CYTOGENETICAL STUDY OF *CLARIAS GARIEPINUS* (BURCHELL 1822) AND *HETEROBRANCHUS BIDORSALIS* (GEOFFROY SAINT-HILAIRE 1809) AND THEIR RECIPROCAL HYBRIDS

Majolagbe F.A.[1], J.I. Awopetu[1] and O.G. Omitogun[2]

[1]*Department of Zoology,* [2]*Department of Animal Sciences, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria.*

Abstract

Specimens of *Clarias gariepinus* and *Heterobranchus bidorsalis* and their reciprocal hybrids were cytologically analysed. The diploid chromosome numbers for *C. gariepinus* and *H. bidorsalis* were 2n = 56 and 2n = 52 respectively, while the average diploid chromosome number in the reciprocal hybrids was 2n = 54. The nombre fundamental (NF) of *C. gariepinus, H. bidorsalis,* ♀ *C. gariepinus* and ♂ *H. bidorsalis* and ♀ *H. bidorsalis* and ♂ *C. gariepinus* were 51, 49, 50 and 52 respectively.

Keywords: Clarias gariepinus, Heterobranchus bidorsalis, diploid chromosome number, reciprocal hybrids

**Corresponding author:* dessybeiby@yahoo.co.uk; Tel: +2348034797058

Introduction

Fishes are the only easily accessible and tameable vertebrate group in the kingdom Animalia that serves man in different forms and they are one of the most important sources of high quality protein. The production of novel high quality but cheap and affordable source of proteinous food can be implemented by the application of the knowledge acquired in genetics and biotechnology. To achieve this, and make it a sustainable means of livelihood in a developing economy like Nigeria, the country has to improve her aquacultural capacities. Among the fish species that are being promoted for aquaculture are *Clarias gariepinus* and *Heterobranchus bidorsalis.* Assessing the genetic diversity in the species is necessary for developing breeding programme that would provide viable, stable and fast growing stocks. Among the most common methods for determining genetic variability in populations is chromosome analysis. Aluko and Awopetu (1995) reported that hybridization between *Oreochromis niloticus* and *Sarotherodon galilaeus* were economical because of the karyotypic similarity (2n = 44) among the species and their hybrids. Ozouf-Costaz, Teugels, and Legendre, (1990) provided the first account of a karyological study of different strains of *C. gariepinus*, while Teugels, Ozouf-Costaz, Legendre, and Parent, (1992), Aluko and Awopetu (1995) called attention to the importance of studying the karyotype of parental species and their hybrids. This according to them ensures a proper understanding of the behaviour of the mitotic chromosomes of the parentals and their hybrids. Teugels, Ozouf-Costaz, Legendre, and Parent, M, (1992) found that hybrids between *C. gariepinus* and *H. longifilis* have a karyotype of 2n = 54 representing the sum of the haploid numbers of *H. longifilis* (2n = 52) and *C. gariepinus* (2n = 56). Awodiran, Aluko, and Adegoke, (2000) reported that the diploid chromosome number of *C. anguillaris* and *H. longifilis* and their hybrid are 2n = 54, 2n = 50 and 2n = 52 respectively.

This study involved the determination of genetic similarities and differences using chromosomal analysis to provide information on the relationship between *Clarias gariepinus* and *Heterobranchus bidorsalis*

with a view to improving the genetic quality of the hybrids and alleviating the problem of short supply of quality fish seeds. No published information concerning the chromosomal analysis of *H. bidorsalis* and the hybrids between *C. gariepinus* and *H. bidorsalis* is available, hence this study.

