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Chromosome diversity in neotropical fishes: NOR studies

PEDRO MANOEL GALETTI, JR.
Departamento de Genética e Evolução,
Universidade Federal de São Carlos,
CP 676, 13565-905 São Carlos SP (Brazil)
e.mail: *galettip@power.ufscar.br*

ABSTRACT

Neotropical fishes present a high chromosome diversity showing a wide diploid number variation range, including different levels of ploidies, sex chromosomes, chromosome supernumeraries, and several cases of polymorphisms, related particularly to heterochromatin and NOR sites. Two main general trends of chromosome diversification can be observed among neotropical fishes. First, several fish groups show a chromosome evolution relatively divergent from the point of view of the karyotypic macrostructure. Sister species show conspicuous differences in karyotype structure and most often also in the number of chromosomes. On the other hand, there are fish groups in which chromosome evolution has been shown to be less divergent, and in this case whole families or even groups of families may share a common karyotype structure and equal number of chromosomes. Several fish groups appear conservative also with respect to the NOR bearing chromosomes. In this case, NOR chromosome location is invariable among species. In contrast , several other groups present wide NOR variability. Sister species may show quite diverse chromosomes bearing nucleolar organizing regions. The NOR and heterochromatin relationship is also very diverse among fishes and this may indicate organizational differences involving these chromosome segments. Thus, neotropical fish fauna presents great chromosome variability, verifiable also by NOR studies.

KEY WORDS: Chromosomes - Karyotype - NORs - Fishes.

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CHROMOSOME DIVERSITY

Neotropical fishes present a high chromosome diversity showing a wide diploid number variation range (see Oliveira *et al.*, 1988, for a revision), including different levels of ploidies (i.e., Morelli *et al.*, 1983b; Almeida-Toledo *et al.*, 1985; Venere & Galetti, 1985; Oliveira *et al.*, 1992), different types of sex chromosome systems (i.e., Galetti *et al.*, 1981; Bertollo *et al.*, 1983; Almeida-Toledo *et al.*, 1984; Galetti & Foresti, 1986, 1987), chromosome supernumeraries (Foresti *et al.*, 1989; Fenocchio & Bertollo, 1990; Maistro *et al.*, 1992; Salvador & Moreira-Filho, 1992), and several cases of polymorphisms, specially related to heterochromatin and NOR sites (i.e., Foresti *et al.*, 1981; Galetti *et al.*, 1984, 1991a).

In contrast to the African fishes, the neotropical fishes are derived of a very small basic stock, mainly represented by two dominant orders: Characiformes and Siluriformes. Despite this, the neotropical fish fauna is very rich in forms and in the Amazon region alone are found about 2500-3000 species.

Two main general trends of chromosome diversification can be observed among neotropical fishes (Galetti *et al.*, 1994). First, several fish groups show a chromosome evolution relatively divergent in a point of view of the karyotypic macrostructure. Sister species show conspicuous differences in karyotype structure and, most frequently, also in the number of chromosomes. In the genus *Astyanax*, a small characid fish, different species can show very diverse karyotypes: *A. bimaculatus*, for instance, has 2n = 50, *A. schubarti* shows 2n = 36 (Morelli *et al.*, 1983a).

In contrast, there are fish groups in which the chromosome evolution has been shown to be less divergent, and in this case whole families or even groups of families may show the same basic karyotypic structure and equal number of chromosomes. In the genus *Schizodon* (Anostomidae, Characiformes), all studied species thus far show the same karyotype pattern, presenting 2n = 54 biarmed chromosomes (Martins, 1997, Master thesis). In fact, different families among Characiformes, such as Anostomidae, Chilodontidae, Curimatidae, Prochilodontidae and others, may show a homogenous karyotype pattern in most of their species (Galetti *et al.*, 1994).

