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The cover. Hybrid half-sibling catfish produced from eggs of a channel catfish (*Ictalurus punctatus*) and sperm from males representing two genera. In each cross the species of the female parent is given first, followed by species of male. Top: channel catfish (*I. punctatus*) × flathead catfish (*Pylodictis olivaris*). Bottom: channel catfish (*I. punctatus*) × blue catfish (*I. furcatus*). See "Inheritance and Variation of Genome Size in Half-Sib Families of Hybrid Catfishes" by T.R. Tiersch and C.A. Goudie, pp. 122–125. Photo by D.F. Oberle.

Inheritance and Variation of Genome Size in Half-Sib Families of Hybrid Catfishes

T. R. Tiersch and C. A. Goudie

We used high-resolution flow cytometry to study variation in genome size within half-sibling families of hybrid catfishes produced from parents representing three genera of the family Ictaluridae. Genome size of hybrid offspring was exactly intermediate to the genome sizes of parental stocks, indicating that nuclear DNA segregates as a function of haploid DNA content and is stable within intergeneric hybrids. Thus, measuring genome size could provide a useful method for identifying hybrids within natural populations of fish or other organisms. Within-group variation in genome size was less than 2.5% for all groups studied, including outbred parental stocks. These values may represent the minimal level of genome size variation detectable within populations by analysis of interphase nuclei. The variation in genome size of ictalurid catfishes is small compared to variation observed in other vertebrates, and may be due to evolutionary conservatism within the catfish genome or to the appearance in other taxa of mechanisms that generate variation in DNA mass.

Genome size (nuclear DNA content) has been estimated for hundreds of plant and animal species. Large variation has been reported within taxa, for example, from less than 1 to more than 125 pg per diploid nucleus among angiosperms (Price 1988), and from less than 1 to more than 200 pg among vertebrates (Olmo et al. 1989). Greatest attention has been given to variation found above the species level. Within-species variation in genome size has been studied in plants, but is not well studied in vertebrates, a group for which the variation among sibiings and the inheritance of genome size are virtually unexplored.

Catfishes of the family Ictaluridae are native to North America and include the channel catfish (*Ictalurus punctatus*), which has become a leading species in the American aquaculture industry and is the only species in the family for which intraspecific variation in genome size has been studied (Tiersch et al. 1990). Hybridization of channel catfish with other catfish species can be accomplished in the laboratory by artificial fertilization. By using several fathers or mothers of different species, half-sib families of different hybrid crosses can be produced, providing unique opportunities for the study of inheritance.

In the study reported here, we evaluated genome size in one intraspecific cross and three interspecific hybrid crosses produced by fertilizing eggs from channel catfish with sperm from males of four species: channel catfish, blue catfish (*I. furcatus*), black bullhead catfish (*Ameiurus melas*), and flathead catfish (*Pylodictis olivaris*). We sought to (a) examine the inheritance of genome size in hybrid catfish produced from parents of different species and genome sizes and (b) evaluate variation of genome size within and among groups of hybrid half-siblings and representative parental stocks. Inheritance of genome size was surprisingly precise; the genome size of hybrid offspring was identical to the sum of the haploid DNA content of the parental species. Variation among half-siblings of any particular cross was minimal, and, overall, these values represent the lowest levels of within-population variation reported for any vertebrate group.

Materials and Methods

Spawning and Verification of Parentage

Half-sib hybrid families were produced by standard spawning procedures described in Goudie et al. (1992). We injected female channel catfish (from a commercial supplier in Mississippi) with human chorionic gonadotropin (1100 i.u./kg) to stimulate ovulation, then paired them with male channel catfish in 80-L aquaria supplied with aerated flow-through well water. Fish

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behavior was monitored, and, following initiation of spawning, females were removed from aquaria and anesthetized in 0.02% tricaine methanesulfonate. We stripped eggs by applying gentle pressure along the abdomen and fertilized them by continuously adding a mixture of sperm obtained from a male of each of the four catfish species (described in Goudie et al., in press). We incubated developing embryos in 8-L hatching troughs and then moved them to 80-L tanks after yolk-sac absorption and initiation of feeding. When two half-sibling groups (from unrelated mothers) were 4 months old, we classified them by external morphology as being channel catfish or one of the three possible hybrid combinations. Protein electrophoresis, performed according to the methods of Liu et al. (1992) was used to verify the morphological classifications.

