Coexistence of cytotypes and chromosomal mosaicism in *Hoplias malabaricus* (Characiformes, Erythrinidae)

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ABSTRACT

Karyotypes of seventeen *Hoplias malabaricus* specimens, collected in the fish culture station of UNOPAR (University of Northern Paraná), were analyzed. The station is in the Claro River system in the Tibagi River basin. Two distinct and coexistent karyotype forms (cytotypes) were identified, comprising either 42 chromosomes (cytotype A) or 40 chromosomes (cytotype C), both presenting metacentric and submetacentric chromosomes. In two specimens, one male and one female, it was not possible to characterize a modal diploid number because different cell lines were observed, with a predominance of $2n=41$ and $2n=42$ chromosomes at a frequency of 38.24% and 41.12%, respectively. The karyotype with $2n=41$ showed some putative monosomic and trisomic chromosomes, while the karyotype with $2n=42$ showed 21 chromosomal pairs, similar to cytotype A. RAPD analysis showed that these two specimens have the same band profile of cytotype A (Nei's genetic identity=92%), discarding a possible hybridization between both cytotypes and supporting the mosaicism hypothesis. These findings corroborate the isolation between cytotypes A and C.

Key terms: *Hoplias malabaricus*, chromosomal mosaicism, coexistence of cytotypes, RAPD, reproductive isolation.

INTRODUCTION

The family Erythrinidae comprises three genera: *Erythrinus*, with two reported species so far (*E. erythrinus* and *E. kessleri*); *Hoplerethrinus*, with three species (*H. unitaeniatus*, *H. cinereus* and *H. gronovii*); and *Hoplias*, composed of nine species and represented in Brazil by *H. brasiliensis*, *H. lacerdae*, *H. macrophthalmus*, *H. microcephalus* and *H. malabaricus* (Oyakawa, 2003). Although a small family, Erythrinids are taxonomically controversial, including several species lacking a precise identification.

Among these species, *Hoplias malabaricus*, commonly known as "traíra"

or dogfish, is a carnivore and predator fish found throughout South America. They are considered an important food source in many regions (Oyakawa, 2003). This species inhabits lentic habitats, such as lakes, reservoirs and fish culture ponds, among others. They commonly occur in fish tanks through transportation of eggs and larvae by the water supply tanks. Supply water is often derived from impounded headwaters or from streams.

Several cytogenetic studies have been performed within this group, which actually represents a species complex (Bertollo et al., 2000). Among *H. malabaricus* populations, several karyotypic forms (cytotypes) were identified regarding

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differences in diploid number and chromosomal morphology, as well as the absence/presence of diverse sex chromosome systems. Based on these features, seven distinct cytotypes (A-G) have been identified, and they have been used for comparative analyses among distinct populations (Bertollo et al., 2000). Some cytotypes, such as A and C, present a wide geographic distribution from Northern to Southern Brazil and also Argentina. In several regions different sympatric cytotypes are found, without any detectable hybrid forms among them (Bertollo et al., 2000).

Because of the remarkable chromosomal variability and the occurrence of different sympatric cytotypes, specimens of *Hoplias malabaricus* were studied in the present work, focusing on both cytogenetic and molecular features, to investigate the genetic events that occur in this population.

MATERIAL AND METHODS

Seventeen specimens of *H. malabaricus* from the fish farm station from UNOPAR (University of Northern Paraná) were collected and analyzed. The water supply for such fish culture is provided by the impounded headwaters of the Claro River, transformed into a lake. The water bodies drained from the tanks belong to the hydrographic system of the Claro River and Tibagi River basin (Tamarana City, Paraná State, Brazil). All analyzed specimens are native to this water system and are absolutely free from any kind of introduction or translocation.

Metaphase chromosomes were obtained through the air drying method (Bertollo et al., 1978) from kidney cells. The chromosomal classification was based on arm ratio, according to Levan et al. (1964).

In addition to the karyotype studies, a genomic DNA analysis using RAPD (Random Amplified Polymorphic DNA) was carried out. Total DNA was taken from muscle tissue samples of twelve specimens (seven from cytotype A, two mosaic specimens and three belonging to cytotype C). After digestion with proteinase K for

twelve hours, phenol/Tris-HCl (pH 8.0) was added, followed by centrifugation and a wash with phenol/Tris-HCl (pH 8.0) and chloroform-isoamyl alcohol. After an additional centrifugation, chloroform-isoamyl alcohol was added and the DNA was precipitated with absolute and cold ethanol for 12 hours at -20°C and eluted in TE 1/10 + RNase.

