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Chromosomal inheritance patterns of intergeneric hybrids of ictalurid catfishes: odd diploid numbers with equal parental contributions

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Hybrid chromosomal compositions of channel catfish *Ictalurus punctatus* × black bullhead *Ameiurus melas* and channel catfish × flathead catfish *Pylodictis olivaris* were analysed by a computer-based method. The karyotype of each hybrid was highly asymmetric, and the diploid numbers and arm numbers were intermediate to the parental types. The hybrid offspring of channel catfish × black bullhead possessed a diploid number of 59 chromosomes, with an arm number estimate of 87. The hybrid offspring of the channel catfish × flathead catfish cross possessed a diploid number of 57 chromosomes, also with an arm number estimate of 87. Nucleolus organizer regions (NORs) were located on a single pair of chromosomes with symmetric staining intensity in channel catfish and in black bullhead, and on a single pair of chromosomes with asymmetric staining intensity in flathead catfish. The channel catfish × black bullhead hybrid had two unpaired chromosomes that stained positively for NORs. The channel catfish × flathead catfish had three unpaired chromosomes that stained positively for NORs. Specific marker chromosomes were identified in each hybrid. There was no evidence of androgenesis, gynogenesis, polyploidy or aneuploidy in the hybrids. Results of this study, plus information reported previously, indicate that chromosomes of ictalurid catfishes are inherited stably in a haploid pattern with an equal contribution to the genomes of F_1 hybrids, even in intergeneric crosses involving divergent numbers of parental chromosomes.

Key words: chromosomes; ictalurid catfishes; intergeneric hybrids; NOR.

INTRODUCTION

An alternative to conventional selective breeding of fishes is the production by interspecific hybridization of different genetic types to yield qualitative or quantitative changes in commercial traits (Chevassus, 1983). Heterosis resulting from hybridization has included improvements in growth (Song, 1987; Kerby & Harrell, 1990), disease resistance (Wu, 1980) and harvest rate (Zhang, 1979). Interspecific hybridization has also been utilized by aquaculturists for production of monosex populations (Lahav, 1990) or sterile populations.

Channel catfish *Ictalurus punctatus* (Rafinesque) is the major food fish species cultured in North America, and an aquaculture industry based on this species is well developed in the south-eastern United States. Production of hybrids between channel catfish and other species of the family Ictaluridae such as blue catfish *I. furcatus* (Lesueur), and white catfish *Ameiurus catus* (L.), has been

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studied (Huner & Dupree, 1984). The channel catfish (female) × blue catfish (male) hybrid has been reported to offer improvements over the parental species including faster growth to marketable size, greater uniformity in growth and higher dress-out percentage (Dunham *et al.*, 1990).

The analysis of genomic structure of hybrid fish provides basic understanding of the newly produced phenotypes, and essential information for breeding studies. The genome of hybrid fishes represents a random recombination of the genetic material present in the parental genomes. Hybridization can result in genome-level alterations including gynogenesis and androgenesis, or in development of different ploidy levels (Chevassus, 1983). For example, in the intergeneric hybrids of rainbow trout *Oncorhynchus mykiss* (Walbaum) × brook trout *Salvelinus fontinalis* (Mitchill) (Chevassus, 1983) or of grass carp *Ctenopharyngodon idella* (Valenciennes) × bighead carp *Aristichthys nobilis* (Richardson) (Beck *et al.*, 1980), all surviving fish were triploid, presumably resulting from a failure to complete the second meiotic division of the maternal genome. In an intergeneric cross of common carp *Cyprinus carpio* L. × grass carp, all hybrids died in the embryonic stages presumably because of the incompatibility of parental genomes and asynchronization in nuclear division (Ye *et al.*, 1989). Yet, in another study of the same hybrid cross, 70% of surviving fish were androgenetic diploids, 28% were normal hybrid diploids, and <2% were gynogenetic diploids (Vasil'ev *et al.*, 1975). Even in diploid hybrids, the chromosomal contribution of parental fish is not necessarily equal, such as in the cross of *Cirrhinus molitorella* (Cuvier & Valenciennes) × *Sinilabeo decorus* (Tungting) where the majority of chromosomes in the hybrid genome were derived from the maternal parent (Zhang *et al.*, 1984).

