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Organization and molecular cytogenetics of a satellite DNA family from *Hoplias malabaricus* (Pisces, Erythrinidae)

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The chromosomes of the primitive South American teleost fish *Hoplias malabaricus* have been analyzed by classical cytogenetic (C-, AgNOR-, Hoechst 33258-, and Q-banding) techniques. A highly repetitive DNA family has been cloned and sequenced. It is a tandemly repeated sequence of about 355 bp, yielding an overall base pair composition of 67% AT with long runs of >50% As and 70% Ts. Analysis of sequence variation has allowed the further categorization of *Hoplias* satellite DNA into two evolutionarily related subfamilies A and B, distinguishable by characteristic insertions and deletions within this 355-bp monomer. Subfamily A satellite is found (in diverged form) at the centromeres of most *H. malabaricus* chromosomes. Sequence variants are clustered on specific chromosomal subsets. Subfamily B satellite is highly specific for the paracentromeric heterochromatin on one particular chromosome pair by fluorescence *in situ* hybridization. These results indicate that the *Hoplias* satellite DNA family has evolved in a concerted manner predominantly via recombination events involving homologous, rather than non-homologous chromosome regions. The clones isolated here may be useful for the molecular, genetic, and cytological analysis of the genus *Hoplias*.

Key words: centromere, concerted evolution, fish cytogenetics, *Hoplias*, satellite DNA

Introduction

The genomes of higher eukaryotes contain various amounts of non-coding satellite DNA sequences located primarily in the (peri)centromeric regions and less frequently in telomeric and interstitial (heterochromatic) regions of metaphase chromosomes. A certain amount of satellite-like repetitive DNA sequences appear to be essential for structural functions at the chromosomal and nuclear level (Singer 1982, Miklos 1985, Haaf and Schmid 1991). Transfection experiments have suggested a role for α-satellite DNA, the centromeric satellite of primate chromosomes, in centromere function (Haaf *et al.* 1992). Satellite DNA sequences are also believed to be a driving force in chromosomal evolution and have been implicated in speciation (Wichman *et al.* 1991).

The monomeric repeat units of different satellite DNA families vary in length from a few base pairs to several hundred nucleotides (Singer 1982, Miklos 1985). In addition, a high degree of sequence divergence of the order of some 10% can exist between monomers of one satellite DNA family within and between (closely related) species. This is, for example, the case with primate α-satellite, one of the most thoroughly studied satellite DNA families. At a second level of hierarchy, sequence variants of α-satellite DNA are organized in tandem arrays that constitute chromosome-specific subsets with clearly definable higher-order repeat units. This (chromosomal) distribution is thought to reflect a concerted mode of sequence evolution among homologous chromosomes (Willard and Waye 1987, Willard 1991). In contrast, sequence variants appear to be completely randomly distributed within *Tenebrio molitor* satellite DNA (Plohl *et al.* 1992), possibly suggesting that, in this case, the process of interchromosomal spreading is faster than the mutation rate and/or intrachromosomal homogenization. Indeed, some satellite DNA families, i.e. *Drosophila melanogaster* and feline satellites, show only low levels of sequence variation between <1% and 3% (Lohe and Brutlag 1986, Fanning 1987), indicating a high rate of homogenization among different (non-homologous) chromosomes.

Satellite DNAs have been extensively studied in invertebrates and mammals (Singer 1982, Miklos 1985). Com-

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pared to other vertebrates, only very limited information is available on sequence and genomic organization of satellite DNAs in fishes (Datta *et al.* 1988, Moyer *et al.* 1988, Wright 1989, Denovan and Wright 1990, Ekker *et al.* 1992), several classes (Osteichthyes, Chondrichthyes, Agnatha) comprising more than 20000 species and representing half of all living vertebrates. Although many fish species possess cytologically detectable constitutive heterochromatin, suggesting the presence of satellite DNA (Haaf and Schmid 1984, Gold *et al.* 1986), the molecular cytogenetics of fish satellite DNAs is currently unknown.

H. malabaricus is a carnivorous fish of the primitive Erythrinidae, widely distributed geographically in all of South America. It is being used as an animal model in biochemical and physiological research (Riggs *et al.* 1979, Machado *et al.* 1989, and references therein). Standard karyotype analyses in the genus *Hoplias* revealed the existence of multiple sex chromosomes of the $X_1X_1X_2X_2$/X_1X_2Y and XX/XY_1Y_2 type (Bertollo *et al.* 1983, Dergam and Bertollo 1990), thus providing a contemporary snapshot of the ongoing processes during the evolution of chromosomal sex-determining mechanisms in primitive vertebrates.

In this report, we describe the nucleotide sequence, genomic structure, and chromosomal localization of a major satellite DNA family from *H. malabaricus*. Cloned monomer variants are clustered on specific chromosome types, indicating that satellite subsets evolve along chromosomal lineages, similar to the situation found in many other satellite DNA families (Miklos 1985, Willard and Waye 1987). The mechanism(s) underlying the (largely) chromosome-specific organization of *Hoplias* satellite is discussed.

Materials and methods

Chromosome and DNA preparation

Six mature specimens (four males and two females) of *H. malabaricus* were caught in the Monjolinho reservoir (Alto Paraná basin, State of São Paulo, Brazil). Mitotic chromosomes were prepared directly from kidney after *in vivo* colchicine treatment (Haaf and Schmid 1984). Genomic DNA was isolated from blood, liver, and spleen according to Blin and Stafford (1976). The specimens analysed here (museum numbers 7161-7165 and 7187) have been preserved at the Biological Department of the Federal University of São Carlos.

