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Overview of Ictalurid Genomes: Nuclear DNA Content, Diploid Chromosome Features, and Physical Mapping of Genes

QUIYANG ZHANG AND RICHARD K. COOPER
Louisiana State University Agricultural Center, Department of Veterinary Science
Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana 70803

TERRENCE R. TIERSCH
Louisiana State University Agricultural Center, Aquaculture Research Station
Louisiana Agricultural Experiment Station

Abstract.—Genome analysis is an essential step for genetic mapping of economically important ictalurid catfish species and provides basic knowledge for breeding and phylogenetic studies. In this review article, we summarize results of studies on nuclear DNA content, chromosome number and structure, karyotype standardization, and physical genetic mapping of catfishes and their hybrids. The genome sizes of four species of ictalurid catfishes representing the genera *Ictalurus*, *Ameiurus*, and *Pylodictis* were 2.11 ± 0.05 picograms (pg) ranging from 2.06 pg (*Ictalurus furcatus*) to 2.20 pg (*Ameiurus melas*) and with a variation of less than 2.5% within a species. This low variation may relate to a low occurrence of constitutive heterochromatin in catfish chromosomes. Nuclear DNA and chromosomes were found to segregate precisely as a function of haploid levels and were stable in genomes of interspecific or intergeneric hybrids. Identification of individual chromosomes has been achieved by different techniques including differential staining for nucleolus organizer regions and heterochromatin, and by restriction enzyme banding and replication banding. Standard karyograms were produced with the assistance of a computer-based image analysis system for morphometric measurements and location of chromosome bands. In situ polymerase chain reaction has been used to identify the chromosomal location of a gene existing in single copy in the haploid genome, which provides a promising method for the physical mapping of catfish genomes. However, supporting techniques must be developed for analysis of chromosome identity after hybridization treatments.

Genetic mapping provides valuable information for detailed analysis and manipulation of organisms where matings and progeny can be accurately monitored (Paterson 1998). Applications of genetic mapping include assignment of genes to specific linkage groups (chromosomes), evolutionary studies, DNA fingerprinting, map-based gene cloning and marker-assisted selective breeding (Liu et al. 1998, 1999).

Mapping using DNA markers has become popular because it is generally easier to isolate and work with DNA than with other genetic markers such as proteins (e.g., allozymes). In ictalurid catfishes, however, DNA sequence information is limited, which has led to difficulties in developing linkage groups with DNA markers. In addition, analysis of linkage groups is also deterred by the long generation interval in catfishes. Thus, instead of analyzing the segregation patterns of DNA markers among offspring, a gene or gene (linkage) group of DNA markers can be assigned to individual chromosomes directly by in situ hybridization (ISH), a form of physical genetic mapping. In addition to localization of single-copy genes, direct mapping of DNA markers can be useful in identifying specific chromosomes (such as sex chromosomes) and quantitative trait loci of economic importance in aquaculture.

Physical mapping requires a basic understanding of genome structure of the species investigated. Although the family Ictaluridae contains the economically important channel catfish *Ictalurus punctatus*, genetic study of this family has lagged well behind that of livestock and other cultured fishes such as salmonids. This paper summarizes available information on nuclear DNA content, chromosomal structure and physical mapping to enhance further genetic mapping in ictalurid species.

Genome Size

Genome size (diploid nuclear DNA content) has been estimated for hundreds of plant and animal species. In ictalurid catfish, nuclear DNA content of erythrocytes of four species representing the genera *Ictalurus*, *Ameiurus*, and *Pylodictis* was estimated by high-resolution flow cytometry to be 2.112 ± 0.047 picograms (pg) (Table 1) (Tiersch and Goudie 1993). Intraspecific variation was less than 2.5% for

TABLE 1. Summary of genomic data for seven species representing four genera of the family Ictaluridae, and four intergeneric hybrids.