On the other hand, features in the ictalurid catfish hybrids studied to date were found mostly to be intermediate to those of the parental species, such as in the morphometric ratios of hybrid offspring in the channel catfish × black bullhead *A. melas* (Rafinesque) cross (Goudie *et al.*, 1993). The nuclear DNA content of blood cells of three hybrid crosses of catfishes was exactly intermediate to that of the parental species (Tiersch & Goudie, 1993). Intermediate chromosome numbers were reported in the channel catfish × white catfish hybrid (LeGrande *et al.*, 1984). However, these data do not necessarily mean that the hybrid genome is composed equally of parental contributions. A closer investigation of chromosome assembly in hybrid genomes is needed to support such an observation.

Overall, cytogenetic analysis of ictalurid hybrids has received little attention (LeGrande *et al.*, 1984). Methods for more detailed analysis of karyotypes such as selective staining for the nucleolus organizer region (NOR) have never been studied in ictalurid hybrids. Intergeneric hybrids between channel catfish and black bullhead catfish, and between channel catfish and flathead catfish were produced. The chromosomal features of these two hybrids were unknown. This article documents the karyograms of these intergeneric hybrids and the NOR phenotypes of the parental and hybrid fishes, analyses the differences among chromosome measurements of the hybrids and parental species, and evaluates chromosomal inheritance in the hybrids.

MATERIALS AND METHODS

SOURCE OF FISH

The F_1 hybrids were produced in May of 1994 by artificial fertilization using eggs from channel catfish and sperm from black bullhead or flathead catfish as described in Tiersch *et al.* (1994). Sperm were collected and stored at 4° C, or cryopreserved before the spawning season. Female fish were induced to spawn by intraperitoneal injection of 100 μg kg^{-1} of synthetic luteinizing hormone-releasing hormone (Busch & Steeby, 1990). After fertilization, the eggs were transferred to incubation troughs. The juvenile fish were reared in a recirculating system until analysis.

CHROMOSOME PREPARATION

Five to 10 fish from each parental species (0·5–1·0 kg) and each hybrid cross (6–8 g) were sampled randomly for cytogenetic analysis. For adult fish, chromosomes were prepared from cultured leukocytes (Zhang & Tiersch, 1995). The leukocytes were isolated from whole blood by gradient centrifugation on ficoll hypaque (Histopaque® – 1·077; Sigma Chemical Company, St Louis, MO, U.S.A.), and were cultured in RPMI medium (Sigma) with the addition of concanavalin A (10 μg ml^{-1}) for stimulation of mitosis. The chromosomes were arrested at metaphase by addition of colchicine (0·5 μg ml^{-1}). For juvenile fish, chromosomes were prepared from kidney tissue collected 1 h after intraperitoneal injection of colchicine (2 μg g^{-1}). The procedures for hypotonic treatment and cold fixation were based on routine methods (LeGrande, 1981).

STAINING OF NUCLEAR ORGANIZER REGIONS (NORs)

Staining of the NOR was based on the procedure of Howell & Black (1980). Slides were covered with a solution of 30% silver nitrate and 1·5% gelatin and incubated at 50° C for 8–10 min. The slides were rinsed briefly with deionized water and dried at room temperature. Letter designations for NOR phenotypes followed guidelines presented in Amemiya & Gold (1988).

COMPUTER-ASSISTED KARYOTYPING

The process of karyotyping was assisted by the Optimas® (Bioscan, Inc., Edmonds, WA, U.S.A.) and Kary® (Pro Data, Oslo, Norway) computer software packages. The programs handled images with a 24-bit video capture board (Imaging Technology Inc., Bedford, MA, U.S.A.) from a light microscope (Microphot-SA, Nikon Inc.) equipped with a high-resolution RGB colour video camera (model A206A; Microimage Video Systems Co., Inc., Boyertown, PA, U.S.A.). Objects of interest in an image were separated from their background by setting appropriate threshold values on a 13-inch medical-grade, high-resolution colour video monitor (PVM1343MD, Sony Corp, Ichinomiya, Japan). Chromosomes that touched one another were separated through the splitting function of the Kary® program. Chromosomes were treated as individual objects and sorted by size. The individual chromosomes were rotated and placed in appropriate positions on a blank template to create karyograms. A good-quality, representative metaphase spread from each parental species and hybrid type was used for preparation of karyograms. The chromosomes were grouped as metacentrics, submetacentrics, subtelocentrics, or telocentrics (as described below) and were arranged by descending size. The entire process took 1–2 h $fish^{-1}$.

Good-quality metaphase spreads (n=61–120) were used for analysis of modal diploid chromosome numbers. Lengths of entire chromosomes, long arms and short arms were measured using high quality metaphase spreads containing the modal diploid number of chromosome, and were expressed as a percentage of total complement length (%TCL). Chromosomes were classified based on their long-arm to short-arm ratios according to Levan *et al.* (1964): values of 1·0–1·7 were classified as metacentric; 1·7–3·0 as submetacentric; 3·1–7·0 as subtelocentric, and >7·1 as telocentric.