Chromosome banding

Chromosomes were C-banded to visualize the constitutive heterochromatin according to Sumner (1972). The nucleolus organizer regions (NORs) were demonstrated using the silver ($AgNO_3$) staining technique of Goodpasture and Bloom (1975). Fluorescent staining with quinacrine mustard was performed according to the method of Caspersson *et al.* (1970), and staining with Hoechst 33258 was according to Jalal *et al.* (1974).

Isolation and cloning of *Hoplias* satellite DNA

Genomic DNA was digested to completion with a panel of 25 restriction endonucleases and run on a 1% agarose gel. When stained with ethidium bromide, a prominent band of approximately 350 bp and additional less prominent bands representing approximate multiples of 350 bp became visible in *Dra*I, *Hind*III, *Hinf*I, and *Mbo*I digests. The approximately 350-bp *Hind*III fragment was purified from low melt agarose, ligated with *Hind*III- digested pUC18, and used to transform *Escherichia coli*. Fifteen recombinant plasmids were recovered. Following hybridization to Southern blots of *Hind*III-digested genomic DNA, inserts of eight independent clones revealed a ladder of hybridizing bands, indicating the presence of a repetitive DNA sequence.

DNA analysis

Genomic DNA was digested with restriction endonucleases according to the recommendations of the suppliers, resolved on 1% horizontal agarose gels in TAE buffer, and Southern blotted. Clones of *Hoplias* satellite DNA were hybridized under high-stringency conditions (at 52°C in 50% formamide, 3 × SSC with a final wash in 0.1 × SSC, 0.1% SDS at 68°C) to the resulting filters. Before reprobing, the Southern blots were washed three times for 20 min each in 0.1 × SSC, 0.1% SDS at 75°C. Nucleotide sequence was determined by the dideoxy-termination method using T7 DNA polymerase (Pharmacia) and ^{35}S-dATP-labelled deoxynucleotide.

In situ hybridization

Probes were labelled with biotin- 11-dUTP using a commercially available nick translation kit (Oncor). For *in situ* hybridization, the slides were treated with 100 μg/ml RNase A in 2 × SSC (pH 7.0) at 37°C for 1 h, rinsed four times in 2 × SSC, and dehydrated in an ethanol series (70%, 80%, 95%). Chromosomal DNA was denatured by immersing the slides for 5 min at 70°C in 70% formamide, 2 × SSC, pH 7.0. The slides were then dehydrated in an alcohol series (70%, 80%, 90%, 95%). The hybridization mixture was composed of 65% (high stringency conditions) or 50% (low stringency) formamide in 2 × SSC, 10% dextran sulphate, 500 μg/ml carrier DNA, and 1.5 μg/ml biotinylated DNA probe. After 5 min denaturation at 70°C, 30 μl of hybridization mixture was applied to each slide under a sealed coverslip. Hybridization was performed overnight in a moist chamber at 37°C. The slides were then washed for 30 min in either 65% formamide, 2 × SSC at 43°C or 50% formamide, 2 × SSC at 37°C, depending on the hybridization conditions, and twice for 5 min at 37°C in 2 × SSC, pH 7.0. Hybridization was detected with fluorescein-conjugated avidin (Vector Laboratories). The signal was enhanced by incubation with biotinylated goat-antiavidin (Vector) followed by fluoresceinated avidin. The chromosomes were counterstained with propidium iodide (1 μg/ml in PBS for 1–5 min).

Results

Ethidium bromide staining of *Hind*III-digested and size-fractionated genomic DNA of *H. malabaricus* revealed a highly repetitive fragment of about 350 bp. This monomer was extracted from the gel and cloned into pUC18 (see Materials and methods). Individual repeat units from eight clones, designated Hop1, Hop3, Hop4, Hop7, Hop9, Hop10, Hop12, and Hop13, all belong to the same satellite DNA family and are the focus of this study.

The complete nucleotide sequences of the eight independently cloned monomers and a consensus sequence have been determined (Figure 1). The monomer length ranged from 333 bp to 366 bp (mean = 355 bp). Direct pairwise sequence comparisons revealed that the cloned monomers are each 62% to 98% identical to each other. It is apparent that these monomers can be arranged into two homology groupings: subfamily A (monomers 1, 3, 7, 9, 10, and 13) and subfamily B (monomers 4 and 12). The level of sequence divergence among monomers within a given subfamily varied from 2% to 11% with an average divergence of 8.1%. Among members of different subfamilies, the sequence divergence varied from 34% to 38% with an average divergence of 35.9%. Compared to subfamily A repeats, monomers 4 and 12 of subfamily B exhibit a characteristic 25-bp insertion (corresponding to positions 63–87 of the consensus sequence) as well as a 25-bp deletion (corresponding to positions 248–272 of the