Species (or hybrid)	Nuclear DNA content (pg)[a]	Base pair ($\times 10^9$)	Diploid number (2N)	Diploid number NOR chromosomes	NOR phenotype[b]	References
Ictalurus punctatus	2.092 ± 0.008	2.050	58	2	DD, paired, symmetric staining	LeGrande 1981; Wolters et al. 1981; Tiersch and Goudie 1993; Zhang and Tiersch 1997
Ictalurus furcatus	2.057 ± 0.012	2.016	58	2	Unknown	LeGrande 1981; Tiersch and Goudie 1993
Ameiurus catus	Unknown	Unknown	48	Unknown	Unknown	LeGrande et al. 1984
Ameiurus melas	2.200 ± 0.012	2.156	60	2	JJ, paired, symmetric staining	LeGrande 1981; Tiersch and Goudie 1993; Zhang and Tiersch 1997
Ameiurus natalis	Unknown	Unknown	62	4	JJKK, paired symmetric staining	LeGrande 1981; this report
Pylodictis olivaris	2.148 ± 0.009	2.105	56	2	DD, paired, asymmetric staining	LeGrande 1981; Tiersch and Goudie 1993; Zhang and Tiersch 1997
Noturus funebris	Unknown	Unknown	44	2	GG, paired, asymmetric staining	This report
I. punctatus x *I. furcatus*	2.076 ± 0.007	2.034	58	2	DH, unpaired	LeGrande et al. 1984; Tiersch and Goudie 1993; Zhang, unpublished data
I. punctatus x *A. catus*	Unknown	Unknown	53	Unknown	Unknown	LeGrande et al. 1984
I. punctatus x *A. melas*	2.146 ± 0.009	2.103	59	2	DJ, unpaired	Tiersch and Goudie 1993; Zhang and Tiersch 1997
I. punctatus x *P. olivaris*	2.118 ± 0.011	2.076	57	3	DDA, unpaired	Zhang and Tiersch 1997

[a] Sample size (N) = 12 to 16; one pg DNA is approximately equal to 0.98 x 10^9 base pairs.
[b] NOR phenotypes (adapted from Amemiya and Gold, 1988): A = terminal on short arm of a medium-sized subtelocentric; D = terminal on short arm of a medium-sized submetacentric, G = terminal on one arm of a large-sized metacentric, H = terminal on short arm of a medium-sized metacentric, J = terminal on short arm of a small-sized submetacentric, and K = terminal on one arm of a small-sized metacentric.

all species studied and was small compared to variation observed in other vertebrates. Furthermore, no significant differences in DNA content were detected among 14 different channel catfish stocks or between males and females (Tiersch et al. 1990). Genome size of hybrid offspring (Table 1) was exactly intermediate to the genome sizes of parental stocks, indicating that nuclear DNA segregated as a function of haploid DNA content and was stable within intergeneric hybrids. Other groups such as cyprinids (Gold et al. 1990) and salmonids (reviewed in Lockwood and Derr 1992) can exhibit within-species and among-species variation larger by an order of magnitude.

The diploid genome of ictalurid catfishes (about 2 pg of DNA) comprises about two billion nucleotide base pairs (bp). Because catfish chromosomes exist in pairs that are essentially identical, about one billion nucleotide pairs (the haploid genome) would need to be sequenced to gain complete information concerning a representative catfish genome. In the human genome (about 7 pg for diploid cells) about 3% of nucleotides code for proteins and the average protein molecule requires approximately 1,000 coding nucleotide pairs (Committee on Mapping and Sequencing the Human Genome 1988). Accordingly, as applied to the ictalurids, catfish genomes should be composed of about 10% protein-coding DNA.