RESULTS

The modal diploid numbers were 58 for channel catfish, 60 for black bullhead, 56 for flathead catfish, 59 for channel catfish × black bullhead hybrids, and 57 for channel catfish × flathead catfish hybrids (Table I). The karyograms of channel catfish (Fig. 1) and black bullhead (Fig. 2) were distinguishable in features other than chromosome number, with the most obvious difference being the lack of small telocentric chromosomes in the channel catfish which had seven pairs of metacentrics (%TCL range 2·1–4·6%), 10 pairs of submetacentrics (%TCL range 2·4–5·1%), 10 pairs of subtelocentrics (%TCL range 3·0–4·2%), and two pairs of telocentrics (%TCL range 3·2–3·5%). Black bullhead had eight pairs of metacentrics (%TCL range 2·7–5·3%), four pairs of submetacentrics (%TCL range 3·1–4·6%) seven pairs of subtelocentrics (%TCL range 2·2–5·2%), and 11 pairs of telocentrics (%TCL range 1·6–5·8%). The most distinguishable feature of the karyogram of the flathead catfish was a large metacentric chromosome (number 1) with a %TCL of 7·5% (Fig. 3). Flathead catfish had nine pairs of metacentrics (%TCL range 2·2–7·5%), five pairs of submetacentrics (%TCL range 2·8–4·6%), six pairs of subtelocentrics (%TCL range 2·6–4·0%), and eight pairs of telocentrics (%TCL range 1·7–4·2%).

The karyogram of the channel catfish × black bullhead hybrids (Fig. 4) was highly asymmetric. It was impossible to classify chromosomes as members of pairs in the hybrid genome. The karyogram consisted of 28 metacentrics and submetacentrics (%TCL range 1·9–4·7%), and 31 subtelocentrics and telocentrics (%TCL range 1·5–5·7%). A large subtelocentric with a %TCL of 5·7%, and several small telocentrics with a %TCL $\leq$ 2·0% were identified as marker chromosomes derived from black bullhead (Fig. 2).

The karyogram of the channel catfish × flathead catfish hybrids was also highly asymmetric (Fig. 5), and consisted of 30 metacentrics and submetacentrics (%TCL range 2·1–7·4%) and 27 subtelocentrics and telocentrics (%TCL range 1·4–4·6%). A large metacentric was found in every hybrid metaphase spread, and was identified as a marker chromosome originating from chromosome 1 of the flathead catfish (Fig. 3).

The NORs of channel catfish were located on a pair of medium-sized submetacentric chromosomes [Fig. 6(a)], and the NORs of black bullhead were located on a pair of small submetacentric chromosomes [Fig. 6(b)]. The staining intensity for the NOR of each homologue was symmetric for these two species. The NORs of flathead catfish were located on a pair of medium-sized submetacentrics, and the staining intensity for the NOR of each homologue was asymmetric [Fig. 6(c)]. The hybrids of channel catfish and black bullhead had two unpaired NORs: one on a medium-sized submetacentric and the other on a small submetacentric chromosome [Fig. 6(d)]. The hybrids of channel catfish and flathead catfish had three NORs: two on medium-sized submetacentric chromosomes and the other on a medium-sized subtelocentric [Fig. 6(e)].

DISCUSSION

In this study, the karyograms of channel catfish × black bullhead, and channel catfish × flathead catfish were documented for the first time. The NOR

TABLE I. Summary of cytogenetic data for channel catfish (*Ictalurus punctatus*), black bullhead (*Ameiurus melas*), flathead catfish (*Pylodictis olivaris*), and hybrid offspring of channel catfish × black bullhead and channel catfish × flathead catfish

Species or hybrid	*n*	2*N*	AN†	%TCL* (max–min)	Formula	Diploid number NOR chromosomes	NOR phenotypes‡
Ictalurus punctatus	10	58	92	5·0–2·0	34 msm, 24 st	2	DD, paired and symmetric staining
Ameiurus melas	5	60	84	5·8–1·6	24 msm, 36 stt	2	JJ, paired and symmetric staining
Pylodictis olivaris	3	56	84	7·5–1·7	28 msm, 28 stt	2	DD, paired and asymmetic staining
Ictalurus punctatus × *Ameiurus melas*	10	59	87	5·7–1·6	28 msm, 31 stt	2	DJ, unpaired symmetric staining
Ictalurus punctatus × *Pylodictis olivaris*	7	57	87	7·4–1·4	30 msm, 27 stt	3	DDA, unpaired symmetric staining

n, Number of specimens; 2*N*, diploid modal count; AN, arm number; %TCL, per cent of total complement length range; Formula, morphological composition of diploid complement; NOR, nucleolus organizer regions.