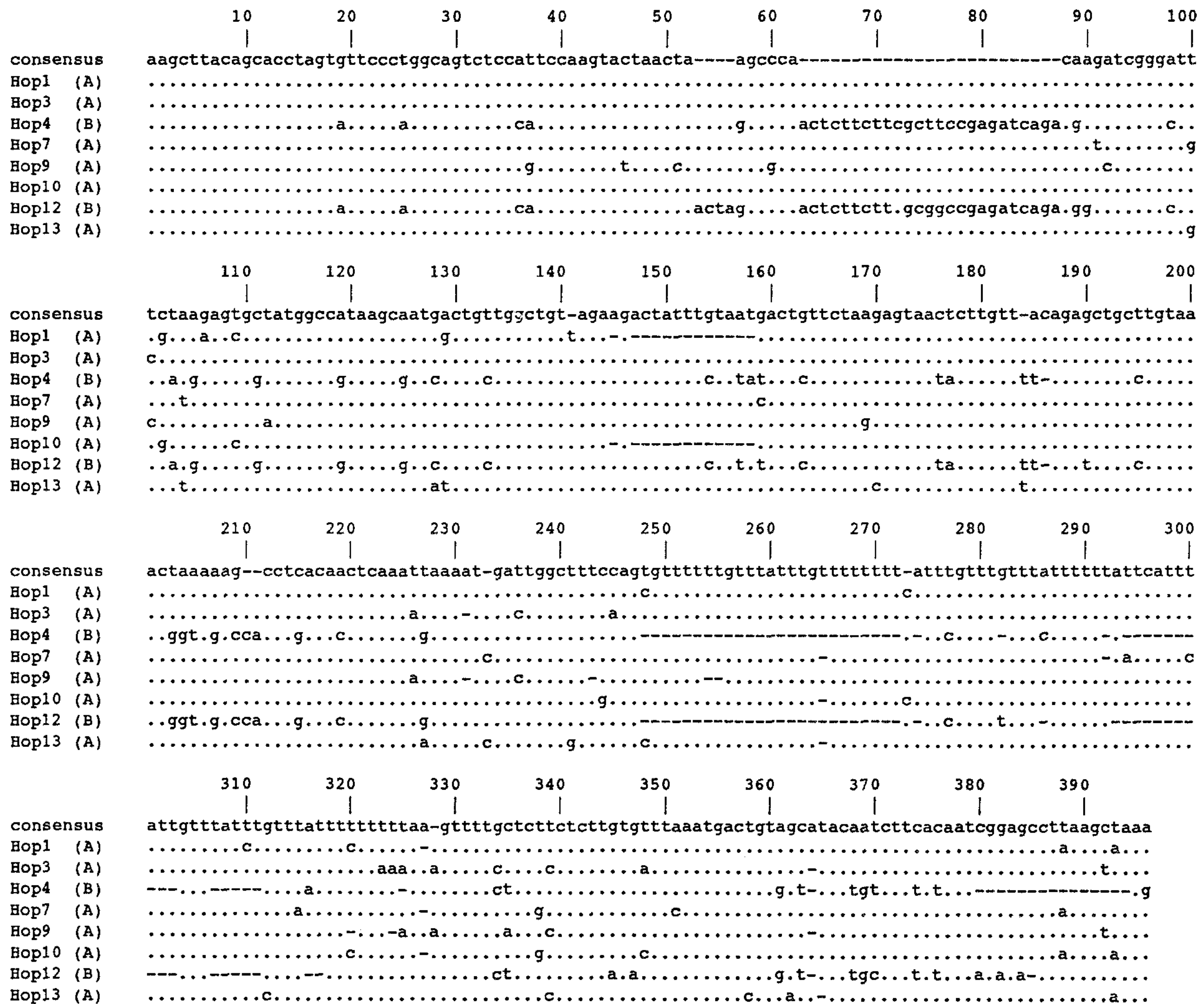
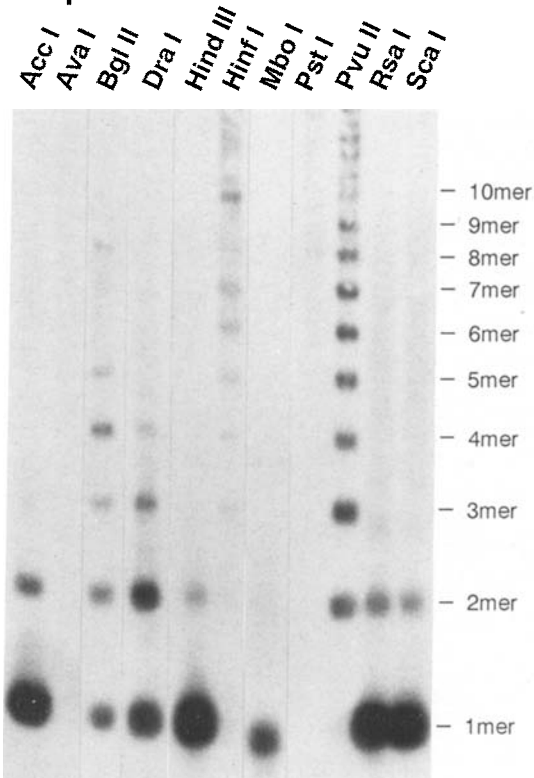
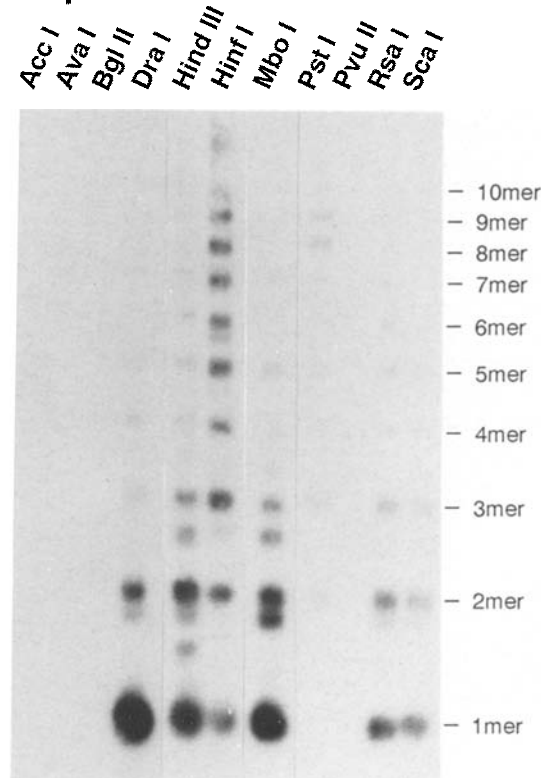
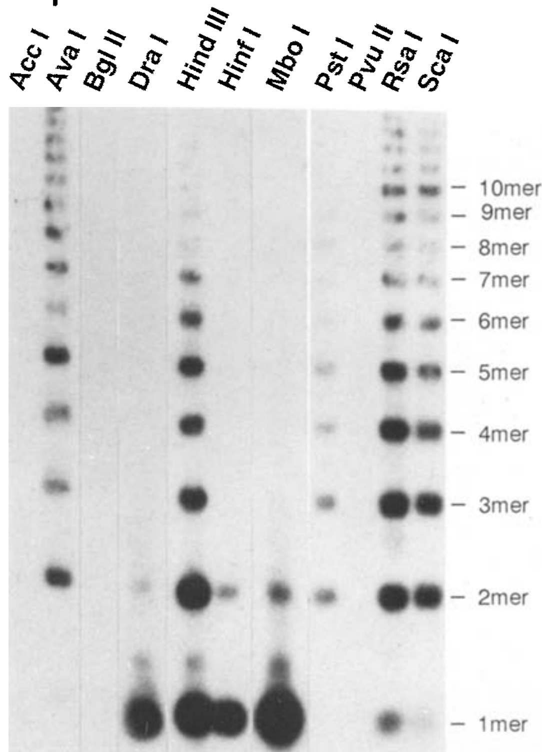