Several cases of sexual chromosomes have already been described among neotropical fishes, from XY or ZW systems involving males or females (Galetti *et al.*, 1981, 1995a; Galetti & Foresti, 1986, 1987; Moreira-Filho *et al.*, 1993), respectively, to multiple systems such as XY_1Y_2 or X_1X_2Y in males (Bertollo *et al.*, 1983; Almeida-Toledo *et al.*, 1984), and ZW_1W_2 in females (Moreira-Filho *et al.*, 1980). A good example of sex chromosomes occurs in the genus *Leporinus*, a characiform of the family Anostomidae. Some species of this genus show a large subtelocentric chromosome, which is unique and characteristics of female karyotypes (Galetti *et al.*, 1981, 1995a; Galetti & Foresti, 1986, 1987). This

W chromosome has been shown to be largely heterochromatic, suggesting that the heterochromatinization was the main chromosomic mechanism involved in the differentiation of this sex chromosome. Base-specific fluorochromes, structural R bands, and replication bands suggest that this W heterochromatin is heterogeneous (Molina, 1995, Master thesis).

Other fishes may show multiple sex chromosomes. Among them, *Apareiodon affinis* (Parodontidae, Characiformes) has a typical ZW_1W_2 system. In this species, while males show 2n = 54 chromosomes, the females show 2n = 55. A centric fission occurring in an element of the first chromosome pair of the karyotype might give rise to both W_1 and W_2 chromosomes (Moreira-Filho *et al.*, 1980). Another characiform, *Hoplias malabaricus* has a typical X_1X_2Y multiple sex chromosomes system, in which females show 2n = 40 and males, 2n = 39 (Bertollo *et al.*, 1983). Synaptonemal complex analysis has confirmed the trivalent formed by X_1-Y-X_2 chromosomes and suggests that a translocation involving the original X_1 and X_2 may have occasioned the development of the Y chromosome in this fish (Bertollo L.A.C., unpubl. data).

Supernumerary chromosomes have been also reported in fishes of the Neotropical region. One of the earliest examples came from studies carried out on a Brazilian species of the family Prochilodontidae, *Prochilodus scrofa* (recently renamed *P. linneatus*). In this species, zero to five B chromosomes varying within and between specimens were initially detected (Pauls & Bertollo, 1983). Latter studies showed up to seven B chromosomes varying among individuals of different populations of this species (Cavallaro, 1992, Master thesis). An other interesting case of B chromosomes was reported in the characin *Astyanax scabripinnis*. (Maistro *et al.*, 1992; Salvador & Moreira-Filho, 1992; Vicente *et al.*, 1996). Different populations of this species may show up to two large B chromosomes, extensively heterochromatic. Recently, repetitive DNA of this fish was identified, cloned and sequenced, showing a monomeric unit of 51 bp. After *in situ* hybridization these repeats were detected throughout almost the entire B chromosome extent, as well as among a few other chromosomes of the normal complement (Mestriner, 1997, Doctoral thesis).

Several chromosome polymorphisms have already been described in neotropical fishes (Bertollo *et al.*, 1979; Oliveira *et al.*, 1990; Moreira-Filho & Bertollo, 1991; Cestari & Galetti, 1992a; Foresti *et al.*, 1992). *Hoplyerythrinus unitaeniatus*, a characiform common in Brazilian rivers, for instance, show extensive chromosome polymorphism in the River Amazon, due to pericentric inversions (Giuliano-Caetano & Bertollo, 1988). Other polymorphisms may be associated with polyploids, in special triploids, a case observed in *Cyphocharax modesta*, a characiform of the family Curimatidae. One triploid individual was detected among diploid forms (Venere & Galetti, 1985). A similar situation has been described for several others fish species (Morelli *et al.*, 1983b; Almeida-Toledo *et al.*, 1985; Giuliano-Caetano & Bertollo, 1990; Fauaz *et al.*, 1994) .

Polymorphisms have been also investigated in heterochromatic segments and nucleolous organizer sites. In some fishes populations, the heterochromatin has a fundamental role in the production of chromosome variants (Molina, 1995, Master thesis). A similar situation has been reported with respect to NOR sites; several fishes may show polymorphisms of these regions (Foresti *et al.*, 1981).