*Per cent of total complement length (%TCL) was calculated by formula: (length of homologous pair/total length of chromosome complement) × 100.

†Metacentric (m) and submetacentric (sm) were counted as two arms, subtelocentric (st) and telocentric (t) were counted as a single arm.

‡NOR phenotypes (adapted from Amemiya & Gold, 1988): A, terminal on short arm of a medium-sized subtelocentric; D, terminal on short arm of a medium-sized submetacentric; J, terminal on short arm of a small-sized submetacentric.

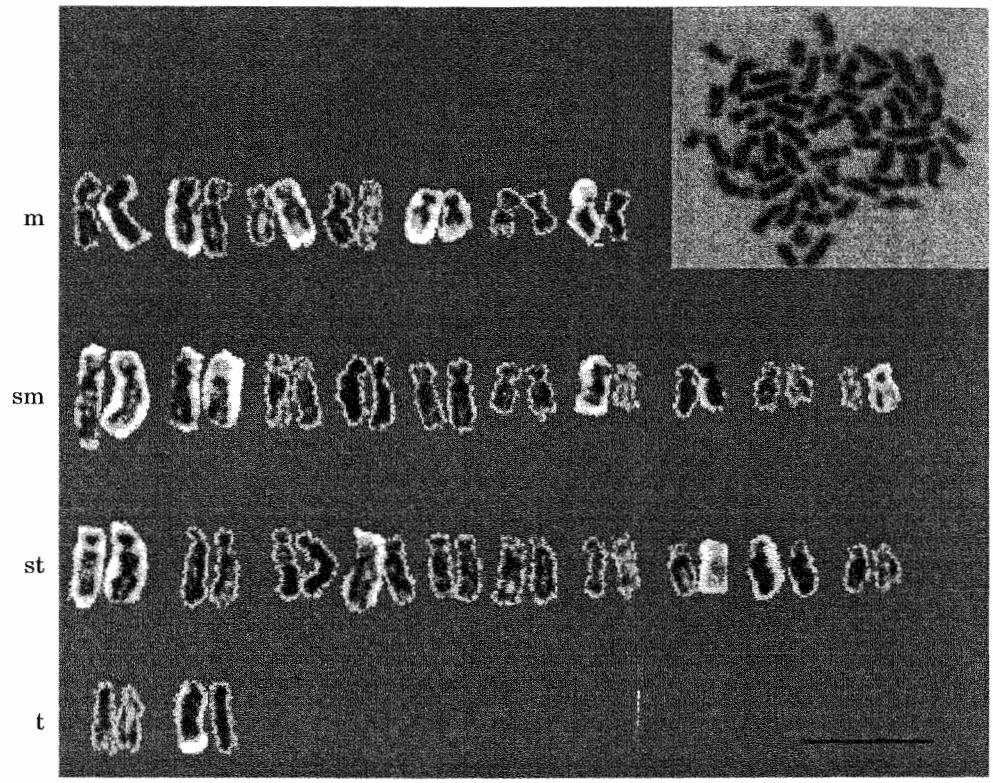

FIG. 1. Representative karyogram of channel catfish. Inset: the original metaphase spread. Bar=5 μm. m, Metacentric, sm, submetacentric; st, subtelocentric; t, telocentric.