Figure 1. Nucleotide sequence (1-396) of cloned *Hoplias* satellite DNA compared with a derived monomer consensus sequence. Hop1, Hop3, Hop4, Hop7, Hop9, Hop10, Hop12, and Hop13 represent eight independent monomers. Capital letters in parentheses reflect the monomer type (subfamily A or B). The consensus position, where identical nucleotides were present in 50% (or more) of sequences, was considered unambiguous. Gaps (—) were introduced by parsimony to improve alignment. Because of frequent insertions and deletions within monomers, the length of the monomer consensus sequence (396 bp) is considerably greater than that of an average cloned monomer (~355 bp). The sequences of clones Hop4 and Hop10 have been deposited into the GenBank/EMBL databases (Accession nos. L11927 and L11928).







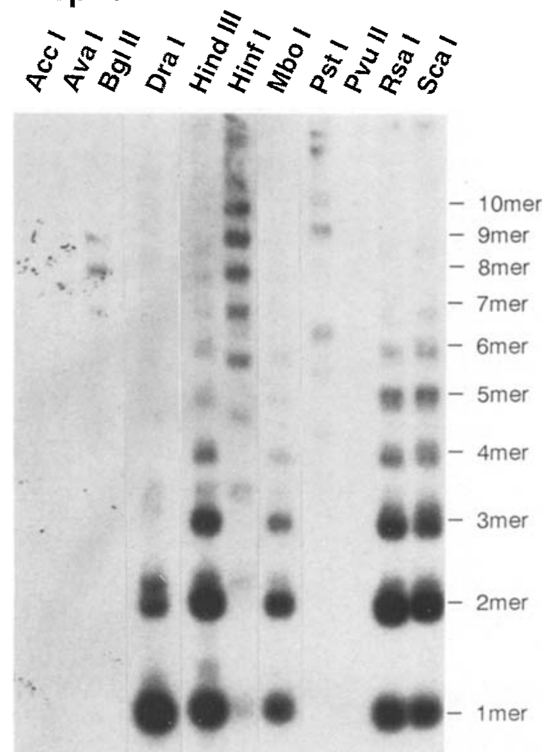


Figure 2. Hybridization of Hop4, Hop7, Hop9, and Hop10 satellite DNA probes to a Southern blot of *H. malabaricus* genomic DNA digested with the indicated restriction enzymes. Probes were hybridized under high-stringency conditions. Relevant lanes from a single gel were assembled. Band sizes are indicated on the right-hand side in multiples of the ~355 bp monomer repeat unit.

consensus), and many other specific base changes and deletions. Nucleotide sequence data have been deposited into GenBank under accession nos. L11927 (clone Hop4) and L11928 (clone Hop10).

The overall sequences are 67% AT-rich. The most striking feature is a cluster (corresponding to positions 199–235 of the consensus) of 53% As, followed by a much larger cluster (corresponding to positions 236–350) of 70% Ts. Significantly, all subfamily B-specific deletions occurred within the extremely T-rich stretch of DNA which may represent a site particularly prone to unequal crossing over and sequence rearrangements. The monomer sequence does not contain obvious direct or inverted repeats, suggesting that the 333-bp to 366-bp monomer represents the smallest basic unit of repetition rather than a multimer of smaller ancestral repeats.