DNA amplification was carried out according to Williams et al. (1990), with some modifications. Overall, sixty primers were examined, comprising 20 OPA, 20 OPB, and 20 OPW types. The primers OPA-07, OPA-09, OPA-11, OPA-12, OPA-15 and OPB-08 generated a higher band polymorphism. The amplification products were analyzed through electrophoresis in agarose gel at 1.8% and stained with ethidium bromide.

The RAPD profiles were scored from the presence and absence of bands, corresponding to each amplified DNA fragment. TFPGA 1.3 software (Miller, 1997) was used to estimate genetic variability based on the rate of polymorphic locus, the heterozygosity and Nei's genetic distance (Nei, 1978).

A dendrogram based on genetic similarity values was obtained using the Jaccard (J) coefficient and UPGMA method using NTSYS-PC software (Rohlf, 1992). The reliability of branches was evaluated through bootstrap, using Boot software, with 1,000 replications (Coelho, 2000).

RESULTS

The chromosomal data obtained in the present work revealed the presence of two coexistent karyotypes in individuals from the UNOPAR fish culture station. Seven specimens (7 females) were classified as cytotype C, according to Bertollo et al. (2000), with a diploid number of $2n=40$ chromosomes m/sm (meta/submetacentric) without sex differentiation, in a total of 175 metaphases analyzed (Fig. 1a), while, based on 200 metaphase counts, eight specimens (5 females and 3 males) were classified as cytotype A, with a diploid number of $2n=42$ chromosomes m/sm for males and females,

without sex differentiation as well (Fig. 1b).

In two specimens, one male and one female, it was not possible to determine a single modal diploid number and two cell lines (with different diploid numbers) were found; one of them with a predominance of $2n=41$ chromosomes and another with $2n=42$ chromosomes, 38.24% and 41.12% in a total of 136 examined cells, respectively (Fig. 2), thus characterizing an intra-individual variation. The cell line with $2n=41$ was also monosomic for some chromosomal pairs, putatively identified as 7, 10 and 14, as well as trisomic in relation to other pairs, putatively identified as 4 and 21 (Fig. 3b).

The cell line with $2n=42$ chromosomes, showed a karyotype composed of m/sm paired chromosomes, as expected for the pattern of cytotype A, described by Bertollo et al. (2000), (Fig. 3a).

RAPD analysis was carried in seven samples of cytotype A, in both mosaic specimens and in three samples of cytotype C. The data showed that cytotypes A and C differed both in number and gel position of polymorphic bands for all primers. The two specimens lacking a specific diploid number presented, invariably, the same band profile of cytotype A individuals for all primers (Fig. 4).

Figure 1: Karyotypes of *H. malabaricus* from UNOPAR: a) Cytotype C ($2n=40$); b) Cytotype A ($2n=42$). Bar= $5\mu\text{m}$.

Figure 2: Frequency histograms of diploid numbers observed in mosaic specimens of *H. malabaricus*, showing a similar frequency of cells with $2n=41$ and $2n=42$ chromosomes.

Figure 3: Karyotypes of the cell lines found within mosaic specimens of *H. malabaricus*: a) karyotype with $2n=42$ chromosomes; b) karyotype with $2n=41$ chromosomes, showing some monosomic and trisomic chromosomes. Bar= $5\mu\text{m}$.

Figure 4: RAPD amplification products of *H. malabaricus* in agarose gel using the primer OPA-12. (bp) base pairs; (Hind III) Hind III Marker; (A) cytotype A samples; (M) mosaic specimens; (C) cytotype C specimens. The arrows indicate the invariable bands of each cytotype. Observe the similarity between band profiles in cytotype A and the mosaic specimens, as well as the presence of distinct bands in cytotype C specimens.

The set of six primers amplified 71 bands (loci), with a polymorphism rate of 23.94% for cytotype A specimens, 8.45% for the mosaic specimens, and 11.26% for cytotype C specimens. The heterozygosity was estimated at 0.118, 0.046 and 0.050, respectively. Values of Nei's genetic distance were 0.083 between cytotypes A and the mosaic specimens, 1.321 between cytotype A and C, and 1.022 between the mosaic specimens and cytotype C. The genetic similarity among individuals, estimated through Jaccard's coefficient, was equal to 92% between the mosaic and cytotype A samples, 26% between cytotypes A and C, and 24% between mosaics and cytotype C individuals. The RAPD-based dendrogram showed a clustering between mosaic and cytotype A specimens, and a differentiation of 100% in relation to the cytotype C sample, supported by bootstrap values (Fig. 5).