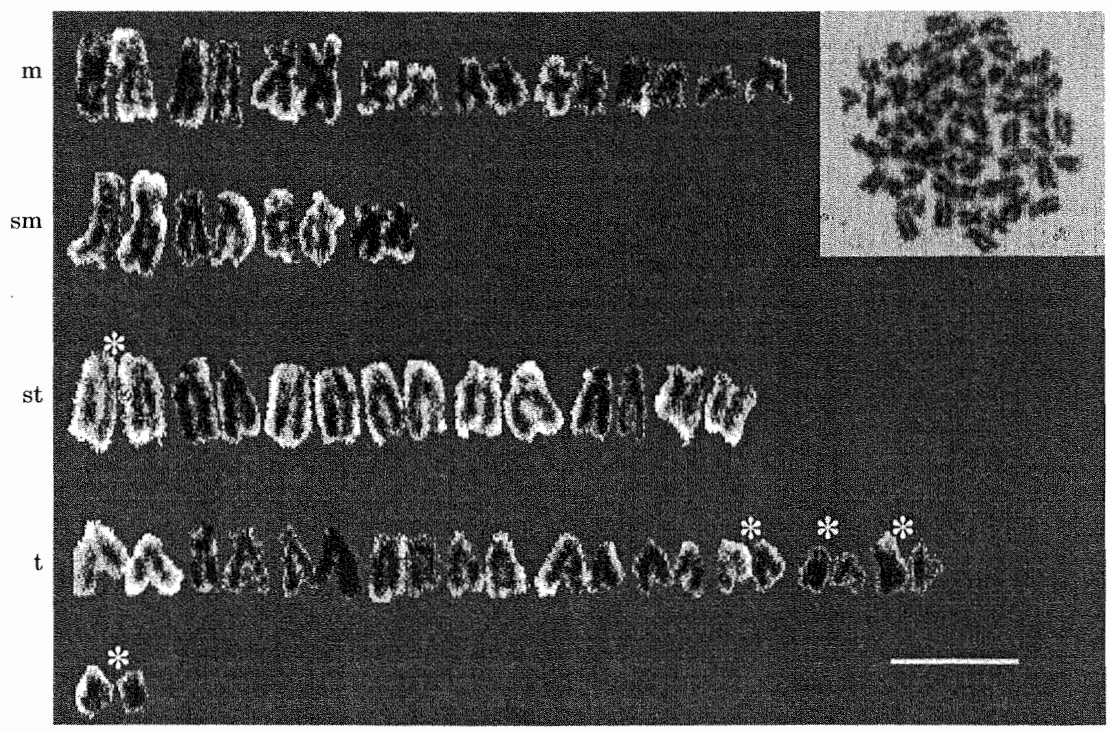

FIG. 2. Representative karyogram of black bullhead. Inset, the original metaphase spread. *Marker chromosomes. Bar=5 μm. m, Metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

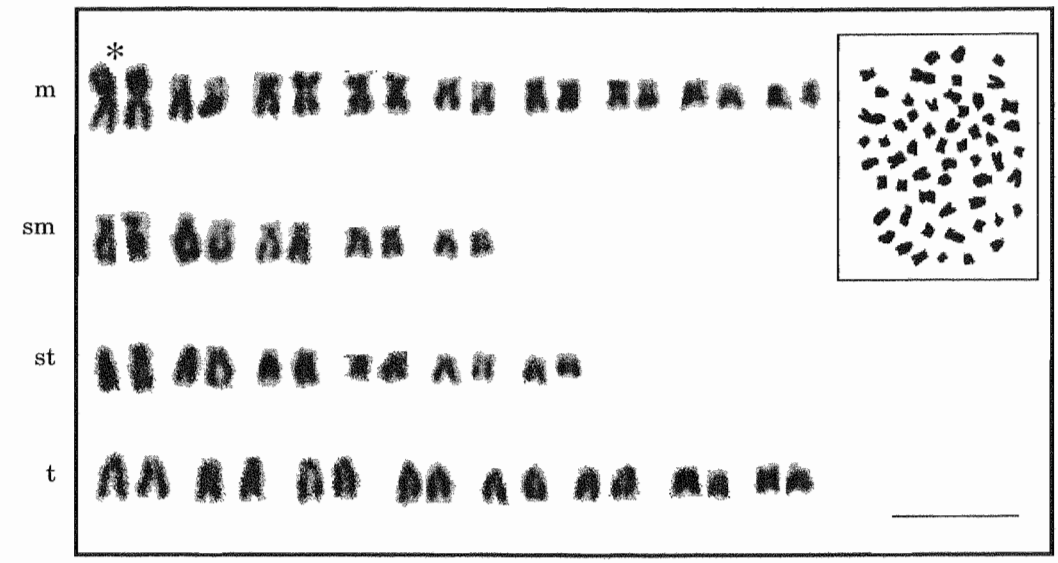

FIG. 3. Representative karyogram of flathead catfish. Inset: the original metaphase spread. *Marker chromosome. Bar=5 μm. m, Metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