A computer-assisted search (Pearson and Lipman 1988) did not reveal any significant homology between the cloned *Hoplias* satellite DNA and sequences recorded in the GenBank/EMBL databases. In addition, hybridization of a *Hoplias* satellite DNA cocktail probe to a zoo blot at low stringency (at 42°C in 50% formamide, 3 × SSC with a final wash in 0.5 M NaCl at 65°C) did not show detectable cross-hybridization with sequences present in the genomes of mammals (human, great apes, mouse, hamster, kangaroo rat), birds (chicken), other fishes (various species of the genera *Leporinus* and *Geophagus*), and *Drosophila* (data not shown).

To determine the genomic organization of *Hoplias* satellite DNA, genomic DNA from *H. malabaricus* was digested with a panel of 25 restriction enzymes and hybridized under high-stringency conditions to clones Hop4, Hop7, Hop9, and Hop10 (Figure 2). A ladder of hybridizing bands corresponding to monomers and oligomers of the *Hoplias* satellite DNA was seen (with the different probes) in *Acc*I, *Ava*I, *Bgl*II, *Dra*I, *Hind*III, *Hinf*I, *Mbo*I, *Pst*I, *Pvu*II, *Rsa*I, and *Sca*I digests. In some digests, the ladder increased (at monomeric increments) to a length of more than 20 oligomers. These results indicate tandem repetition of the monomer unit with loss of restriction site(s) due to mutation in some repeats.

It is important to note that each clone analysed produced a distinct hybridization pattern. This possibly reflects differences in the (higher order) genomic organization of distinct satellite DNA subsets homologous to Hop4, Hop7, Hop9, and Hop10. The pattern given by Hop4, for example, is characterized by very prominent bands at the monomer position in *Acc*I, *Mbo*I, *Rsa*I, and *Sca*I digests. Some repeats homologous to Hop4 contain restriction sites for *Bgl*II and *Pvu*II not found in other *Hoplias* satellite DNA subsets. On the other hand, restriction sites for *Hinf*I and *Pst*I are very rare in Hop4-like repeats. In contrast, clone Hop9 hybridized predominantly to the monomer band in *Hinf*I digests, indicating the presence of a *Hinf*I restriction site in the majority of Hop9-like repeats. The subset homologous to Hop9 also contains (diagnostic) *Ava*I restriction sites virtually absent in other repeats. The pattern given by Hop7 shows characteristic intermediate bands in *Hind*III and *Mbo*I digests. It follows that multiple restriction sites for these enzymes are present in some Hop7-like repeats. Hybridization of Hop10 to *Hind*III, *Mbo*I, *Rsa*I, and *Sca*I digested DNA produced (equally) strong bands at the monomer and dimer positions. Consistent with the results of nucleotide sequence analysis, genomic analysis therefore strongly suggests the existence of different subsets of *Hoplias* satellite DNA.

Hybridization with cloned *Hoplias* satellite DNA generally revealed ladders of bands with the intensity of the hybridization signal decreasing regularly as the number of monomers in the oligomer increased. A 'reversed' ladder of hybridizing bands was evident in *Hinf*I digests hybridized to Hop4 and Hop10 (Figure 2). These hybridization patterns suggest sequence divergence among monomers (of a given satellite DNA subset) with restriction sites (dis)appearing in a stochastic manner. Defined higher order restriction enzyme

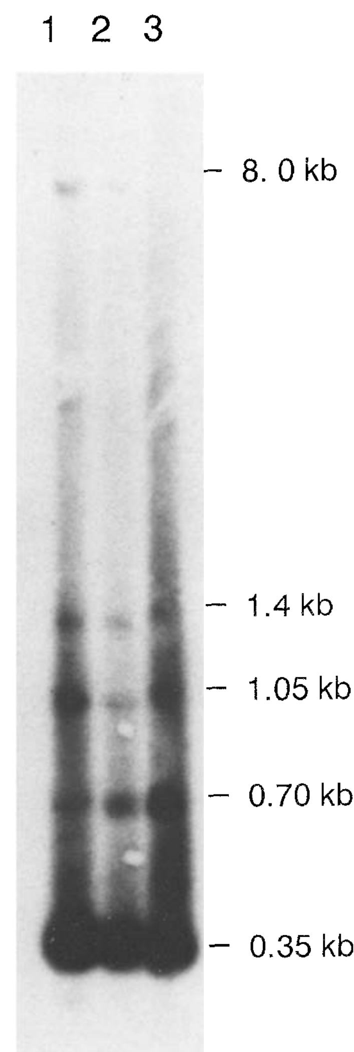


Figure 3. Detection of a *Hoplias* satellite DNA polymorphism. Genomic DNA from three specimens was digested with *Dra*I and hybridized with Hop10. Band sizes are indicated at the right in kb. An approximately 8-kb polymorphic fragment was detected in two of three specimens analysed.

periodicities indicating the existence of prominent higher order repeating structures could not be observed with the enzyme panel tested.

The cloned satellite DNA sequences exhibit polymorphisms detectable by conventional agarose-gel electrophoresis and may thus serve as valuable genetic markers. Hop10, for example, detects a polymorphism consisting of an approximately 8 kb band when hybridized to *Dra*I-digested genomic DNA. This specific fragment was present in two of three specimens so analysed (Figure 3).