MATERIALS AND METHODS

Metaphase chromosomes were freshly prepared from newly hatched larvae as described by Aluko and Awopetu (1995) though slightly modified by reducing the number of hours in Colchicine (BH15 1TD, England) solution (Olaniyi, 2008). One hour old embryos were put in 0.01% Colchicine solution for 3 h and for another 1h in distilled water before fixing in 3:1 ethanol-acetic acid solution and kept in the refrigerator until use. The tails of the hatchlings containing mitotic cells were severed and minced in 50% ethanol- acetic acid solution (freshly prepared) to form a cell suspension. Two drops of the cell suspension were put on a clean slide which had been dried on a slide dryer. Slides were stained with a drop of FLP-orcein solution for 10 minutes.

The material was further squashed by laying a piece of filter paper on the cover slip and pressing firmly with the thumb to achieve a good spread of the cells and the chromosomes and also to remove excess stain which was absorbed by the filter paper. A binocular research microscope was used for the microscopic examination of the slides. The stained slides were scanned for cells in mitotic metaphase from one end of the cover slip to the other in a meticulous and methodical manner under the × 40 objective of the microscope. Only the cells with well-spread out chromosomes were selected for chromosome counting and for photomicro-graphs under × 100 objective (oil immersion). Photomicrographs were then taken with a digital microscope (Olympus Trinocular Microscope, Model XSZ-156). The metaphase chromosomes of the parentals and reciprocal hybrids were classified into four groups, namely metacentrics, submeta-centrics, subtelocentrics and acrocentrics, according to the method described by Aluko and Awopetu (1995).

RESULTS AND DISCUSSION

Table 1 shows the range of diploid numbers observed, modal diploid number and the number of spread observed in each genetic group. A modal diploid chromosome number of 2n = 56 and 2n = 52 were recorded for C. *gariepinus* and *H. bidorsalis*, respectively (Table 1 and Plate 1) while both hybrids show a modal chromosome complement of 2n = 54 representing the sum of the haploid numbers of the parentals. The parental chromosome numbers are relatively close to each other. Plates 2-3 and Table 2 show the karyotypes and *nombre fondamental* (NF) of the four crosses. The metaphase chromosomes of the parentals and reciprocal hybrids were metacentrics, sub-metacentrics, sub-telocentrics and acrocentrics. *C. gariepinus* consisted of 3m + 6sm + 14st + 5a with NF = 51; while that of *H. bidorsalis* were 6m + 1sm + 16st + 3a and NF = 49. For the hybrids, in the ♀C. g x ♂H. b cross, the karyotypes and NF values were made up of 3m + 2sm + 18st + 4a with NF = 50 while the reciprocal, ♀H. b x ♂ C. g had 3m + 2sm + 20st + 2a and NF = 52.

One of the factors which influence the amount of genotypic diversity generated in a species is the number of chromosomes in the genome (Avers, 1980). Genetic variation among catfishes capable of interbreeding determines their adaptive features and the cytological investigation revealed genetic variation in the parental species. Klinkhardt (1998) reported that the family Clariidae has a range of diploid chromosome numbers of between 50 and 58. The difference in karyotypes according to Klinkhardt (1998) may be due to chromosome polymorphism which took place independently in several families of Siluriformes in the course of their evolution. These figures furthermore corroborate the earlier study by Ozouf-Costaz *et al.* (1990), Teugels *et al.* (1992) and Eyo (2005) that *C. gariepinus* had a diploid

chromosome number of 56. A karyotype of 2n = 54 representing the sum of the haploid numbers of *H. longifilis* (2n = 52) and *C. gariepinus* (2n = 56) was recorded for the hybrids between *C. gariepinus* and *H. longifilis* by Teugels *et al.* (1992). Teugels *et al.* (1992) also reported that *C. anguillaris* and *C. gariepinus* have the same diploid chromosome number of 56 (2n = 56) and a nearly identical chromosome formula, and *H. longifilis* has diploid chromosome numbers of 52 chromosomes, 2n = 52.

However, chromosome complements of 2n = 50 for *H. longifilis* (Olufeagba, Aluko and Omotosho, 1999 and Awodiran *et al.*, 2000) and 2n = 54 for *C. anguillaris* (Awodiran *et al.*, 2000) have been reported while Eyo (2005) reported a karyotype of 2n = 56 for *C. anguillaris*. No report was found on the modal chromosome number of *H. bidorsalis*.