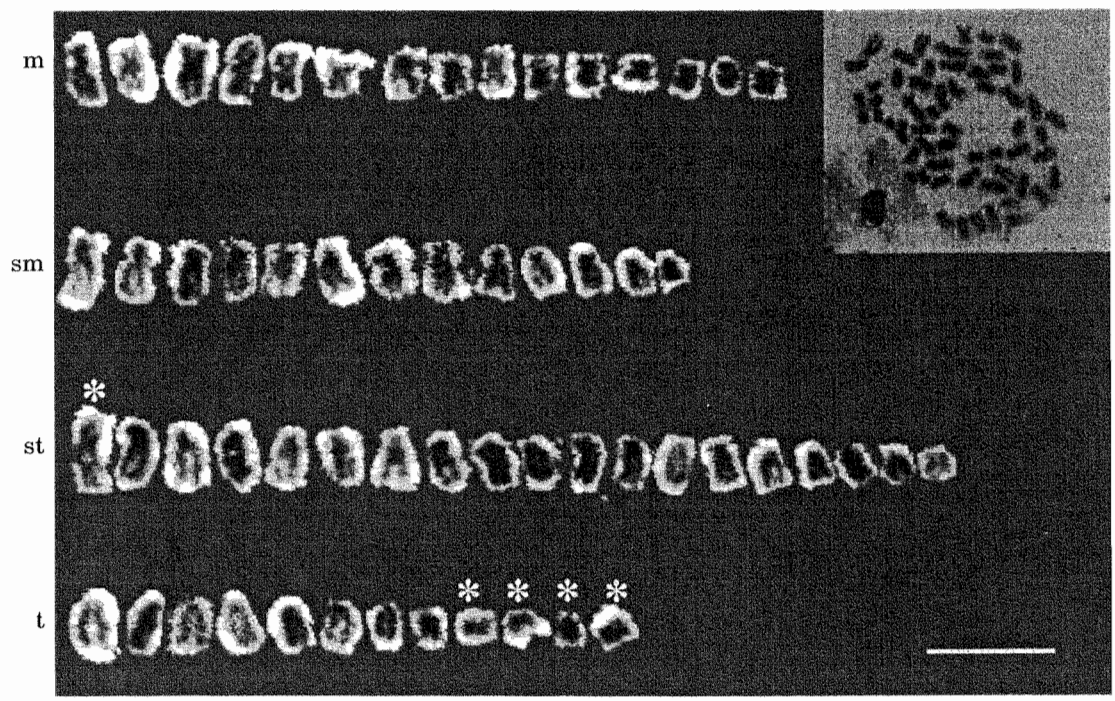

FIG. 4. Representative karyogram of channel catfish × black bullhead hybrid. Inset, the original metaphase spread. *Marker chromosomes from black bullhead. Bar=5 μm. m, Metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

phenotypes of channel catfish, black bullhead, flathead catfish, channel catfish × black bullhead hybrids, and channel catfish × flathead catfish hybrids are also described for the first time. The modal diploid chromosome numbers of parental species were found to be consistent with those reported by LeGrande (1981) and Wolters *et al.* (1981) for channel catfish, with those reported by