Diploid Chromosome Number

Studies focusing on catfish chromosomes began in the 1970s (Hudson 1976), although reports including catfish can be found earlier (Muramoto et al. 1968). One important contribution of these studies was to use chromosomal data for analysis of phylogenetic relationships within the family (LeGrande 1981). Diploid chromosome number (2N) within Ictaluridae varied among different genera: *Ictalurus* with 56–58, *Ameiurus* with 48–62, *Pylodictis* with 56, and *Noturus* with 40–72 (LeGrande 1981; Wolters et al. 1981). Although great differences exist in chromosome numbers and structures among species of different genera, hybrids have been successfully produced between channel catfish (CCF) and blue catfish *I. furcatus*, CCF and black bullhead *A. melas*, and CCF and flathead catfish *P. olivaris* (LeGrande et al. 1984; Zhang and Tiersch 1997). The CCF x white catfish *A. catus* (WCF) cross represents an extreme example with parental values of 58 (CCF) and 48 (WCF) yielding a hybrid with 53 chromosomes (LeGrande et al. 1984). The haploid inheritance pattern was found to be common to intergeneric hybrids of the family Ictaluridae (LeGrande et al. 1984; Zhang and Tiersch 1997). The diploid numbers of hybrid fish were found to be the average of the diploid numbers of the parental species. The occurrences of each chromosome type (e.g., metacentric) were also close to the average occurrence of these chromosome types within the parental species (Zhang and Tiersch 1997). These results further testify that the genome of ictalurid catfishes segregates as a function of haploid chromosome number and nuclear DNA content, and is stable in interspecific and intergeneric hybrids.

Chromosome Banding

Differential staining of chromosomal structures such as nucleolus organizer regions (NOR) and constitutive heterochromatin (C-banding) has been applied successfully to catfish chromosomes (Zhang and Tiersch 1998a). All ictalurid species examined so far have one or two pairs of metacentric or submetacentric chromosomes bearing NOR on their short arms (Table 1). In channel catfish, the NOR phenotype was stable among different tissue types (Zhang and Tiersch 1998a)

The constitutive heterochromatin of channel catfish chromosomes was found to be sparse and limited to centromeric regions (Zhang and Tiersch 1998a). Distinct secondary bands were absent on most channel catfish chromosomes, unlike results observed in cyprinids (Gold et al. 1986) and many salmonids (Phillips and Hartley 1988) where noncentromeric C-banding was prominent and useful for identifying homologous chromosomes. The low abundance of heterochromatin may explain the stable genome size found in the ictalurid catfishes as described above and, in contrast to cyprinids and salmonids, reflect evolutionary conservatism within the genomes of ictalurid catfishes.

The NOR-staining and C-banding techniques are useful to identify chromosomes with special structures and to identify homologous pairs. However, these techniques do not readily distinguish among chromosomes of similar size and centromeric position. In higher vertebrates, individual chromosomes are easily identified by linear structural banding methods such as Giemsa (G) and reverse (R) banding (Schwarzacher and Wolf 1974). However, chromosomes of catfishes are not only numerous, small, and homogeneous in size, but are also relatively monotonous in structure. Therefore, catfish chromosomes were not differentiated by application of standard treatments such as trypsin, heat, or fluorochromes (Zhang 1996).

Fortunately, other techniques such as restriction enzyme (RE) banding and replication banding were capable of generating multiple bands, allowing differentiation of morphologically similar chromosomes (Zhang and Tiersch 1998a; Zhang et al. 1998). Each of these techniques deals with the DNA rather than the protein components of chromosomes. Digestion of whole chromosomes by REs causes removal of DNA fragments, and Giemsa staining can be used to reveal the relative amount and location of the remaining DNA, yielding reproducible patterns. For example, the restriction enzyme *Hind* III (which has a recognition sequence of the nucleotides AAGCTT) produced 3–9 bands per channel catfish chromosome, with a total of 161 bands for the entire haploid set (Zhang and Tiersch 1998a) (Figure 1).