DISCUSSION

The three Erythrinidae genera (*Hoplias*, *Hoplerythrinus* and *Erythrinus*) show wide

karyotypic diversity, comprising different cytotypes in *Hoplias malabaricus* (Bertollo et al., 2000), *Hoplerythrinus unitaeniatus* (Giuliano-Caetano et al., 2001; Diniz and Bertollo, 2003) and *Erythrinus erythrinus* (Bertollo et al., 2004). In the mentioned species, the cytotypes can be differentiated either by diploid number and/or chromosomal morphology, representing distinguishable and easily identified karyotypic forms. Furthermore, in *H. malabaricus* and *E. erythrinus*, the presence of distinct single or multiple sex chromosome systems leads to a precise identification of the several cytotypes observed.

Sympatry for the occurrence of distinct cytotypes has already been observed in some natural fish populations, such as *Astyanax scabripinnis* (Souza et al., 1995; Maistro et al., 2000; Fernandes and Martins-Santos, 2005), *Laetacara* cf *dorsigera* (Martins-Santos et al., 2005) and *Hoplerythrinus unitaeniatus* (Giuliano-Caetano, 1988; Diniz and Bertollo, 2006). Different sympatric cytotypes have been reported in nearly nine distinct populations of the so-called *H. malabaricus* throughout

Brazil and Argentina. Two such populations are characterized by the sympatry occurrence of cytotypes A ($2n=42$) and C ($2n=40$), one of them in the Igarapé Mindú - Manaus/AM system (Bertollo et al., 1997; Bertollo et al., 2000), and another in the Aguapey River - Corrientes/Argentina system (Lopes et al., 1998). Hybrid forms between cytotypes were not detected in any of the sympatric conditions, suggesting they are reproductively isolated. This can be considered a strong feature towards the characterization of distinct species.

In the present study, the coexistence of cytotypes A and C in the fish farm station was also ascertained. Most of the analyzed specimens presented karyotypes that allowed us to define them as representatives of each cytotype. Initially, the specimens in which the diploid number could not be defined, i.e., they presented a high number of both 41 and 42 chromosomes in metaphase spreads, might suggest they would be a result of hybridization events

between the cytotypes A and C. However, an alternative hypothesis should be considered, related to non-disjunction events in cytotype A individuals, giving rise to cell lines bearing trisomies and monosomies and hence leading to cells with 41 chromosomes.

Torres et al. (2002) detected a drastic reorganization in the karyotype of *Trichomycterus paolence*, with diploid numbers ranging from 54 to 57 chromosomes in kidney cells. They observed the presence of normal-sized chromosomes and microchromosomes, probably resultant from chromosomal breakages and consequent formation of acentric fragments that would be lost during the cell cycle. In another species of the same genus, *T. davis*, three cell lines with different diploid numbers ($2n=54$ to 56) were also identified, probably derived from both mitotic non-disjunction ($2n=55$) and chromosome fission ($2n=56$) (Borin and Martins-Santos, 2000).

Figure 5: Genetic similarity dendrogram, based on RAPD profiles, in *H. malabaricus*. Observe the clustering between mosaic specimens (B) and those belonging to cytotype A, as well as the distance in relation to cytotype C specimens.

The distinct band profiles obtained by RAPD analyses provided a reliable identification of cytotypes A and C, revealing a genomic differentiation between both cytotypes and their putative reproductive isolation. These data corroborate the hypothesis that the species *H. malabaricus* must represent a species complex with different cytotypes (Bertollo et al., 2000). Nonetheless, the two mosaic specimens showed a similar RAPD pattern obtained within cytotype A sample, which weakens the hypothesis that they originated through hybridization between the two cytotypes. Therefore, the different cell lines appear to be a consequence of chromosomal alterations (non-disjunction) in the early mitotic divisions of zygotes belonging to the cytotype A. Moreover, the similarity dendrogram based on RAPD profiles also reinforces the close relationship between cytotype A and mosaic specimens, as well as their higher distance in relation to cytotype C. Nevertheless, the precise causes for the mosaicism observed in this study cannot be addressed, suggesting that further studies are still required.

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