Aquaculture, 87 (1990) 15–22
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Stability of Genome Size Among Stocks of the Channel Catfish

TERRENCE R. TIERSCH[a], BILL A. SIMCO[a], KENNETH B. DAVIS[a], ROBERT W. CHANDLER[b,*], STEPHEN S. WACHTEL[b] and GARY J. CARMICHAEL[c]

[a]*Ecological Research Center, Department of Biology, Memphis State University, Memphis, TN 38152 (U.S.A.)*
[b]*Division of Reproductive Genetics, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, TN 38163 (U.S.A.)*
[c]*U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Stoneville, MS 38776 (U.S.A.)*

(Accepted 1 September 1989)

ABSTRACT

Tiersch, T.R., Simco, B.A., Davis, K.B., Chandler, R.W., Wachtel, S.S. and Carmichael, G.J., 1990. Stability of genome size among stocks of the channel catfish. *Aquaculture*, 87: 15–22.

Nuclear DNA content of erythrocytes from male and female channel catfish (*Ictalurus punctatus*) was determined by flow cytometry. Fourteen stocks of catfish were studied ($n=115$), including domesticated and wild fish, fish from sex-reversed populations and those produced by gynogenesis. Mean DNA content was 1.977 ± 0.010 (SD) pg DNA per cell, and mean within-stock variation was 1.28%. The stocks had an average difference of 0.19% from the species mean; no significant differences in DNA content were detected among the stocks or between males and females. The intraspecific variation among the channel catfish stocks examined is lower than that reported for other fish species. This may reflect artificial stabilization of genome size by human intervention, or alternatively, evolutionary conservatism within the genome of the channel catfish.

INTRODUCTION

Channel catfish (*Ictalurus punctatus*) is the major species of fish cultured for food production in North America. The native distribution of channel catfish included a broad geographic region, encompassing the central United States from southern Canada to northern Mexico and from the Rocky Mountains in the West to the Appalachians in the East (Glodek, 1980). These fish have been introduced elsewhere, including the east and west coasts of North America and several other continents (Hallerman et al., 1986). Native northern and south-

*Present address: Puckett Laboratory, 4200 Mamie St., Hattiesburg, MS 39402 (U.S.A.).

0044-8486/90/$03.50

TABLE 1

Source of experimental animals, origin of channel catfish stocks and date of recorded domestication. Information concerning domestication dates and sources is from Dunham and Smitherman (1984) and from personal communications with the suppliers of the stock

Stock	Source of channel catfish	Geographic origin (date of domestication)
Auburn	Auburn University, Auburn, AL	Rivers in AR, KS, TX and OK (1956)
Albino-Florida	U.S. Fish and Wildlife Service, Marion, AL	Richloam State Fish Hatchery, Webster, FL (unknown)
Albino-Tifton	U.S. Fish and Wildlife Service, Marion, AL	Tifton, GA (1969)
(Auburn × Rio Grande) × (Marion × Kansas)	Auburn University, Auburn, AL	Rio Grande River, TX (1970); other parental stocks as given
Kansas	Auburn University, Auburn, AL	Ninnescah River, Kansas State Fish Hatchery, Pratt (1911)
Marion	Auburn University, Auburn, AL	Rivers in AL, Marion National Fish Hatchery, AL (1970)
Minnesota × Kansas	Auburn University, Auburn, AL	St. Louis River, MN (unknown) and Ninnescah River, KS (1911)
Mississippi	U.S. Dept. of Agriculture, Stoneville, MS	Mississippi River, commercial aquacultural strain (unknown)
Norris	U.S. Dept. of Agriculture, Stoneville, MS	Black River, AR and Lake Erie, OH (1963)
Red River	U.S. Dept. of Agriculture, Stoneville, MS	Red River, ND (1988)
Santee-Cooper	Auburn University, Auburn, AL	Santee-Cooper River, SC (1975)
Tishomingo	Auburn University, Auburn, AL	Various rivers, OK (1930s)
Gynogenetic	U.S. Fish and Wildlife Service, Marion, AL	Produced by meiotic cold shock (1986)
Sex-reversed	U.S. Fish and Wildlife Service, Marion, AL	Produced by androgen treatment of fry (1980)

