particular model for evolutionary studies (Cioffi et al., 2012a,b).

In higher eukaryotes, the ribosomal RNA genes (rDNA) comprise two repetitive DNA families, the 45S and the 5S rDNA. The 45S rDNA is formed by tandemly repeated units composed by three transcribed regions, the 18S, 5.8S, and 28S rRNA generating regions separated by internal transcribed spacers (ITS 1 and ITS 2) and by non transcribed spacer (NTS) sequences (Long and David, 1980; Pendás et al., 1993). These clusters are usually located in the secondary constrictions that are visible in one or more chromosomes of the karyotype with conventional staining (Pendás et al., 1994; Alonso et al., 1999; Galetti Jr., 1998). The location of the 45S rDNA clusters on the chromosomes can be correctly investigated by FISH (fluorescence in situ hybridization), using 18S rDNA probes. $AgNO_3$ staining can also be utilized for evidencing the 45S sites in the karyotypes but, in this case, only the sites whose transcription occurred during the preceding interphase are revealed.

The 5S rDNA is formed by tandemly repeated units of a coding region for the 5S rRNA and a non transcribed spacer (NTS) (Long and David, 1980; Pendás et al., 1993). The 5S clusters do not form visible structures like the 18S, what makes FISH the only possible methodology for its physical mapping (Pendás et al., 1994).

The 5S and 18S sites can be syntenical, i.e., located on the same chromosome pair or, alternatively, on different chromosome pairs. Diniz et al. (2009) have reported the synteny of these genes in *Triportheus nematurus* after double-FISH experiments. No double-FISH experiments were performed on other *Triportheus* species, making it impossible to conclude whether the condition found in *T.*

* Corresponding author. Tel.: +55 16 3351 8431; fax: +55 16 3351 8377.
E-mail address: mbcioffi@ufscar.br (M.d.B. Cioffi).

0968-4328/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.micron.2012.11.008

Table 1
Triportheus species analyzed, with the respective collection sites and sample sizes.

Species	Locality	Sample size
T. nematurus	Rio Piracicaba (SP)	10♂ 06♀
T. trifurcatus	Rio Araguaia (MT)	09♂ 08♀
T. auritus	Rio Negro (AM)	07♂ 08♀
T. signatus	Rio Piranhas-açu (RN)	11♂ 09♀
T. angulatus	Rio Negro (AM)	10♂ 07♀
T. guentheri	Rio São Francisco (MG)	08♂ 07♀
T. albus	Rio Negro (AM)	10♂ 13♀

AM, Amazonas; SP, São Paulo; MT, Mato Grosso; MG, Minas Gerais Brazilian states.

nematurus is unique to this species or shared by other species of this genus.

Thus, in this study, we investigated the distribution of the 18S and 5S rDNA sites in seven *Triportheus* species (*Triportheus albus*, *Triportheus angulatus*, *Triportheus auritus*, *Triportheus guentheri*, *Triportheus nematurus*, *Triportheus signatus* and *Triportheus trifurcatus*), by means of double-FISH experiments. The analysis performed showed that the synteny of the ribosomal 18S and 5S genes is not a synapomorphic condition for *Triportheus*, whereas it is probably a basal characteristic for this group.

2. Materials and methods

2.1. Mitotic chromosome preparation

Samples from both sexes of five *Triportheus* species (*T. albus*, *T. auritus*, *T. guentheri*, *T. nematurus* and *T. trifurcatus*) and from females of other two species (*T. angulatus* and *T. signatus*) were analyzed (Table 1). Fig. 1 indicates the collection sites, which belong to distinct Brazilian hydrographic basins.

Mitotic chromosomes were obtained from cell suspensions of the anterior kidney using the conventional air-drying method (Bertollo et al., 1978). The experiments followed ethical conducts, and anesthesia was used prior to sacrificing the animals.

2.2. Fluorescence in situ hybridization (fish)

2.2.1. Probes

Two tandemly arrayed rDNA sequences isolated from the genome of the Erythrinidae species *Hoplias malabaricus* were used. The first probe contained a 5S rDNA repeat copy and included 120 bp of the 5S rRNA transcribing gene and 200 bp of the non transcribed spacer (NTS) (Martins et al., 2006). The second probe corresponded to a 1400-bp segment of the 18S rRNA gene obtained via PCR from nuclear DNA (Cioffi et al., 2009). The 5S and 18S rDNA probes were cloned into plasmid vectors and propagated in DH5α *Escherichia coli* competent cells (Invitrogen, San Diego, CA, USA).