Flow Cytometry

The two families, consisting of half-siblings and corresponding parental stocks, were analyzed separately. We collected blood samples in sodium citrate (Becton-Dickinson vacutainer 4606), then refrigerated, coded, and interspersed them systematically (Hurlbert 1984), and analyzed them as described previously (Tiersch et al. 1989). Blood cells of the catfish under study and blood cells of triploid hybrid (*I. punctatus* × *I. furcatus*) catfish (used as an internal reference) were suspended as a mixture in 0.5 ml of lysis-staining buffer containing 25 μg buffered RNase, 0.1% sodium citrate, 0.1% Triton X100, and 25 μg propidium iodide.

We estimated DNA content of the cells with a PROFILE flow cytometer (Coulter Electronics, Hialeah, Florida), with the argon-ion laser operated at a wavelength of 488 nm. Fluorescence values of at least 40,000 propidium-iodide-stained nuclei were digitized individually and used to calculate DNA content in relation to a value of 7 pg DNA assigned for fresh human leukocytes. In each test the value of the internal reference was canceled during the calculation of DNA content, according to the following formula: nuclear DNA (pg) $= 7 \times C/R \times R/H$, where C, R, and H are, respectively, the fluorescence values for the nuclei of catfish, internal reference, and human.

Nuclear Volume

We measured nuclear volume for two parental species and the hybrid offspring. Blood samples from *I. punctatus*, *A. melas*, and *I. punctatus* × *A. melas* hybrids were collected in heparinized capillary tubes, and cells were suspended in 10 ml of buffered saline containing 1 drop of lysing reagent (Baxter B3157-15). We estimated nuclear volume using a Coulter Multisizer (Coulter Electronics, Hialeah, Florida) equipped with a 70-μm orifice, and calibrated with five sizes of latex microspheres ranging from 2.02 to 42.16 μm in diameter. We used volume estimates of at least 20,000 nuclei from each animal to generate frequency histograms that supplied mode (peak channel) values.

Data Analysis

The offspring of the channel catfish × channel catfish cross represented their parents in comparisons with their half-siblings. Wild-caught flathead catfish (from the Mississippi River), and laboratory stocks of blue catfish (from the Mississippi River) and black bullhead catfish (from Spirit Lake, Iowa) were the sources of the males that provided sperm, and were used to represent the parental species in comparisons with hybrids.

We compared values of genome size for members of each family and for representative parental stocks by one-way analysis of variance (StatView 512+, software for Macintosh, Brainpower Inc.) and Scheffe's multiple range test; $P < .05$ was chosen as the level for statistical significance. The same procedures were used for comparisons of nuclear volume.

Results

The grand mean of all values of genome size observed in this study ($N = 204$) was 2.112 ± 0.047 (±SD) pg DNA (Table 1). The range between the lowest (2.027 pg) and highest (2.233 pg) values was 0.206 pg, or 9.75% of the mean. Values for Family 1 and parental stocks (2.119 ± 0.047 pg) were significantly larger (ANOVA, $F_{203} = 4.47$, $P = .0358$) than the values for Family 2 and parental stocks (2.105 ± 0.046). Comparison of the DNA values of six flathead catfish included in both analyses revealed that, on average, values from the first analysis were 0.008 pg (0.37%) greater than the values obtained for the same fish in the second analysis, which suggests a small but systematic difference between the values of the two analyses.

Values for genome size were tightly clustered for each hybrid or parental group and were significantly different within each family (ANOVA, $F_{6,101} > 359.53$, $P < .001$ for each). The range of values was less than 0.2 pg per group within each analysis, allowing all groups within an analysis to be differentiated from each other (Table 1) with only one exception: in Family 1, values for flathead catfish were not different from those of the channel × black bullhead hybrids.

The genome size of offspring of the three hybrid combinations in each family was intermediate to the genome sizes of the parental stocks (Table 2). The mean difference (±SD) between predicted genome size of the hybrids, calculated as the sum of the mean parental values divided by two, and the actual genome size of the hybrids was 0.002 pg, or about 0.1% for all groups.