NOR POLYMORPHISMS IN NEOTROPICAL FISHES

Two main patterns of NOR distribution may be observed among fishes. Some groups have their ribosomic cistrons located in a simple chromosome pair (Galetti *et al.*, 1984; Moreira-Filho *et al.*, 1984; Feldberg & Bertollo, 1985; among others), while other ones show NOR sites spread on several chromosomes of the karyotype (Galetti *et al.*, 1985; Foresti *et al.*, 1989; Cestari & Galetti, 1992a, b; among others). These sites have been currently identified by silver staining, as well as base specific fluorochromes, such as chromomycin and mythramycin, and more recently through *in situ* hybridization using rDNA 18 and 28S probes.

Independently of the number of NOR bearing chromosomes, sometimes a whole family or even distinct families appear conservative with respect to NOR bearing chromosomes. In this case NOR chromosome location among species is invariable. An interesting example has been described in the genus *Brycon*, a characid of the subfamily Bryconinae, in which all species investigated thus far show a common large submetacentric NOR bearing chromosome (Margarido & Galetti, 1996), whose NOR sites, located at the end of the long arm, are often quite visible through silver staining, mithramycin and rDNA *in situ* hybridization (Wasko & Galetti, unpubl. data).

In contrast, several other groups present wide NOR variability among species. Sister species may show quite diverse chromosomes bearing nucleolar organizing regions. In *Leporinus*, for instance, two morphologically criptical species are easily differentiated through the NOR bearing chromosomes (Galetti *et al.*, 1984). *Leporinus elongatus* shows ribosomic cistrons located at the end of the long arm of a medium-sized submetacentric, while *Leporinus obtusidens* presents these sites on the short arm of a large metacentric. In fact, NOR variability is quite pronounced in *Leporinus*. Previous studies in *Leporinus friderici* using silver staining have always detected only one pair of chromosomes bearing NOR sites (Galetti *et al.*, 1984). However, in an exceptional situation, other chromosomes have recently been observed bearing silver positive regions, suggesting the occurrence of secondary NOR sites (Galetti *et al.*, 1991a). Newer studies using mithramycin and rDNA *in situ* hybridization confirm the presence of these secondary sites. Moreover, these investigations detected a remarkable NOR variability, within and between individuals, and the occurrence of post-zygotic modifications involving these chromosome sites has been suggested (Galetti et al., 1995b).

Several fish groups have NOR sites distributed in multiple chromosomes along the karyotype. In piranhas, *Serrasalmus spilopleura*, for instance, which shows 2n = 60 chromosomes, Ag-NOR sites were detected on the short arm of ten acrocentric chromosomes of the karyotype (Galetti *et al.*, 1985). Intra- and inter-individual variations in those silver-stained sites are frequent and probably are related to genetic regulation of these NOR cistrons

Fishes, in general, represent an excellent material for studies concerning the relationship between heterochromatin and NOR sites. Several groups show the C-banded heterochromatin to be strikingly associated with NORs (Almeida-Toledo *et al.*, 1981; Galetti *et al.*, 1991b; among others). Consequentely NOR stainability with GC-specific fluorochromes (as mithramycin and chromomycin) sometimes has been interpreted as a heterochromatin effect, whose segments should be interspersed within the NOR sites (Pendás *et al.*, 1993). This idea is strongly corroborated by some non NOR-associated heterochromatins which appear brightly fluorescent after either mithramycin or chromomycin staining (Margarido V. P. & Galetti Jr. P. M., unpubl. data).

The present data, however, do not allow a more conclusive understanding of this subject. There are several fishes in which the NOR sites, apparently free of even interspersed heterochromatin are still brightly fluorescent after mithramycin or chromomycin. In *Liposarcus anisitsi* for instance, a large catfish, NORs are detected by GC-specific fluorochromes, but C-banded heterochromatin is only detected adjacent to the NOR and not throughout these sites (Artoni , 1996, Master thesis). At least in these cases, the fluorochrome stainability is exclusively related to NOR sites, independently of any heterochromatin segment.

It appears quite evident, however, that NOR sites can vary greatly among fishes, and chromosome studies on this subject may disclose much more than chromosome markers in cytotaxonomic and phylogenetic approaches. The molecular organization of NORs itself might be better understood through fish studies, and the neotropical fauna represents excellent material for this proposal.

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