The four female *H. malabaricus* specimens examined here displayed a diploid chromosome number of $2n = 40$ (Figure 4). This is consistent with the findings of Dergam and Bertollo (1990), who reported a female diploid number $2n = 40$ and a male diploid number $2n = 39$ for the Monjolinho population, suggesting an $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome mechanism. (Because of the inferior metaphase quality of the two male specimens analysed, the presence of sex chromosomes in *H. malabaricus* from the Monjolinho reservoir could not be confirmed with certainty). All chromosomes had a submetacentric to metacentric appearance. One animal exhibited one or two supernumerary B chromosomes in a percentage of metaphase cells (see Figure 5C). In the C-banded karyotype of *H. malabaricus*, constitutive heterochromatin is located at the centromeres of all chromosomes as well as in the telomeric regions of some chromosomal arms (Figure 4a). Silver staining demonstrated that nucleolus organizer regions (NORs) are located in the distal long arms of two chromosome pairs (Figure 4b). The AT-specific fluorescent dyes Hoechst 33258 and quinacrine mustard did not show any labelling of note on the cloned AT-rich satellite DNA. All *Hoplias* chromosomes fluoresced with uniform intensity (Figure 4c, d).

Fluorescence *in situ* hybridization of biotinylated *Hind*III inserts to denatured metaphase spreads was used to localize regionally the cloned satellite DNA on *Hoplias* chromosomes (Figures 5 and 6). Under low stringency conditions, subfamily A probes (i.e. Hop1, Hop9, and Hop10) detected homologous sequences on at least 11 chromosome pairs (Figures 5A and 6A). Specific labelling was confined to the (peri)centromeric regions of the target chromosomes. At high stringency, each subfamily A clone analysed hybridized predominantly to the centromeric regions of a much smaller subset of chromosomes. *In situ* hybridization with Hop1 and Hop10 (which are 98% identical in sequence) resulted in

Figure 4. Karyotype of (female) *H. malabaricus*. The chromosomes ($2n = 40$) were numbered and arranged into karyotypes following the system proposed by Bertollo *et al.* (1983). (**a**) C banding. Note the large amounts of (peri)centromeric heterochromatin in all chromosomes and the additional telomeric C bands in some chromosomal arms. (**b**) Silver (AgNOR) staining. The distal long arms of chromosome pairs 9 and 15 are endowed with nucleolus organizer regions. (**c**) Hoechst-33258 fluorescence. (**d**) Q banding.