The chromosomes are variable numerically in the parentals and in the reciprocal hybrids. This could explain the ability of the Clariids to adapt to different environmental conditions, while the autopolyploidy observed in the species and their hybrids could have adaptive significance on the hybrids in the subsequent generations. Eyo (2005) reported that in nature, the occurrence of chromosome number around modal values among the Clariids may suggest that chromosomal changes may be associated with the process of speciation within the group, possibly through high rate of hybridization. Karyological evidences have been employed in solving problems relating to chromosome number, functional arm, phyletic relationship, the taxonomic status as well as possibility of speciation among the studied *Clarias* species. For instance, the wide dispersal of chromosome number around modal value (2n = 56) among the Clariids suggested possibilities of the species undergoing speciation (Eyo, 2005).

Strickberger (2000) suggested that genetic variability expressed in a population by the existence of two or more genetically distinct forms may include the maintenance of different kinds of chromosomal anomalies e.g. inversions, translocations, and extra-chromosomes.

The mosaicism evident in the hybrids may be due to the unbalanced parental haploid chromosomes. Awodiran *et. al.*, (2000) reported that the unequal parental haploid numbers might result in difficulties in chromosome pairing during meiosis accounting for differences and wide ranging chromosome complement and possible aberrations. However, the inclusion of the parental species under the same family therefore, seems justified by the cytological evidence which showed how close the chromosome numbers are.

Acknowledgments

I am grateful to Prof. (Mrs.) O.G. Omitogun, Head of the Biotechnology Laboratory of the Department of Animal Sciences and Dr. (Mrs) Adedeji of the Department of Botany of Obafemi Awolowo University for making the photomicroscopy possible.

References

Aluko, P.O. and Awopetu, J.I. (1995). Karyotype of two cichlid fishes: *Oreochromis niloticus, Sarotherodon galilaeus* and their hybrids- *O. niloticus* x *S. galilaeus. Nigerian Journal of Genetics.* 10: 87-91.

Avers, C.J. (1980). *Genetics.* Revised Edition. D.Van Nostrand Company, New York. P. 606

Awodiran, M.O., Aluko, P.O. and Adegoke, J.A. (2000). Cytogenetic Study of *Clarias anguillaris* (Linnaeus, 1758), and *Heterobranchus longifilis* (Valenciennes, 1840) and their reciprocal hybrids. *Nigerian Journal of Genetics* 15(2000); 47-52.

Eyo, J.E. (2005). Cytogenetic variations in *Clarias* species (Clariidae: Siluriforms) of the Anambra river using leucocytes culture techniques. *Animal Research International.* 2(1):275 – 286.

Klinkhardt, M. (1998). Some aspects of karyoevolution in fishes. *Animal Research and Development.* 47: 7-36.

Olaniyi, W.A. (2008). Induction of triploidy, gynogenesis and androgenesis in African giant catfish (*Clarias gariepinus* Burchell). *M. Sc. Thesis.* Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife. 99 pp.

Olufeagba, S.O., Aluko, P.O. and Omotosho, J.S. (1999). Karyomorphology of African catfish, *Heterobranchus longifilis* (Valenciennes, 1840) (Pisces-Clariidae). *Nigerian Journal of Genetics*. 14: 53-58.

Ozouf-Costaz, C., Teugels, G.G., and Legendre, M. (1990). Karyological analysis of three strains of the African catfish, *Clarias gariepinus* (Clariidae) used in aquaculture, *Aquaculture*. 87: 271-277.

Strickberger, M.W. (2000). Evolution, 3rd Edition. Jones and Bartlett Publishers Sudbury, Massachusetts. Pp.228-232.