The 18S rDNA probe was labeled with biotin-14-dATP using the kit Biotin-Nick Translation Mix (Roche), while the 5S probes were labeled with digoxigenin-11-dUTP, using the kit DIG-Nick Translation Mix (Roche), according to manufacturer's instructions.

2.2.2. Slides preparation, hybridization and signal detection

The slides containing the metaphase plates were initially incubated with RNAse (40 mg/ml) for 1 h at 37 °C in a moist chamber. After denaturing the chromosomal DNA for 3 min in 70% formamide/2× SSC, the slides were dehydrated in an ethanol series of 50%, 70% and 100%, 3 min in each bath. The hybridization mixtures containing 100 ng of the denatured probe (2.5 ng/μl of DNA, 50% deionized formamide, 10% dextran sulfate and 2× SSC at 37 °C for 18 h), were heated at 85 °C for 5 min and then applied to the slides. Hybridization was performed with 77% stringency at 37 °C in a moist chamber. The post-hybridization washes were performed in 2× SSC and in 1× SSC for 5 min each and at 42 °C. The detection of the probes was performed with Streptavidin-Cy3 (Sigma) for the 18S rDNA probe and anti-digoxigenin-FITC (Roche) for the 5S rDNA probe. The chromosomes were counterstained with DAPI (1.2 g/ml) in Antifade solution (Vector Laboratories). Approximately 30 metaphases were analyzed per species.

3. Results

We verified the already described cytogenetic features of the *Triportheus* species, all presenting $2n = 52$ chromosomes and a ZZ/ZW sex chromosome system. All species showed evident signals for both the 18S and the 5S rDNAs probes. All of them presented a characteristic 18S rDNA site on the W chromosome. *T. angulatus* presented also a clear 18S site located on the Z chromosome. Concerning autosomes, two chromosome pairs bear these sites in *T. trifurcartus*, *T. auritus* and *T. angulatus*, in contrast to *T. nematurus*, *T. signatus*, *T. guentheri* and *T. albus*, which display only one chromosome pair with these sites (Figs. 2 and 3). All species have only one autosomal 5S rDNA site, with exception of *T. auritus*, which shows a total of ten 5S rDNA positive signals, while all the other species show only a chromosome pair bearing these sites (Figs. 2 and 3).

Synteny between the 18S and 5S rDNA sites was found in five of the seven analyzed species (*T. angulatus*, *T. auritus*, *T. nematurus*, *T. signatus* and *T. trifurcatus*). All these species have synteny of the 18S and 5S rDNA sites, which are adjacently located on the short arms of the chromosome pair number 2 (Figs. 2 and 3), easily recognized by its conspicuous secondary constriction. In the other two species (*T. albus* and *T. guentheri*) the 18S and 5S rDNA sites were not collocated. The FISH results are summarized in Table 2 and Fig. 4.

4. Discussion

All the analyzed species provided clear results concerning the number and location of the ribosomal sites in the chromosomes, which are in agreement with some previous single FISH experiments (Diniz et al., 2009). The number and distribution of the 18S and 5S rRNA genes are not conserved among the species of *Triportheus*. Indeed, some species, such as *T. trifurcatus*, *T. auritus*, and *T. angulatus*, have a greater number of rDNA sites than the other ones. Particularly in relation to 5S rDNA, *T. auritus* is the most diversified species, with 5 chromosome pairs bearing these sites in contrast to only one pair found in the other species. Although the occurrence of more than two 5S rDNA sites has been reported for many fish species (Fujiwara et al., 1998; Murakami and Fujitani, 1998; Martins and Galetti, 1999), it is not the predominant situation in *Triportheus* (Diniz et al., 2009). Some studies suggest that transposable elements might play an important role on the spreading of rDNA sequences over the genome (Raskina et al., 2008; Zhang et al., 2008; Cioffi et al., 2010). Some classes of transposons appear to be able to "capture" entire genes and move them to other parts of the genome (Jiang et al., 2004; Lai et al., 2005). At the moment, we have no information on the possible role of transposable elements in increasing the number of the 5S rDNA sites in *T. auritus*, which will be the goal in further investigations. Furthermore, some clusters are localized on the short arms of the chromosomes, while others are on the long arms, thus evidencing that chromosomal rearrangements have played a role in their distribution in the karyotype. This way, the cytogenetic mapping of rDNA classes provides informative chromosomal markers to reveal genomic differentiation that took place during the evolutionary events in *Triportheus*.

Another interesting feature of this group is the ZZ/ZW sex chromosome system that appears to be a particular synapomorphy of *Triportheus* among the Characidae family. Chromosome painting has showed that the Z chromosome shows a conservative nature