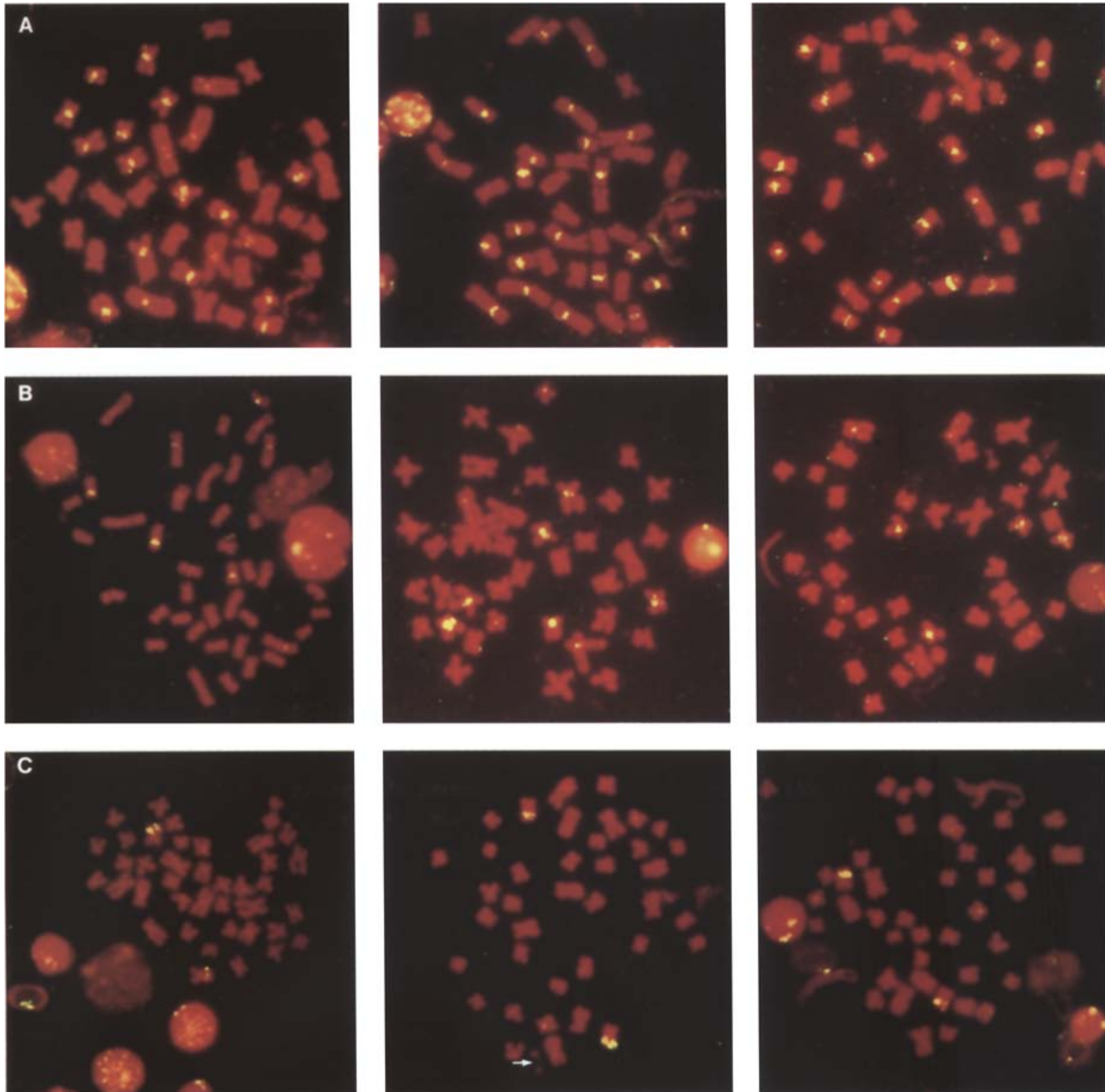


Figure 5. Fluorescence *in situ* hybridization using biotinylated *Hoplias* satellite against metaphase spreads from a female specimen. (**A**) Under low-stringency conditions, hybridization signals of a subfamily A satellite cocktail probe are detectable at the centromeres of most chromosomes. (**B**) Under high-stringency conditions, Hop1 detects homologous sequences on only three chromosome pairs. (**C**) A single pair of chromosomes shows hybridization signals (at high stringency) with subfamily-B satellite, i.e. Hop4 and Hop12. The arrow in (C) indicates two supernumerary B chromosomes found in some metaphase cells from specimen #7165.

strong labelling on two to three chromosome pairs; only weak signals were detected at other centromeres (Figures 5B and 6B). This indicates that sequence variants homologous to Hop1 or Hop10 are clustered on specific chromosomes. Subfamily B monomers (Hop4 and Hop12) were highly localized in the large paracentromeric region of chromosome pair 6 (Figures 5C and 6C); even under low stringency conditions, very minor or no hybridization signals were seen on other chromosomes.

Discussion

We have isolated and characterized a satellite DNA family from the fish *H. malabaricus*. The cloned satellite has a monomer length of about 355 bp and is 67% AT-rich. Since sequence (and genomic) analysis of the large monomers did not reveal the presence of an underlying small oligonucleotide consensus sequence, *Hoplias* satellite DNA seems to be

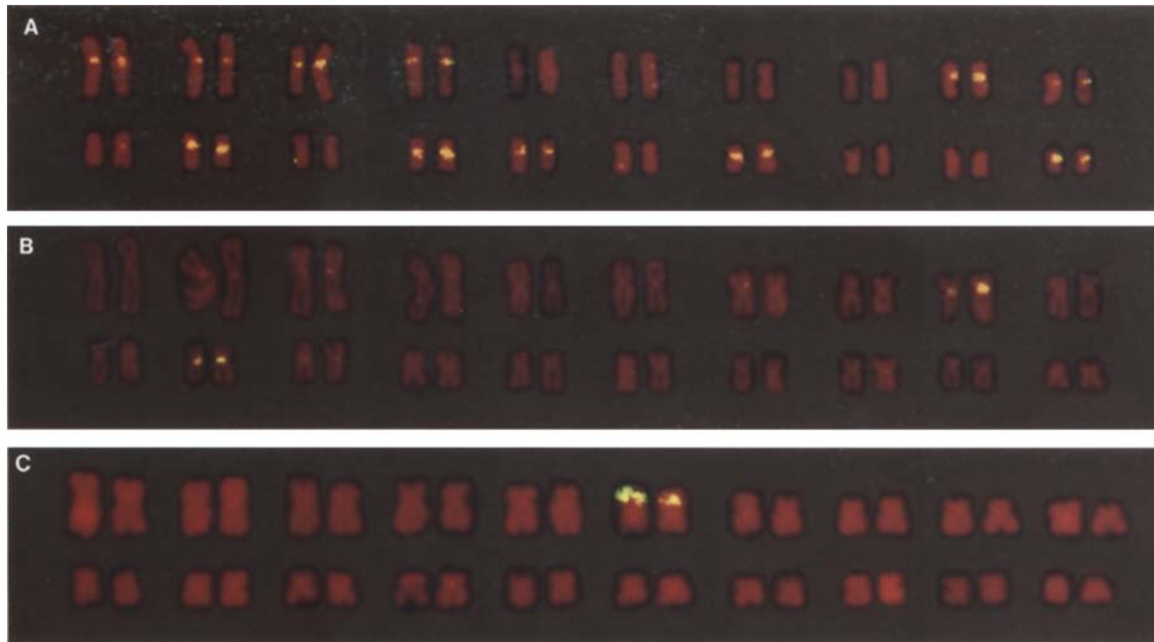


Figure 6. (**A, B**) Karyotypes of *H. malabaricus* hybridized to Hop10 under low-stringency (A) and high-stringency (B) conditions respectively. Subfamily A satellite DNA sequences cross-hybridizing with Hop10 are present in the centromeric regions of at least 11 chromosome pairs (A). The Hop10 subset of subfamily A is highly enriched in two chromosome pairs (B). (**C**) Specific localization of Hop4-like sequences (subfamily B) to the paracentromeric region of chromosome pair 6.

based on a fundamental repeat unit of approximately 355 bp that has been extensively amplified and diverged. Up to now, only few other fish satellite DNAs have been identified: their monomer repeat units were found to be 165 bp (in *Brachydanio rerio*; Ekker *et al.* 1992), 174 bp (in *Notropis lutrenensis*; Moyer *et al.* 1988), 200 bp (in *Pollachius virens*; Denovan and Wright 1990), 237 bp (in *Oreochromis mossambicus/hornorum*; Wright 1989), and 245 bp (in *Cyprinus carpio*; Datta *et al.* 1988) in length and their base composition varied from 53% to 68% AT. Thus, to the extent it is known, fish satellites seem to be composed of relatively large monomeric repeat units of AT-rich DNA.