ern populations of channel catfish differ in performance traits such as annual spawning period and age at sexual maturity (Broussard and Stickney, 1981). Ashley et al. (1981) reported differences in back-calculated growth rates for channel catfish from widely separated geographic areas. Channel catfish have been domesticated for hatchery production for over 70 years (Hallerman et al., 1986) and various levels of artificial selection have been employed by fish breeders to obtain fish with desirable aquacultural traits (Dunham and Smitherman, 1987). Many stocks have been maintained and crossbred repeatedly with broodstock from wild and domesticated populations.

Intraspecific variation in nuclear DNA content (genome size) has been described in two fish families – salmonids (Johnson et al., 1987) and cyprinids (Gold and Amemiya, 1987). Flow cytometry has been used for quantification of nuclear DNA in fishes (Thorgaard et al., 1982; Allen, 1983; Johnson et al., 1987; Gold et al., 1988), and has been used routinely to discriminate between males and females of human and horse on the basis of small differences in DNA content in the sex chromosomes (Elias et al., 1988; Kent et al., 1988).

Protein electrophoresis has been used to characterize strains or stocks of channel catfish that may vary in mean heterozygosity, percentage of polymorphic loci and number of alleles per locus (Hallerman et al., 1986). However, variation in genome size has not been determined in these fish. The objectives of the present study were to measure, by flow cytometry, the nuclear DNA content in male and female channel catfish, and to characterize the degree of intraspecific variation in genome size among wild, domestic, gynogenetic and sex-reversed stocks.

MATERIALS AND METHODS

Fourteen stocks of channel catfish ($n=115$) were examined (Table 1). Twelve of these stocks originated in a large geographic range representing much of the native distribution of the channel catfish. Two other stocks were produced from fish of unknown geographic origin. Fish from an all-female population were gynogenetically produced from cold-shocked eggs that had been activated with irradiated milt. The cold shock was administered at the time of meiosis II (C.A. Goudie et al., unpublished data). The other unspecified stock consisted of sex-reversed, "XY-type" phenotypic females produced by feeding androgen to swim-up fry (Goudie et al., 1983; Davis et al., in press).

Blood samples, collected by caudal puncture, were refrigerated until analysis. Channel catfish erythrocytes were mixed with those from domestic chicken (*Gallus gallus*) in 0.5 ml of a 50-mg/l solution of propidium iodide containing 25 μl RNAase (1.0 mg/ml), 0.1% sodium citrate, and 0.1% Triton X-100. After mixing, the samples were filtered through a 20-μm nylon mesh and were analyzed within 15 min.

Samples were analyzed in randomized order with a Coulter EPICS V flow

cytometer according to Tiersch et al. (1989). The Coulter PARA 1 program (Coulter Electronics, Hialeah, FL, U.S.A.) was used to calculate the fractional mode channel to four decimal places for the fluorescence peaks of each chicken and catfish mixture. Nuclear DNA content of channel catfish was expressed in relation to an assigned value of 7.0 pg DNA per cell for fresh human leukocytes (based on Shapiro, 1976) analyzed as a mixture with the chicken cells. Nuclear DNA content was calculated according to the formula

$$\text{pg DNA} = 7.0\ (A/B)(C/D)$$

where A, B, C, and D are fractional mode channel values, and B and C represent the value of the chicken internal reference analyzed as a mixture with (A) channel catfish or (D) fresh human leukocytes. The criteria for inclusion of histograms in data analyses were: (a) coefficient of variation at half-maximum height of the erythrocyte fluorescence peaks of less than 4%; and (b) no overlap of erythrocyte histogram peaks large enough to affect the fractional mode channels.

Statistical analyses comparing DNA content values among stocks and between the sexes of the various stocks were performed using one-way analysis of variance and the non-parametric Mann–Whitney U test; $P < 0.05$ was chosen as the level of statistical significance.

