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Evaluation of *Clarias gariepinus*, *Heterobranchus bidorsalis* and Their Hydridsploidy

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Abstract
This study investigated the chromosome numberof *Clariasgariepinus*, *Heterobranchus bidorsalis,* their hybrid *C. gariepinus* × *H. bidorsalis* and reciprocal hybrid *H. bidorsalis* × *C. gariepinus*. The chromosome preparation through mitotic cells stimulation, harvesting, fixation, and spreading were carried out following modification of the standard methods. Cytological examination confirmed the established karyotype of*C. gariepinus* having 2n=56 while *H. bidorsalis* recorded 2n=52. Both hybrids revealed intermediate karyotype of 2n=54 as sum of haploid numbers of chromosome from the parents. The closeness of the chromosome numbers explains the successful hybridization even in nature. However, the aneuploidy of the hybrids suggests its non-suitability for breeding program for commercial purposes.

Keywords: Chromosome number, *C. gariepinus, H.bidorsalis,* hybrids, aneuploidy

Introduction

Cytogenetic studies present valuable data for the taxonomic and evolutionary studies of fish species. The numbers of chromosomes present in fish can be used as a valuable indicator to assess closeness of the species interrelationship within the families. Karyotype analysis also helps to predict fertility or sterility of hybrids by comparing with the quantity and morphology of the chromosomes of parental species. Chromosome analysis or the assessment of the ploidy level of diploid or polyploidy individuals (triploid or tetraploid), gynogenetic and androgenetic as well as haploid follows after a successful hybridization or chromosomal manipulation procedures. Ploidy levels can be assessed by a variety of techniques including indirect methods such as measurement of erythrocyte nuclear or cellular volumes, nucleolar counting, electrophoresis of protein and chromosome morphology examination. The direct methods are chromosome counting, and DNA content determination by flow cytometry (Chourrout, 1987; Purdom, 1993; Zhang and Arai, 1996). Chromosome counting is an accurate, direct method of ploidy assessment but time consuming. Well spread preparations of chromosomes are possible from embryos because of their high mitotic indices (Hoornbeek and Burke, 1981; Thorgaard *et al*., 1981; Yamazaki *et al.,* 1981).

The African catfish *C. gariepinus* and *H. bidorsalis* are economically important species. Many recent breeding works are progressing on their hybridization but little is known about their genetic status and the karyotype. The cytogenetic study is needed for proper identification of these hybrids and to further determine their genetic relationship. Although, some parameters such as growth rate have been used in the past to identify these two species, but more specific tool such as karyological analysis is needed for more and better concise differentiation.

The study of cytogenetic variation in *C. gariepinus* and *H. bidorsalis* will help to improve production of their hybrids and in resolving some taxonomic problems and related issues that may be present in the family *Clariidae*, such as speciation, phyletic relationship, identity of sex determining chromosome, and for both interspecific and even intergeneric hybrids.

Therefore, the objective of the study was to investigate the chromosome number of *C. gariepinus*, *H. bidorsalis s*and their hybrids that may have implication for breeding programs.

Materials and Methods

Location and Samples: The experiment was conducted at the Wet and Biotechnology Laboratories, Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The fish samples used for this research were table sized fish of hybrids catfish species, *C. gariepinus* and *H. bidorsalis* of 600±100g.

Chromosome preparation: Chromosome was prepared from 18 samples of catfish following the methods of Klingerman and Bloom (1977) and Henegariu *et al*. (2001) with some modifications. The fish were injected with yeast suspension intraperitoneally at 1 ml/100g. After completion of mitosis stimulation period (24 h), the fish was injected intraperitoneally, with a 0.025 % colchicine solution at 1 ml/100 g body weight and left for 1hrthereafter before they were sacrificed. The gill and kidney tissues were dissected and transferred separately into 10 ml hypotonic solution of 0.56 % KCl and 0.8 % trisodium citrate and then made to homogenous suspension. The suspensions were incubated at 30-35^{o}C for 20 min. Drops of 3-5freshly prepared fixative (methanol 3:1 glacial acetic acid) were added to it and further re-suspended. The solution was then centrifuged at 900 rpm for 10

min and the supernatant was discarded. Thereafter, 1 ml of the freshly fixative was added, and the cells were re-suspended. This final suspension was the used for slide preparation.

Slide preparation and chromosome analysis: Cells suspension were evenly dropped and distributed on several locations on glass slide, and then dried at ambient temperature. After the surface became grainy, the slide was briefly steamed and 4 – 6 droplets of acetic acid were placed on the slide. After the acetic acid slowly spread and covered the surface, the slide was then quickly dried at >65 °C. The dried slides were then stained with 8% Giemsa stain, pH 6.8 for 15 min, rinsed with water and then quickly dried at >65 °C. The coded slides were screened for good metaphase spreads under biological computer-aided light microscope (Leica Galen III) mounted with digital camera (AmScope MT, Version 3.0.01).

Results and Discussion

Frequency of chromosome numbers: Table 1 shows the frequency distribution of diploid chromosome number of *C. gariepinus*, *H. bidorsalis* and their hybrids; while Plate 1 (a-d) depicts the representatives of the metaphase chromosome spread of the specimen.