Variation in genome size for the hybrid and parental groups of each family ranged from 1.16% to 2.32%, in relation to the mean (Table 3). The difference between the smallest range (0.024 pg) of genome size values for a group and the largest range (0.051 pg) was 0.027 pg.

With respect to nuclear volume, the nuclei of blood cells of black bullhead catfish were 12.4% larger than those of channel catfish (Table 4). The nuclear volume of hybrids was intermediate to the nuclear volumes of the parental stocks, and within 0.06 μm^3 (0.56%) of the predicted value. Values for genome size were more precise than values for nuclear volume, in which within-group variation ranged from 21.7% of the mean (black bullheads) to 24.2% (channel catfish).

Discussion

In contrast to the situation described for hybridization of some plants and animals, there seems to be strong fidelity in the inheritance of DNA content in hybrid catfish. Price et al. (1983) reported that values for genome size of offspring were scattered and did not cluster around the midpoint of the values of the parental species in crosses between two plant species of the genus *Microseris*. In similar fashion, increased within-individual variation in cellular DNA content, as measured by the percentage coefficient of variation (%CV), was reported for intraspecific hybrids collected from a contact zone between two populations of the white-footed mouse (*Peromyscus leucopus*) (Baker et al. 1991). It was suggested in the above studies that when parental genomes are combined in a hybrid, rearrangements such as amplification, deletion, or translocation of certain (perhaps transposable) DNA sequences could be triggered. We found no evidence for this in our study. Genome size

Table 1. Genome size (diploid nuclear DNA content, or pg DNA) of half-sibling hybrid catfish and parental stocks

Group	Family 1 N	Family 1 Mean ± SD	Family 1 Min.-Max.[a]	Family 2 N	Family 2 Mean ± SD	Family 2 Min.-Max.[a]
Parental species						
Channel catfish	16	2.092 ± 0.008a	2.080-2.113	15	2.083 ± 0.008a	2.073-2.099
Blue catfish	15	2.057 ± 0.012b	2.043-2.088	15	2.041 ± 0.006b	2.027-2.053
Black bullhead catfish	15	2.200 ± 0.012c	2.182-2.233	15	2.180 ± 0.007c	2.164-2.193
Flathead catfish	12	2.148 ± 0.009d	2.132-2.166	12	2.141 ± 0.010d	2.125-2.164
Hybrids						
Channel catfish × blue catfish	14	2.076 ± 0.007e	2.061-2.085	15	2.060 ± 0.008e	2.048-2.073
Channel catfish × black bullhead catfish	15	2.146 ± 0.009d	2.132-2.167	15	2.127 ± 0.011f	2.110-2.140
Channel catfish × flathead catfish	15	2.118 ± 0.011f	2.096-2.144	15	2.110 ± 0.011g	2.096-2.130
Mean		2.119 ± 0.047			2.105 ± 0.046	
Grand mean			2.112 ± 0.047			

Homogeneous groupings were made by Scheffe's multiple range test; values sharing a letter within a family were not significantly different.
[a] Minimum and maximum values observed for sample.

Table 2. Predicted and actual values of genome size (pg DNA) for half-sibling hybrid catfish

Group	Family 1 Predicted[a]	Family 1 Actual	Family 2 Predicted[a]	Family 2 Actual
Channel catfish × blue catfish	2.074	2.076	2.062	2.060
Channel catfish × black bullhead catfish	2.147	2.146	2.132	2.127
Channel catfish × flathead catfish	2.120	2.118	2.112	2.110

[a] Predicted values were obtained by dividing by two the sum of the mean values of the parental species.

Table 3. Variation in genome size (pg) observed among half-sibling hybrid catfish and parental stocks

Group	Family 1 N	Family 1 Range (pg)	Family 1 Difference (%)[a]	Family 2 N	Family 2 Range (pg)	Family 2 Difference (%)[a]
Parental species						
Channel catfish	16	0.033	1.58	15	0.026	1.25
Blue catfish	15	0.045	2.19	15	0.026	1.27
Black bullhead catfish	15	0.051	2.32	15	0.029	1.33
Flathead catfish	12	0.034	1.58	12	0.039	1.82
Hybrids						
Channel catfish × blue catfish	14	0.024	1.16	15	0.025	1.21
Channel catfish × black bullhead catfish	15	0.035	1.63	15	0.030	1.41
Channel catfish × flathead catfish	15	0.048	2.27	15	0.034	1.61
Mean ± SD[b]		0.039	1.83		0.030	1.42

[a] Percentage of difference was calculated by dividing the range by the mean value of the sample.
[b] Mean ± SD for combined families was 0.034 ± 0.009 pg and 1.62% ± 0.40% difference.

variation of hybrid populations was not different from the variation of parental populations, and the values for %CV were low (mean ± SD, 2.52 ± 0.49; range, 1.82-3.91) and not different between hybrids and parental stocks.