RESULTS

Nuclear DNA content averaged 1.977 ± 0.010 (SD) pg per cell (Table 2), and the difference between the individuals with the highest DNA content (1.995

TABLE 2

Mean (± SE) and range of nuclear DNA content (pg per cell) in 14 stocks of channel catfish

Stock	n	DNA content	Range
Auburn	5	1.984 (0.005)	1.967-1.995
Sex-reversed	2	1.984 (0.010)	1.974-1.994
Kansas	10	1.981 (0.003)	1.963-1.993
(Auburn × Rio Grande) × (Marion × Kansas)	7	1.980 (0.002)	1.973-1.990
Red River	10	1.979 (0.003)	1.967-1.994
Albino-Tifton	10	1.979 (0.004)	1.962-1.995
Tishomingo	6	1.978 (0.002)	1.972-1.986
Marion	10	1.978 (0.003)	1.964-1.992
Norris	10	1.978 (0.002)	1.970-1.987
Santee-Cooper	11	1.976 (0.003)	1.962-1.990
Mississippi	9	1.973 (0.004)	1.957-1.990
Albino-Florida	10	1.972 (0.004)	1.956-1.988
Gynogenetic	5	1.972 (0.008)	1.956-2.002
Minnesota × Kansas	10	1.970 (0.003)	1.951-1.978

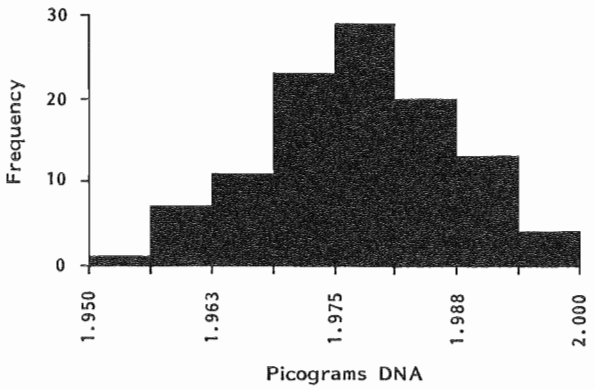

Fig. 1. Frequency distribution of nuclear DNA values for 108 channel catfish from 12 diverse stocks.

pg) and the lowest (1.951 pg) was 2.26% of the grand mean for the species. Gynogenetic and sex-reversed fish were excluded from these numbers. The average difference from the grand mean of the species was 0.19% among all stocks; no significant differences were detected among the stocks. Within-stock variations (as expressed by range) averaged 1.28% of the stock mean. The sex-reversed XY females averaged 1.984 ± 0.013 (SD) pg of DNA per cell. Gynogenetic fish had an average of 1.972 ± 0.019 (SD) pg of DNA per cell.

No difference was observed in DNA content of male and female cells for all the stocks combined, or for any individual stock. The values for DNA content for the combined male and female populations (excluding the gynogenetic and sex-reversed fish) were distributed normally (Fig. 1).

DISCUSSION

Other procedures have been used to estimate the nuclear DNA content of channel catfish erythrocytes, although the analysis of intraspecific variation was limited by small sample sizes. Muramoto et al. (1968), using microdensitometric analyses of Feulgen-stained nuclei, reported the DNA content of a single channel catfish as 30% of that of human lymphocytes (ca. 2.1 ± 0.02 (SD) pg DNA per cell). A similar value of 1.72 ± 0.27 pg DNA per cell for eight channel catfish was obtained by colorimetric analysis of erythrocyte DNA following perchloric acid extraction (Hudson, 1976). Whether the differences between our value (1.98 ± 0.01 (mean $\pm$ SD) pg DNA per cell) and the DNA content reported by other workers are due to actual differences in genome size or to disparate methodologies is problematic. The absence of sex differences in DNA content of channel catfish supports previous cytogenetic observations that they lack morphologically differentiated sex chromosomes (LeGrande, 1981; LeGrande et al., 1984).