Teugels, G. G., Ozouf-Costaz, C., Legendre, M. and Parent, M. (1992). A karyological analysis of the artificial hybridization between *Clarias gariepinus* Burchell, 1822 and *Heterobranchus longifilis* Valenciennes. 1840 (Pisces: Clariidae). *Journal of Fish Biology*. 40: 81 – 86.

Table 1: Diploid chromosome numbers of C. gariepinus, H. bidorsalis and their reciprocal hybrids

Crosses	*Number of spreads*	*Range of diploid chromosome number*	*Modal diploid chromosome number*
♀C. g x ♂ C. g	50	54 -58	56
♀C. g x ♂H. b	50	50-54	54
♀H. b x ♂ C. g	50	50-54	54
♀H. b x ♂H. b	50	48-52	52

C. g = *C. gariepinus*, H. b = *H. bidorsalis*

Table 2: Chromosome types of C. gariepinus, H. bidorsalis and their reciprocal hybrids

Crosses	*Metacentric chromosomes (m)*	*Sub-metacentric chromosomes (sm)*	*Sub-telocentric chromosomes (st)*	*Acrocentric chromosomes (a)*	*NF*
♀C. g x ♂ C. g	1-3	4-9	10-23	24-28	51
♀C. g x ♂H. b	1-3	4-5	6-23	24-27	50
♀H. b x ♂ C. g	1-3	4-5	6-25	26-27	52
♀H. b x ♂H. b	1-6	7	8-23	24-26	49