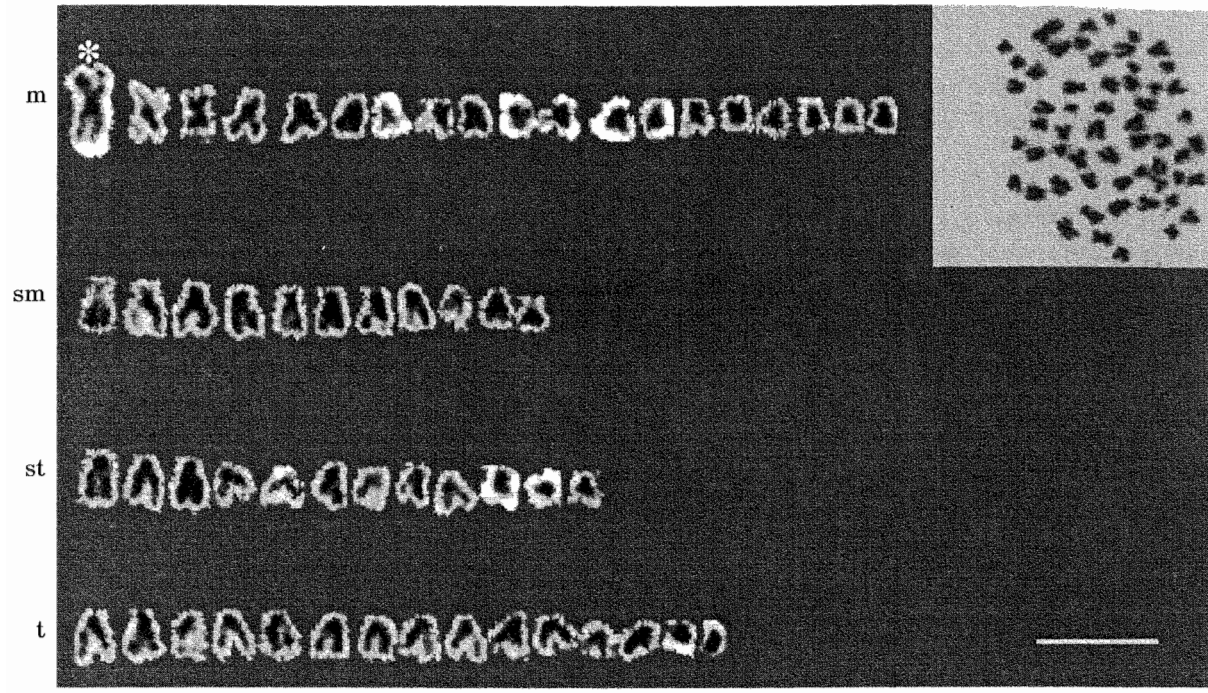

FIG. 5. Representative karyogram of channel catfish × flathead catfish hybrid. Inset, the original metaphase spread. *Marker chromosome (number 1) from flathead catfish. Bar=5 μm. m, Metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

Hudson (1976), LeGrande (1981), and Clark & Mathis (1982) for black bullhead, and with that reported by LeGrande (1981) for flathead catfish.

The haploid inheritance pattern was found to be common to intergeneric hybrids of the family Ictaluridae. The diploid numbers of the channel catfish × black bullhead hybrid and the channel catfish × flathead catfish hybrid were found to be the average of the diploid numbers of the parental species. The occurrences of each chromosome type (e.g. metacentric) in the genomes of the hybrids varied within two pairs of the averaged occurrence of these chromosome types in the parental species (our technical limit of detection). An intermediate diploid number ($2N=53$) was reported previously in hybrids of channel catfish and white catfish ($2N=48$), species that differ significantly in their chromosome numbers (LeGrande *et al.*, 1984). The genome sizes (diploid nuclear DNA content) of three different interspecific hybrids of ictalurid catfishes (channel catfish crossed with black bullhead, flathead catfish or blue catfish) were reported to be exactly intermediate to those of the parental groups (Tiersch & Goudie, 1993). The present study found no evidence for androgenesis, gynogenesis, polyploidy or aneuploidy in the hybrids. Taken together, this information suggests that the genome of ictalurid catfishes segregates as a function of haploid chromosome number and DNA content, and is stable in interspecific and intergeneric hybrids.

The measurements of chromosomal length and centromere location yielded detailed information of the parental and hybrid genomes. The accuracy and repeatability of these data were improved with the use of image analysis. The hybrid karyograms in this study displayed features in arm number (87 for each hybrid) and relative chromosome sizes (1·6–5·7% for the channel catfish × black