Larger variations in genome size among the stocks examined might have been expected, given the intense artificial selection many channel catfish populations have undergone and the broad geographic range over which they are distributed. The Kansas stock, the oldest domesticated population, was established in 1911 (Dunham and Smitherman, 1987), yet the DNA content of the Kansas stock differs by less than 0.2% from DNA content in wild fish collected 77 years later from the Red River (North Dakota). Common practices such as crossing various populations (Dunham and Smitherman, 1987) and promiscuous introductions, may have resulted in a tendency toward "intermediate" DNA content in this species that has opposed any tendency for divergences normally associated with reproductive isolation, genetic drift or founder effects. Alternatively, the species has been conservative in stabilizing genome size over its evolutionary history. Examination of additional fish from wild and domesticated stocks could address this topic.

Channel catfish possess little variation in genome size compared to species of pocket gophers (Genus *Thomomys*) in which intraspecific differences as high as 35% have been reported (Sherwood and Patton, 1982). Variations of genome size of from 0.40% to 15.40% (with mean value of 5.49% $\pm$ 2.81% (SD)) have been reported in salmonids and cyprinids. In order to directly compare our results with the results of these studies, we calculated all values as the population range divided by the population mean, in accordance with the use of range as a measure of within-population variability in the previous studies. We calculated a mean of 4.81% within-population variation using the data from the study of 14 populations of 12 salmonid species by Johnson et al. (1987); and a mean of 5.76%, on the basis of the study of 20 populations ($n=10$, for each) of 20 cyprinid species by Gold and Amemiya (1987). In our study, the overall intraspecific variation was 2.26% of the grand species mean ($n=108$), based on combined data from 12 diverse stocks. Only two salmonid populations have been described with variation lower than the intraspecific variation we observed in channel catfish; and these populations had sample sizes of $n=3$ and $n=4$ (Johnson et al., 1987). Tetraploid animals, such as the salmonids, which are actively involved in "rediploidization" processes (Allendorf and Thorgaard, 1984), may have intrinsically high levels of genomic size variation. Certain cyprinids (Gold and Price, 1985) and pocket gophers (Sherwood and Patton, 1982) possess large variations in chromosomal heterochromatin content, which could explain the variability of genome size in these groups.

Because genomic size differences are subtle or absent among stocks of channel catfish, nuclear DNA content is not useful presently as a tool for intraspecific genetic studies. Estimation of nuclear DNA content may enable differentiation among channel catfish, other ictalurids, and interspecific hybrids. "Genomic size markers" might be created for stock identification through nonrandom mating of individual fish with high or low nuclear DNA content. Perhaps estimation of genomic size could then be used in combination with other

techniques for stock characterization as suggested by Johnson et al. (1987) for populations of kokanee salmon (*Oncorhynchus nerka*). The apparent stability of the genome of channel catfish may allow development of genetic tools to study this important aquacultural species.

ACKNOWLEDGEMENTS

The authors thank Nick Parker for providing fish from Marion, AL, and Rex Dunham, of Auburn University, for providing catfish and critically reading the manuscript. This work was supported in part by USDA grant 88-34123-3504, NIH grant AI-23479, and a Van Vleet Memorial fellowship (Memphis State University). We are grateful to Dawn James and Tammy Oliver for their help in preparing the manuscript.

REFERENCES

Allen, S.K., 1983. Flow cytometry: assaying experimental polyploid fish and shellfish. Aquaculture, 33: 317-328.

Allendorf, F.W. and Thorgaard, G.H., 1984. Tetraploidy and the evolution of salmonid fishes. In: B.J. Turner (Editor), Evolutionary Genetics of Fishes. Plenum Press, New York, NY, pp. 1-53.

Ashley, K.W., Garling, D.L. and Ney, J.J., 1981. Use of the age-length relationship as a marker for differentiating geographical stocks of adult channel catfish. North Am. J. Fish. Manage., 1: 77-79.

Broussard, M.C. and Stickney, R.R., 1981. Evaluation of reproductive characteristics for four strains of channel catfish. Trans. Am. Fish. Soc., 110: 502-506.