C. g = C. gariepinus, H. b = *H. bidorsalis*, NF = *Nombre fundamental*

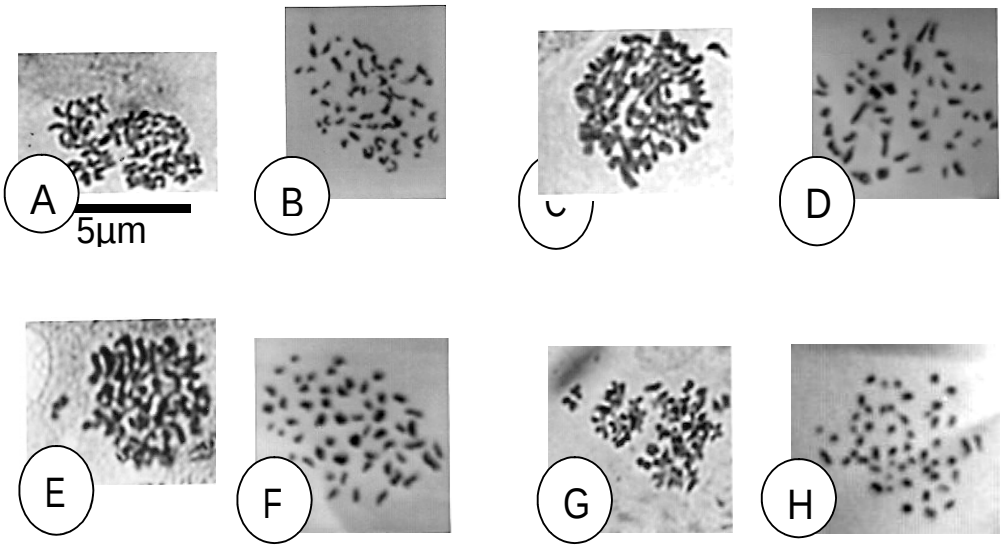


Plate 1: Mitotic metaphase (x 1000) chromosomes of the four crosses

A= Metaphase chromosomes of *C. gariepinus*, 2n=56

B=Edited metaphase chromosomes of *C. gariepinus*

C= Metaphase chromosomes of ♀*C. gariepinus* x ♂ *H. bidorsalis*, 2n=54

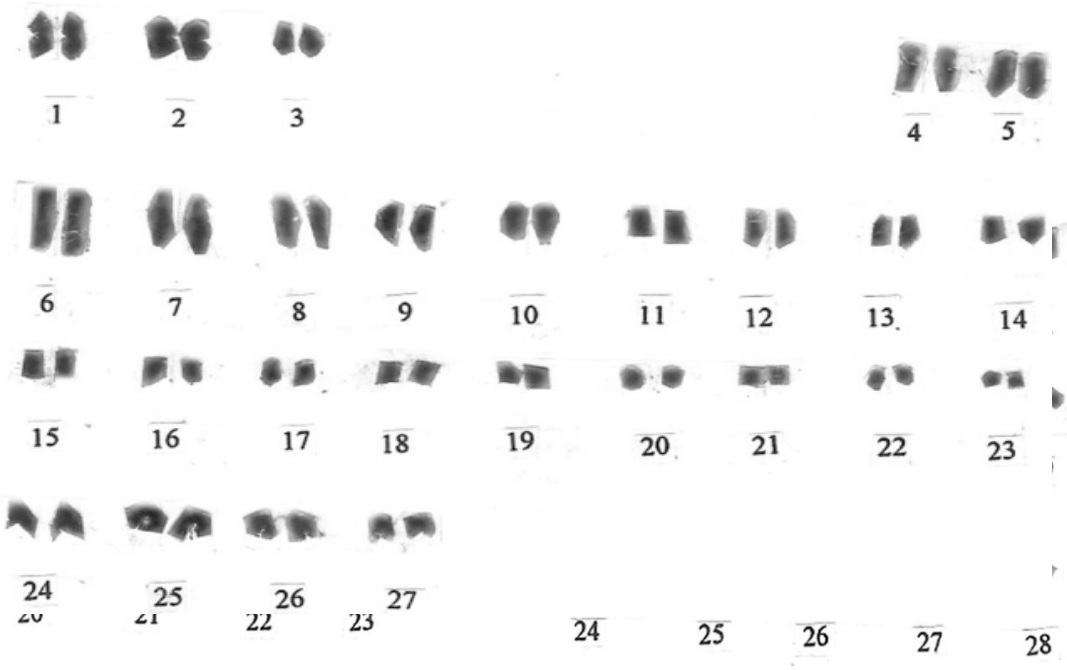
D= Edited metaphase chromosomes of ♀ *C. gariepinus* x ♂ *H. bidorsalis*

E= Metaphase chromosomes of ♀*H. bidorsalis* x ♂*C. gariepinus,* 2n=54

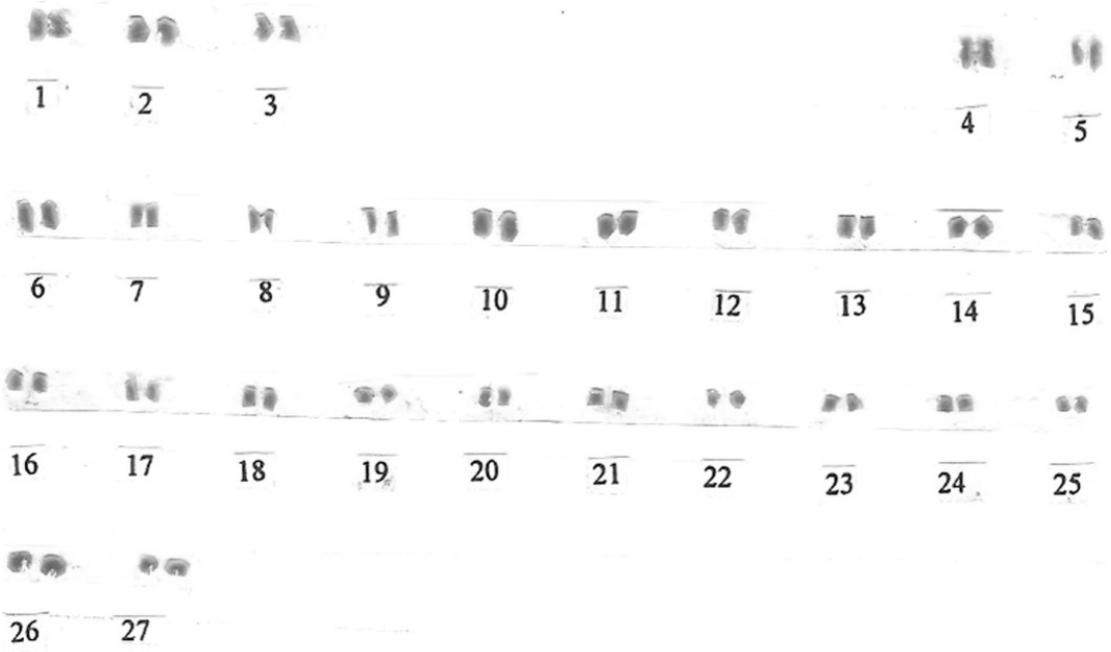
F= Edited metaphase chromosomes of ♀*H. bidorsalis* x ♂*C. gariepinus*