In a study of minnows of the genus *Phoxinus* (Dawley and Goddard 1988), genome size of diploid hybrids (3.45 pg) was intermediate to the genome sizes of the parental species, *P. eos* (3.28 pg) and *P. neogaeus* (3.65 pg). This, in connection with the results reported in this article, would suggest that in fishes genome size segregates as a function of haploid DNA content and is stable in hybrids, despite the unbalanced condition that could result from intergeneric hybridization and chromosomal differences between parental species. In the fishes studied, genome size of offspring was unimodal and exactly intermediate to the values of the parental species, a finding that would be expected in organisms that lack heteromorphic sex chromosomes. Accordingly, inheritance of genome size would be expected to be bimodal in organisms with heteromorphic sex chromosomes (e.g., Tiersch and Mumme, in press). This idea is supported by the observation that spermatozoa can be sorted by flow cytometry based on the difference in DNA mass of the X and Y chromosomes (Johnson et al. 1987; Johnson and Pinkel 1986).

Chromosome numbers have been reported for channel (58), blue (58), black bullhead (60), and flathead (56) catfish (LeGrande 1981; LeGrande et al. 1984). In general, genome size in the study reported here seemed to be related to chromosome number, although the species with the second-largest genome (flathead catfish) had the fewest chromosomes. Hybrid offspring of the channel catfish × blue catfish cross have 58 chromosomes (LeGrande et al. 1984). The number of chromosomes of the other hybrid combinations in this study is unknown, but it could be predicted, based on the inheritance of genome size, that the progeny of the channel catfish × flathead catfish cross would have 57 chromosomes, and the progeny of the channel catfish × black bullhead catfish cross would have 59 chromosomes. It should be noted that in crosses between channel catfish and *A. catus*—the white catfish, which has 48 chromosomes—the hybrid offspring had 53 chromosomes, a number intermediate to the parental numbers (LeGrande et al. 1984).

Intraspecific variation in genome size of ictalurid catfishes is low in comparison to the variation observed in other vertebrates. Variation was less than 2.5% for over 100 fish representing 14 populations of channel catfish (Tiersch et al. 1990), and was less than 2.5% for all stocks examined in this study, which included outbred populations of blue, black bullhead, and flathead catfish. Yet, intraspecific variation, as measured by microdensitometry of Feulgen-stained nuclei, averaged 4.86% for 49 species of minnows of the family Cyprinidae (Gold et al. 1990), and variation averaged 10.6% for 19 populations of 17 species of fish of the family Salmonidae (reviewed in Lockwood and Derr 1992).

Variation within eight species of neo-

Table 4. Predicted and actual values for nuclear volume (μm^3) of channel catfish, black bullhead catfish, and their hybrid progeny

Species	*N*	Mean ± SD	Min.-Max.[b]
Channel catfish	15	9.92 ± 0.66	9.17-11.57
Black bullhead catfish	15	11.32 ± 0.71	10.12-12.58
Hybrid			
Predicted[a]		10.62	
Actual	15	10.68 ± 0.66	9.43 ± 11.94

[a] Predicted values were obtained by dividing by two the sum of the mean values of the parental species.
[b] Minimum and maximum values observed.

tropical bats averaged 21.7% (Burton et al. 1989), and variation as large as 35% was observed within species of pocket gophers of the genus *Thomomys* (Sherwood and Patton 1982). Moreover, the average variation in genome size attributable to sex alone was greater than 2.5% between male and female of 29 species of birds with heteromorphic sex chromosomes (Nakamura et al. 1990). Conservatism during evolutionary history could account for the low variation in genome size observed for ictalurid catfishes in relation to other taxa. Alternatively, mechanisms such as differential accumulation and loss of heterochromatin (such as in minnows and pocket gophers), diploidization of a recently duplicated, tetraploid genome (as in salmonids), or development of heteromorphic sex chromosomes (as in birds) could serve to generate variation in genome size higher than that observed in catfishes.