Replication banding is based on the incorporation of the base analog bromodeoxyuridine (BrdU) during DNA replication which results in BrdU-rich and BrdU-poor regions detectable by ultraviolet irradiation coupled with fluorochrome and Giemsa staining. In channel catfish, 5–13 bands were generated on each chromosome by this technique, with a total of 215 bands for the entire haploid set (Figure 1) (Zhang et al. 1998). These techniques did not

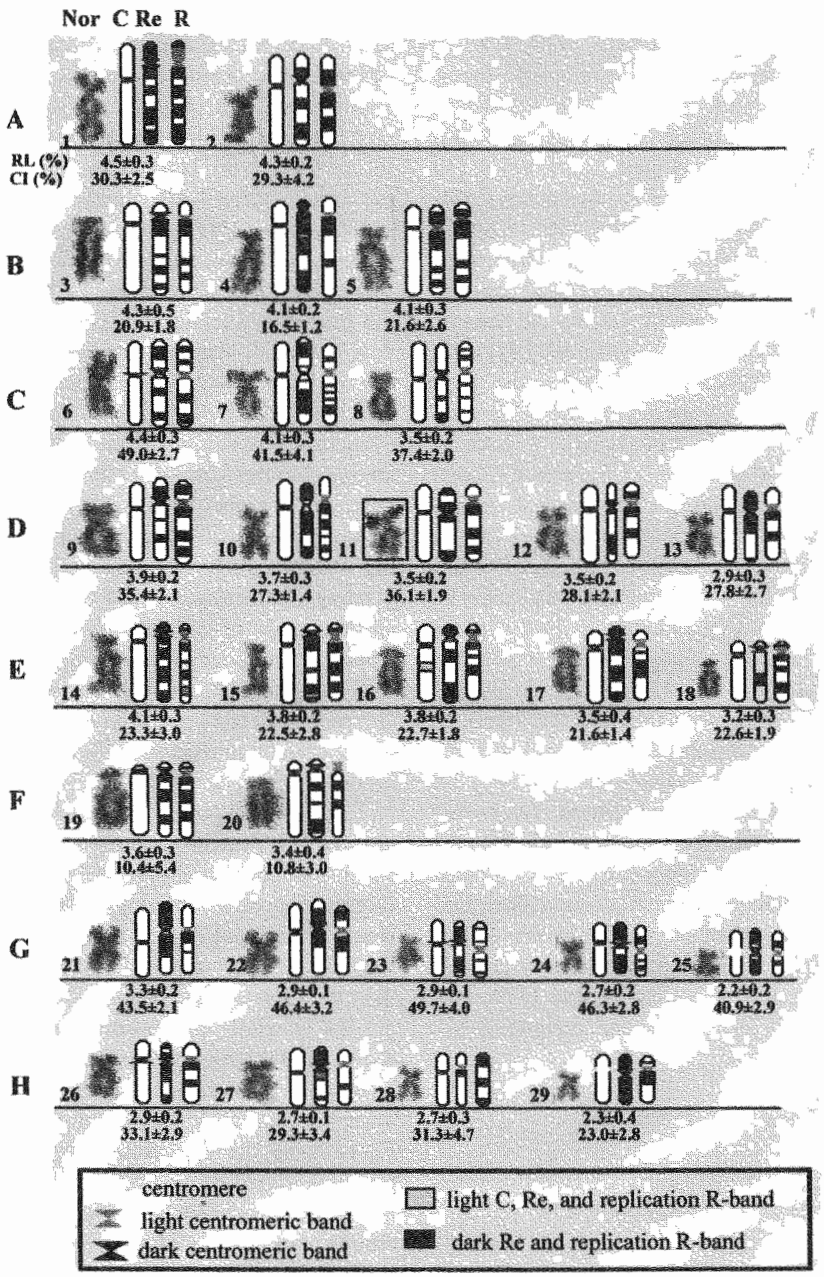

FIGURE 1. A comprehensive idiogram (adapted from Zhang 1996) of channel catfish chromosomes treated by silver staining for nucleolus organizer regions (NOR), C-banding (C), restriction enzyme digestion with *Hind* III (Re), and replication R-banding (R). Chromosomes were sorted by centromeric index (CI) and relative length (RL), and divided into eight groups: A, large submetacentric; B, large subtelocentric; C, large and medium metacentric; D, medium submetacentric; E, medium subtelocentric; F, telocentric; G, small metacentric, and H, small submetacentric chromosomes. Inset: the NOR-bearing chromosomes. Measurements of CI and RL for each chromosome were derived from analysis of 20 spreads obtained from 5 channel catfish, and were calculated using the following formulae:

RL (%) = (length of the chromosome pair/total complement length) × 100;

CI (%) = (length of short arm/total length of the chromosome) × 100

produce robust bands along the chromosomes seen in most other teleost species, and analysis of banding patterns required use of a computer-based method described below.

Karyotype Standardization and Banding Pattern Analysis

Relative length (RL) and centromeric index (CI) are two parameters used frequently for characterization of mammalian chromosomes (Levan et al. 1964). However, manual measurement of catfish chromosomes does not yield consistent results. In our studies, chromosomal data were recorded and analyzed with a computer-based image analysis system (Zhang and Tiersch 1998b). The morphometric measurements and karyotyping were handled by the Optimas® (Bioscan, Inc., Edmonds, Washington) and Kary® (Pro Data, Oslo, Norway) computer software packages, allowing the process to be semiautomated, which increased consistency and minimized errors. In channel catfish, the 29 pairs of homologous chromosomes were divided into eight groups based on relative length and centromeric position (Figure 1). The NOR were located on a pair of medium-sized submetacentric chromosomes (identified as D-11, Figure 1, inset).

Application of the image analysis system enabled identification of weak chromosome bands produced by RE-banding and replication-banding techniques (Zhang and Tiersch 1998b). The dark-and-light banding patterns of chromosomes were analyzed by densitometry and expressed as a luminance plot. An idiogram was prepared based on the plot for each chromosome using the Microsoft Powerpoint® computer software (Figure 1).

Physical Mapping

Although in situ hybridization (ISH) techniques have worked successfully for highly repeated sequences and genes with multiple copies in the haploid genome (Phillips and Reed 1996), these techniques have limited capability to identify target sequences existing as a single-copy in haploid genomes. The newly emerged technology of in situ polymerase chain reaction (ISPCR) allows multiplication of target DNA sequences to increase efficiency of hybridization and detection (Gu 1994). In the ISPCR technique, denatured DNA is amplified (copied) using primers (short nucleotide sequences) labeled with biotin, digoxigenin, or protein molecules that allow direct visualization of the target sequences after amplification. In one study, we examined nuclear and chromosomal location of the channel catfish *Ig H* gene (Wilson et al. 1990) which encodes a portion of the immunoglobulin heavy chain (Zhang et al. 1997; Zhang et al. 1999). The *Ig H* gene was detected at a telomeric position of a chromosome with relative length of 3.2 ± 0.2%, which was identical to the results obtained from fluorescent in situ hybridization (FISH). In other studies, the 28S ribosomal RNA gene (28S rDNA) of channel catfish was cloned and partially sequenced (Zhang et al., in press). Chromosomal location detected by ISPCR of the channel catfish 28S rDNA was found to be associated with the NOR (Zhang et al., in press), an association recognized in most animal species including humans. These results indicate the validity of the ISPCR techniques for use in chromosomal mapping.

However, there is one problem to overcome before ISPCR can be used routinely in physical mapping. The identity of the chromosomes bearing target genes is difficult to establish because visibility of replication R-bands of catfish chromosomes is reduced by the heat and salt treatments of ISPCR and FISH (Zhang et al. 1997). Although procedures have been developed for simultaneous detection of replication R-bands and hybridization signals in mammalian species, these techniques are not applicable for channel catfish chromosomes. Potential solutions for the low visibility of chromosomal banding patterns include densitometric analysis or sequential treatment with restriction enzymes. Another promising method is chromosome painting by repetitive DNA probes (Schrock et al. 1996), which could be an alternative to replication banding for use with ISPCR.

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