Davis, K.B., Simco, B.A., Goudie, C.A., Parker, N.C., Cauldwell, W. and Snellgrove, R., in press. Hormonal sex manipulation and evidence for female homogamety in channel catfish. Gen. Comp. Endrocrinol.

Dunham, R.A. and Smitherman, R.O., 1984. Ancestry and breeding of catfish in the United States. Ala. Agric. Exp. Stn., Auburn Univ., Circ. 273, Auburn, AL, 93 pp.

Dunham, R.A. and Smitherman, R.O. (Editors), 1987. Genetics and breeding of catfish. South. Coop. Ser. Bull. 325, Ala. Agric. Exp. Stn., Auburn Univ., Auburn, AL, 20 pp.

Elias, S., Chandler, R.W. and Wachtel, S., 1988. Relative DNA content of interphase leukocytes by flow cytometry: a method for indirect diagnosis of chromosomal abnormalities with potential for prenatal diagnosis. Am. J. Obstet. Gynecol., 158: 808-818.

Glodek, G.S., 1980. *Ictalurus punctatus* (Rafinesque), channel catfish. In: D.S. Lee et al. (Editors), Atlas of North American Freshwater Fishes. North Carolina State Mus. Nat. Hist., Raleigh, NC, p. 446.

Gold, J.R. and Amemiya, C.T., 1987. Genome size variation in North American minnows (Cyprinidae). II. Variation among 20 species. Genome, 29: 481-489.

Gold, J.R. and Price, H.J., 1985. Genome size variation among North American minnows (Cyprinidae). I. Distribution of the variation in five species. Heredity, 54: 297-305.

Gold, J.R., Amemiya, C.T., Karel, W.J. and Iida, N., 1988. The karyotype and genome structure of the pirate perch *Aphredoderus sayanus* (Aphredoderidae: Teleostei). Experientia, 44: 68-70.

Goudie, C.A., Redner, B.D., Simco, B.A. and Davis, K.B., 1983. Feminization of channel catfish by oral administration of steroid sex hormones. Trans. Am. Fish. Soc., 112: 670–672.

Hallerman, E.M., Dunham, R.A. and Smitherman, R.O., 1986. Selection or drift – isozyme allele frequency changes among channel catfish selected for rapid growth. Trans. Am. Fish. Soc., 115: 60–68.

Hudson, R.G., 1976. A comparison of karyotypes and erythrocyte DNA quantities of several species of catfish (Siluriformes) with phylogenetic implications. Unpublished PhD Dissertation, North Carolina State University, NC.

Johnson, O.W., Utter, F.M. and Rabinovitch, P.S., 1987. Interspecies differences in salmonid cellular DNA identified by flow cytometry. Copeia, 1987: 1001–1009.

Kent, M., Chandler, R.W. and Wachtel, S., 1988. DNA analysis by flow cytometry. Cytogenet. Cell Genet., 47: 88–89.

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

LeGrande, W.H., Dunham, R.A. and Smitherman, R.O., 1984. Karyology of three species of catfishes (Ictaluridae: *Ictalurus*) and four hybrid combinations. Copeia, 1984: 873–878.

Muramoto, J.S., Ohno, S. and Atkin, N.B., 1968. On the diploid state of the fish order Ostariophysi. Chromosoma, 24: 59–66.

Shapiro, H.S., 1976. Deoxyribonucleic acid content per cell of various organisms. In: G.D. Fasman (Editor), Handbook of Biochemistry and Molecular Biology, Vol. 2. CRC Press, Cleveland, OH, pp. 284–306.

Sherwood, S.W. and Patton, J.L., 1982. Genome evolution in pocket gophers (Genus *Thomomys*). Chromosoma, 85: 163–179.

Thorgaard, G.H., Rabinovitch, P.S., Shen, M.W., Gall, G.A., Propp, J. and Utter, F.M., 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture, 29: 305–309.

Tiersch, T.R., Chandler, R.W., Wachtel, S.S. and Elias, S., 1989. Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry, 10: 706–710.