Our use of half-sib families might have contributed to the low level of variation observed in this study. Based on observations of thousands of animals from hundreds of species, we suggest that these values represent the minimal level of genome size variation detectable within populations by analysis of interphase nuclei by flow cytometry. In addition, nuclear volume, estimated by a technique with markedly lower resolution than flow cytometry, confirmed our observation of intermediate genome size in hybrids and suggests a strong relationship, even in intergeneric hybrids, between the size of the genome and the volume of the nucleus.

References

Baker RJ, Porter CA, Hanks BG, and Bickham JW, 1991 Increased variation in cellular DNA content at a hybrid zone: hybrid breakdown in *Peromyscus leucopus*. J Hered 82:27-30.

Burton DW, Bickham JW, and Genoways HH, 1989. Flow-cytometric analyses of nuclear DNA content in four families of neotropical bats. Evolution 43:756-765.

Dawley RM and Goddard KA, 1988. Diploid-triploid mosaics among unisexual hybrids of the minnows *Phoxinus eos* and *Phoxinus neogaeus*. Evolution 42:649-659.

Gold JR, Ragland CJ, and Schliesing LJ, 1990. Genome size variation and evolution in North American cyprinid fishes. Genet Sel Evol 22:11-29.

Goudie CA, Simco BA, Davis KB, and Parker NC, 1992. Reproductive performance of pigmented and albino female channel catfish induced to spawn with HCG or ovaprim. J World Aquacult Soc 23:138-175.

Goudie CA, Tiersch TR, Simco BA, Davis KB, and Liu Q, in press. Early growth and morphology among hybrids of ictalurid catfishes. J Applied Aquacult.

Hurlbert SH, 1984. Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54:187-211.

Johnson LA, Flook JP, Look MV, and Pinkel D, 1987. Flow sorting of X and Y chromosome bearing spermatozoa into two populations. Gamete Res 16:1-9.

Johnson LA and Pinkel D, 1986. Modification of a laser-based flow cytometer for high-resolution DNA analysis of mammalian spermatozoa. Cytometry 7:268-273.

LeGrande WH, 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtoms, *Noturus*. Copeia 1981:33-52.

LeGrande WH, Dunham RA, and Smitherman RO, 1984. Karyology of three species of catfishes (Ictaluridae: *Ictalurus*) and four hybrid combinations. Copeia 1984: 873-878.

Liu Q, Goudie CA, Simco BA, Davis KB, and Morizot DC, 1992. Gene-centromere mapping of six enzyme loci in gynogenetic channel catfish. J Hered 83:245-248.

Lockwood SF and Derr JN, 1992. Intra- and interspecific genome-size variation in the Salmonidae. Cytogenet Cell Genet 59:303-306.

Nakamura D, Tiersch TR, Douglass M, and Chandler RW, 1990. Rapid identification of sex in birds by flow cytometry. Cytogenet Cell Genet 53:201-205.

Olmo E, Capriglione T, and Odierna G, 1989. Genome size evolution in vertebrates: trends and constraints. Comp Biochem Physiol (B) 92:447-453.

Price HJ, 1988. Nuclear DNA content variation within angiosperm species. Evol Trends Plants 2:53-60.

Price HJ, Chambers KL, Bachmann K, and Riggs J, 1983. Inheritance of nuclear 2C DNA content variation in intraspecific and interspecific hybrids of Microseris (Asteraceae). Am J Bot 70:1133-1138.

Sherwood SW and Patton JL, 1982. Genome evolution in pocket gophers (genus *Thomomys*): II. Variation in cellular DNA content. Chromosoma 85:163-179.

Tiersch TR, Chandler RW, Wachtel SS, and Elias S, 1989. Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry 10:706-710.

Tiersch TR and Mumme RL, in press. An evaluation of the use of flow cytometry to identify sex in the Florida scrub jay. J Field Ornithol.

Tiersch TR, Simco BA, Davis KB, Chandler RW, Wachtel SS, and Carmichael GJ, 1990. Stability of genome size among stocks of the channel catfish. Aquaculture 87:15-22.