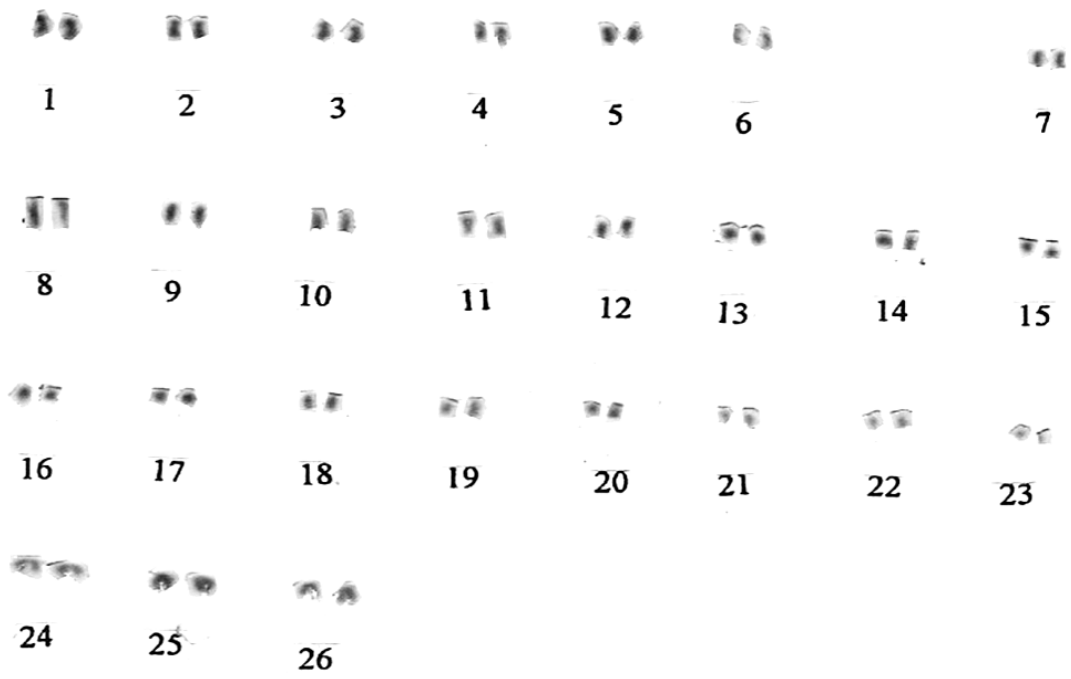
G= Metaphase chromosomes of *H. bidorsalis*, 2n=52

H=Edited metaphase chromosomes of *H. bidorsalis*



B Plate 2: Karyotypes of
A*: C. gariepinus*
B: ♀ C. gariepinus x ♂ H. bidorsalis





A

B

Plate 3: Karyotypes of
A: ♀H. bidorsalis x ♂ C. gariepinus
B: H. bidorsalis