Evidence has been presented that subsets of *Hoplias* satellite DNA are quite divergent in sequence and are, in consequence, largely specific to the chromosome(s) in origin. Diverged *Hoplias* satellite sequences were found at the centromeres of more than half of the chromosome complement. Chromosomes lacking detectable hybridization signals on their centromeric heterochromatin may contain very diverged subsets of the same *Hoplias* satellite DNA family or, alternatively, a different satellite DNA family(ies). As discussed previously (Willard 1990, Haaf *et al.* 1992), the lack of sequence conservation among centromere-specific satellite DNAs as well as the high degree of sequence variability among the monomers of one satellite DNA family need not exclude the possibility that satellite DNA sequences are the primary determinant in the centromere of higher eukaryotes.

Clustering of sequence variants on particular chromosomes was observed within subfamily A satellite by fluorescence *in situ* hybridization. Similarly, subfamily B sequences were highly localized in the paracentromeric heterochromatin of one chromosome pair. In this light, it seems that in the case of *H. malabaricus* genomic turnover processes homogenizing individual chromosomal subsets are relatively more efficient than processes spreading sequence variants throughout the family (Ohta and Dover 1983). Turnover of satellite DNA sequences may occur suddenly by disproportionate replication/gene amplification (Schimke 1984) or slowly by continuous mechanisms such as unequal crossing over between repeats of sister chromatids (Smith 1976) and gene conversion (Baltimore 1981). Studies on human α-satellite DNA suggest that homogenization processes proceed in a localized, short-range fashion leading to formation of large domains of sequence identity (Warburton and Willard 1990).

In many satellite DNA families, including primate α-satellite, individual copies of a fundamental repeat unit (monomers) are further organized hierarchically into multimeric higher order repeat units. It is the concerted evolution at the level of the higher order repeat unit that confers on α-satellite the observed chromosome specificity (Willard and Waye 1987, Willard 1991). Genomic analysis did not reveal defined higher order repeating structures within *Hoplias* satellite DNA. Although the definition of such higher order repeat units depends on the adventitious

use of a restriction endonuclease(s) that cuts once per higher order repeat unit (Willard and Waye 1987) and, hence, conclusions need to be made with some caution, our results argue in favour of the notion that *Hoplias* satellite DNA is characterized by amplification at the level of monomers rather than oligomers and that these monomeric repeat units have been homogenized in a largely chromosome-specific fashion during recent evolution of this satellite DNA family. Characteristic insertions/deletions at particular base pair positions within the monomer may account for the chromosome specificity of a given monomer variant detectable by fluorescence *in situ* hybridization. Within subfamily A, for example, monomers Hop1 and Hop10 are distinguishable by a deletion corresponding to positions 145 to 158 of the consensus and by additional diagnostic base changes. Subfamily B monomers which are highly specific for the paracentromeric heterochromatin on chromosome pair 6 share a large 25-bp insertion, a 25-bp deletion, and several smaller deletions.

In summary, we have characterized a major family of tandemly repeated DNA from the teleost fish *H. malabaricus*. Two *Hoplias* satellite subfamilies have been delineated that include subsets at the centromeres of most *H. malabaricus* chromosomes. The observed chromosome specificity reflects a concerted mode of genome evolution that results in a high degree of intrachromosomal—relative to interchromosomal—sequence homogeneity. The overall properties of *Hoplias* satellite including its chromosomal distribution and polymorphic nature are similar to those exhibited by primate α-satellite and many other mammalian satellite DNA families.

Our data strongly suggest that sequence evolution of *Hoplias* satellite DNA has occurred in a concerted fashion on homologous chromosomes. This reflects a major, although not exclusive, mode of genome evolution. In this context, it is worth emphasizing that concerted evolution can also operate across non-homologous chromosomes. *In situ* hybridization data from the genera *Reithrodontomys* (Hamilton *et al.* 1990), *Peromyscus* (Hamilton *et al.* 1992), and *Equus* (Wichman *et al.* 1991) indicate that in some species there is tremendous intragenomic movement of satellite DNA 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(s). Molecular cloning and genomic analysis of the many varied satellite DNA families may eventually provide better insights into the possible structural/functional tasks that repetitive DNA sequences fulfill in the various genomes as well as into evolutionary mechanisms that shape chromosomes and genomes.

The genus *Hoplias* poses considerable taxonomic problems. A precise definition at the species level is often difficult and superficially similar or indistinguishable populations may well represent more than one taxon (Bertollo *et al.* 1983, Dergam and Bertollo 1990). Since satellite DNA subsets are not only chromosome specific but also species specific as well (as a consequence of concerted evolution), cryptic species can be discriminated on the basis of the cloned satellite DNA sequences. Our results demonstrate that satellite DNA sequences constitute a very useful class of marker for the genetic, molecular, and cytogenetic analysis of fish genomes.

Acknowledgements

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