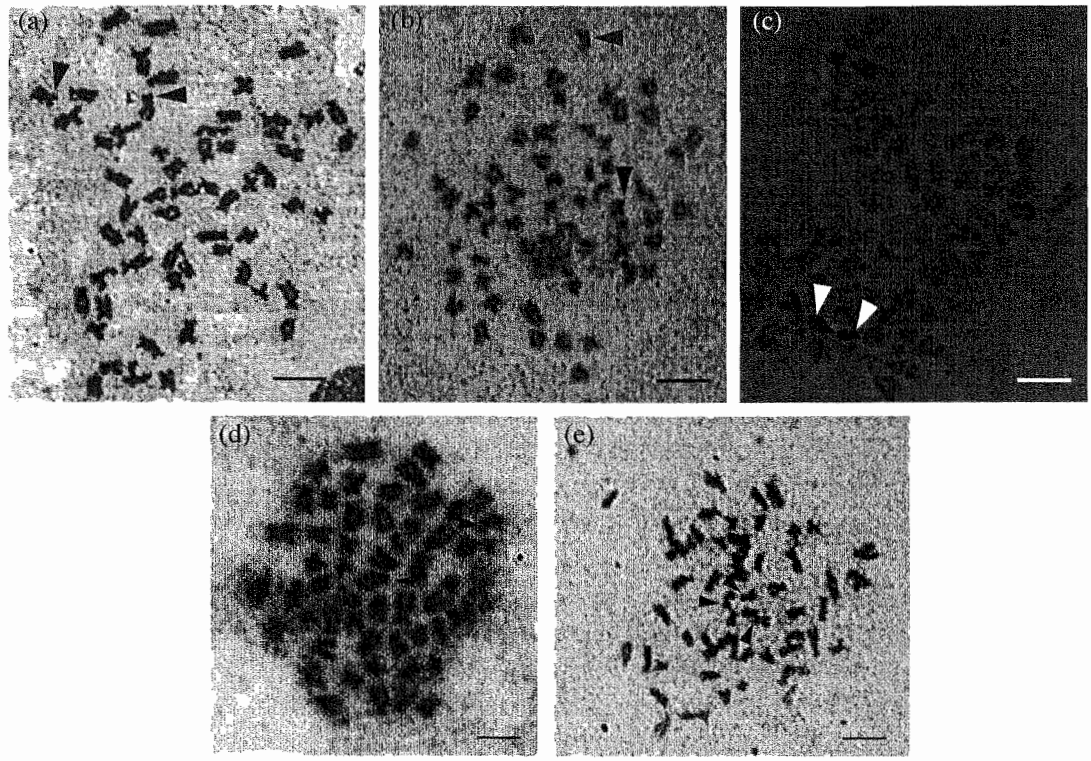

FIG. 6. Silver-stained metaphase spreads of (a) channel catfish, (b) black bullhead, (c) flathead catfish, (d) channel catfish × black bullhead, and (e) channel catfish × flathead catfish. Nucleolus organizer regions of chromosomes are indicated by arrowheads. Bar = 5 μm.

bullhead hybrid, and 1·4–7·4% for the channel catfish × flathead catfish hybrid) distinct from those observed in the parental species. This provides evidence supporting a balanced and stable pattern of chromosome segregation in hybrid ictalurid catfishes. Thus, chromosome number could be used as a method for verification in cases of suspected hybrid identity, although study of arm number, relative chromosome size, and marker chromosomes (e.g. Li *et al.*, 1991) would provide a more definitive identification of hybrid karyograms. This is especially pertinent to the present study because the diploid numbers of the hybrid fishes deviated only by one chromosome from those of the parental species, and chromosome counts can be influenced by preparation technique.

Marker chromosomes were identified based on computation of long-arm to short-arm ratios and the relative sizes of individual chromosomes. These chromosomes identified in the genomes of flathead and black bullhead catfish have distinctive features in size and centromeric position. No comparable chromosomes could be found in the genome of channel catfish in our study or in published reports for this species (LeGrande, 1981; LeGrande *et al.*, 1984). The NORs of parental fish and their hybrids provided additional features for analysis of hybrid genomes. The unpaired and heteromorphic NORs in the channel catfish × black bullhead hybrid were located on a medium-sized submetacentric (presumably originating from the channel catfish), and a small submetacentric (black bullhead). In the channel catfish × flathead catfish hybrid, one medium-sized NOR-bearing submetacentric was presumably contributed by the channel catfish, and the other medium-sized submetacentric was contributed by the

flathead catfish. The source of the additional NOR-bearing medium-sized subtelocentric, however, remains unknown. Because the target molecules of silver staining are proteins associated with transcriptional activity of ribosomal RNA genes, variation in NOR phenotypes can be caused by the functional state of NOR sites. Non-dysjunctional segregation can also lead to the additional NOR chromosome observed in the hybrid genome of the channel catfish × flathead catfish cross, as reported in the hybrid of *Cirrhinus molitorella* × *Sinilabeo decorus* (Zhang *et al.*, 1984). The assignment of specific identity to most of the chromosomes in the hybrid karyograms will require development of techniques for differential chromosome banding or *in situ* hybridization with DNA probes, but unfortunately, these techniques are not available at this time for most fish species, including catfishes.

The chromosomal differences between channel catfish and black bullhead, or channel catfish and flathead catfish were not only in chromosome number, but also in arm number and distribution of relative chromosome sizes. Thus chromosome pairing during meiosis could be affected in F_1 hybrids and result in the production of aneuploid gametes. This, in turn, could result in sterility of F_1 individuals. Indirect evidence, such as skewed sex ratios, has been reported in hybrids of channel catfish × flathead catfish (Goudie *et al.*, 1993), and channel catfish × white catfish (LeGrande *et al.*, 1984). Assessment of the effects of aberrant chromosome pairing on fertility will require study of meiotic chromosomes and breeding trials.

The production of sterile hybrids could benefit study and application of gene transfer in catfish species. Sterility can reduce the potential for inadvertent transfer of foreign or altered genes into wild populations. Sterile hybrid catfishes could supplement recreational fisheries for stocking in natural environments. With respect to aquaculture, the production of hybrid catfishes has been studied for more than three decades for improvement of traits such as growth rate (Giudice, 1966). Interspecific hybridization has been identified as a method for introduction of specific characters such as disease resistance (Wolters *et al.*, 1996), into the genome of cultured catfishes. Currently, most studies have focused on hybrids between channel catfish female and other ictalurid species. The potential for generating desirable culture traits through reciprocal crosses (channel catfish male × other ictalurid species) or crosses between species other than channel catfish needs to be explored.

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