Ploidy evaluation: The diploid chromosome number of all the species crosses were evaluated and revealed in Plate 1(a-d). The diploid *C. gariepinus* (Plate 1a) gave chromosome number 2n = 54 to 56 with modal number of 56; while *H. bidorsalis* gave 48 to 52 with modal number of 52 (Plate 1b). The hybrids[1] of *C. gariepinus* × *H. bidorsalis* gave 52 to 54 with modal number being 54; and *H. bidorsalis* × *C. gariepinus* with 50 to 54 having modal number of 54. The evaluation of ploidy and cytogenetic analysis of *C. gariepinus* in this study supported the earlier studies (Eyo, 2005; Majolagbe *et al.,* 2010; Ojo *et al.,* 2011). It is therefore in line with the foremost findings of Ozouf-Costaz *et al.* (1990), supporting Teugels (1982) study that *C. gariepinus* is synonymous to *C. lazera, C. mosambicus* due to the same chromosomal number of 2n = 56. While Richter *et al.* (1987) found 2n = 54numbers of chromosomes, Ergene *et al.* (1999) reported that fish chromosomes are of different variations which lead to various difficulties in karyotypical analysis. From his works on the karyotypes of *C. lazera*, its diploid chromosome number is approximately 2n = 56. Some variations (that is between 48 and 56 among different specimens) in this chromosome number and its morphology was due to what he viewed as a small part of the main population, thereby accepts the highest value obtained. However, in order to ascertain the synonymous characterization of these species (*C. lazera, C. mosambicus and C. gariepinus*) as well as other associate strains, it must be determined whether they can breed true viable offspring.

Fewer karyological studies are on *Heterobranchus* species. Majolagbe *et al.* (2010) reported 2n=52 for *H. bidorsalis* while Teugels *et al.* (1992) reported 2n=52 for its congener *H. longifilis.* In this study, 2n varied between 48 and 52 having mostly 52. The hybrids *C. gariepinus*×*H. bidorsalis* and *H. bidorsalis* × *C. gariepinus* were both 2n=54. This value represented the mean number of the sum of diploid parents species ploidy levels, *C. gariepinus* (2n=56) and *H. bidorsalis* (2n=54). Teugels *et al.* (1992) reported karyotype of 2n=54 for the *C. gariepinus* versus *H. longifilis* and same number was also recorded by Majolagbe *et al.* (2010) for hybrids of *C. gariepinus* and *H. bidorsalis*. The intermediate value of the parents as recorded by the hybrid karyotype indicated total addition of the haploid chromosome numbers of the parents, that is, each chromosome with its complement (homology) and extra two unpaired chromosomes, hence aneuploid. This might explain underdeveloped gametes of the hybrids. Moreover, despite the aneuploidy case, hybrids are not completely sterile but develop abnormal gonads with low fertile gametes compared to the parents' species (Teugels *et al.,* 1992).

The present study has revealed the closeness of the chromosome numbers of the *Clariid* species of *C. gariepinus* and *H. bidorsalis* which explains their successful hybridization, even in nature; however, the aneuploidy make-up of the hybrids suggests its non-suitability in breeding programs for commercial purposes. Further studies especially on genomic may be carried out in addition to karyotyping that will show the chromosome mapping to provide more information.

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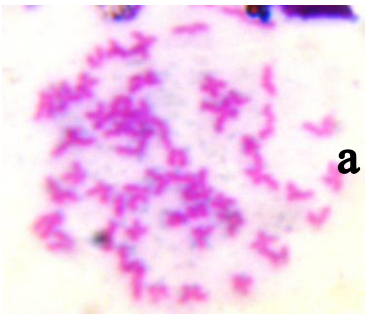
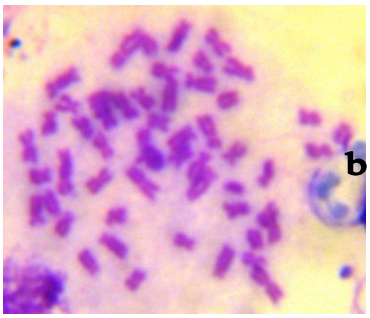
Table 1.Frequency distribution of the diploid chromosome number of catfish species of *Clarias gariepinus Heterobranchus bidorsalis* and their hybrids

Species	*Specimen*		*Diploid chromosome number (2n)*								
	Male	Female	48	49	50	51	52	53	54	55	56
C. gariepinus	2	2	-	-	-	-	-	-	2	-	15
H. bidorsalis	2	3	6	-	5	1	11	-	-	-	-
C. gariepinus × *H. bidorsalis*	2	3	-	-	-	-	3	-	17	-	-
H. bidorsalis × *C. gariepinus*	2	2	-	-	1	1	2	2	11	1	-





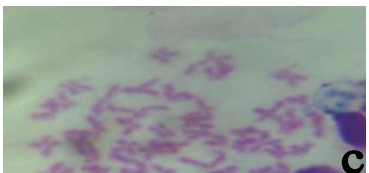


Plate 1(a-d): Representative metaphase chromosome spread from catfish species. (a) *C. gariepinus*, 2n=56; (b) *H. bidorsalis*, 2n=52, (c) Hybrid*C. gariepinus*×*H. Bidorsalis*, 2n=54 (d) Reciprocal hybrid, *H. bidorsalis*× ♂*C. gariepinus*, 2n=54.