

PLOIDY EVALUATION OF TRIPLOID HYBRID SPECIES OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS BIDORSALIS*

W.A. Olaniyi^{**}, J.A. Adefolaju^{*}, J.O. Aniyi^{*}, O.Olusegun^{*} and O.G. Omitogun^{*}

^{*}Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria; ^{*}Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria; ^{*}Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

^{*}Corresponding author: wasiu.olaniyi@aaua.edu.ng; waolaniyi@gmail.com; +234 803 3799 789

ABSTRACT

This study was undertaken to investigate the ploidy levels of the triploid hybrid catfish species between *Clarias gariepinus* and *Heterobranchus bidorsalis*. Samples of triploid hybrid ($\text{♀}C. \text{gariepinus} \times \text{♂}H. \text{bidorsalis}$) and its reciprocal ($\text{♀}H. \text{bidorsalis} \times \text{♂}C. \text{gariepinus}$) were prepared for ploidy assessment. Cytological assessment showed varying chromosome numbers. Most of the counts for triploid hybrid showed modal outcome of $3n=84\pm2$ while its reciprocal triploid gave $3n=80\pm2$. The occurrence of more chromosome numbers around the modal value might be caused by chromosome polymorphism which may suggest ongoing speciation of the two fish species.

Keywords: ploidy, *Clarias gariepinus*, *Heterobranchus bidorsalis*, polymorphism, speciation

INTRODUCTION

Genomic technologies have been used to develop fisheries and aquaculture production especially in breeding and production systems (Olaniyi and Omitogun, 2012). Cytogenetics provides good confirmation of induction of polyploidy techniques that reveals the success of the technology via the genomic evaluation of the chromosome numbers among other analysis. Chromosome counting, though laborious, is an accurate and direct method of ploidy assessment; and this is best achieved through a very well spread preparations of chromosomes from embryos because of their high mitotic indices (Hoorbeek and Burke, 1981; Thorgaard *et al.*, 1981; Yamazaki *et al.*, 1981, Olaniyi and Omitogun, 2012, 2014). The technology helps in the rapid improvement and development of genomics in aquaculture.

The improvement of the two *Clariid* species of *Clarias gariepinus* and *Heterobranchus bidorsalis* through genetic manipulation and hybridization techniques have been established in our previous studies (Olaniyi *et al.*, 2018; Olaniyi and Omitogun, 2018, 2020). The present study was undertaken to investigate the ploidy levels of the triploid hybrid catfish species between *C. gariepinus* and *H. bidorsalis*.

MATERIALS AND METHODS

Location: The research was carried out at the Departments of Animal sciences and Zoology, Obafemi Awolowo University (O.A.U) Ile-Ife, Nigeria.

Sample collection: Twenty (20) hybrid triploid catfish species ($600\pm100\text{g}$) between *C. gariepinus* and *H. bidorsalis* and their reciprocal were obtained from the department of Animal Science, O.A.U Ile Ife, and used for this study.

Preparation of chromosomes: The hybrid triploid catfish species were injected with yeast suspension intraperitoneally at 1 ml/100 g. Thereafter, at the completion of mitosis stimulation period (24 h), the samples were injected intraperitoneally, with a 0.025 % colchicine solution at 1 ml/100 g body weight and left for a period of 1hr before being slaughtered. The gill and kidney fragments of these sampled species were transferred separately into 10 ml hypotonic solution of 0.56 % KCl and 0.8 % trisodium citrate and then squashed into homogenous suspension. The homogenized suspensions were incubated at 30-35°C for 20 min. 3 – 5 drops of freshly 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 and used for slide preparation.

Slide preparation and chromosome analysis: The cells suspension was evenly dropped and distributed on different locations on glass slide, and allowed to dry at ambient temperature. The

surface of the glass slide was allowed to become grainy, and briefly steamed with 4 – 6 droplets of acetic acid being placed on the slide. After the acetic acid was slowly spread and covered the surface, the slide was then quickly dried at $>65^{\circ}\text{C}$. The dried slides were then stained with 8% Giemsa stain, pH 6.8 for 15 min, rinsed with water and immediately dried at $>65^{\circ}\text{C}$. The coded slides were screened for good metaphasic spreads under biological computer-aided light microscope (Leica Galen III) mounted with digital camera (AmScopeMT, Version 3.0.01) and photographed. The slides with good chromosome spread were analyzed for the frequency of occurrence.

RESULTS AND DISCUSSION

Frequency of chromosome numbers: Table 1 shows the frequency distribution of ploidy chromosome numbers of the triploid hybrids of *C. gariepinus* and *H. bidorsalis*; while Plate 1 (a and b) depicts the representatives of the metaphase chromosome spread of the samples.

Ploidy evaluation: In our previous study (Olaniyi and Omitogun, 2018), the mean number of the sum of diploid species ploidy levels respectively recorded for *C. gariepinus* and *H. bidorsalis* were $2n=56$ and $2n=54$; while the hybrids¹ *C. gariepinus* $\times$ *H. bidorsalis* were both $2n=54$. The present study recorded successful presence of triploid sample species with varying frequency of chromosome numbers. The triploid chromosome number of the samples were evaluated [Plates 1 (a and b)]. The triploid hybrids of *C. gariepinus* $\times$ *H. bidorsalis* gave varying chromosome numbers from 81 to 84 with modal number being 82 and even high values >85 ; and for hybrid triploids of *H. bidorsalis* $\times$ *C. gariepinus* having modal number of 80 with its variations (Table 1). Countable numbers of cytogenetics works have been carried on *C. gariepinus*, however very few studies have been recorded on *Heterobranchus* species. The triploid hybrid $\text{♀}C. \text{gariepinus} \times \text{♂}H. \text{bidorsalis}$ revealed varying chromosome numbers. Most of the counts for triploid hybrid $\text{♀}C. \text{gariepinus} \times \text{♂}H. \text{bidorsalis}$ showed $3n=84\pm2$ while the reciprocal triploid hybrid $\text{♀}H. \text{bidorsalis} \times \text{♂}C. \text{gariepinus}$ gave $3n=80\pm2$. This might be caused by chromosome polymorphism which has been a concern in cytogenetic (Ozouf-Costaz *et al.*, 1990).

CONCLUSION AND RECOMMENDATION

The present study has shown possible chromosomal polymorphism in the studied species. Further studies employing molecular methods, chromosomes' karyotypical and morphological analysis will give deeper insight.

REFERENCES

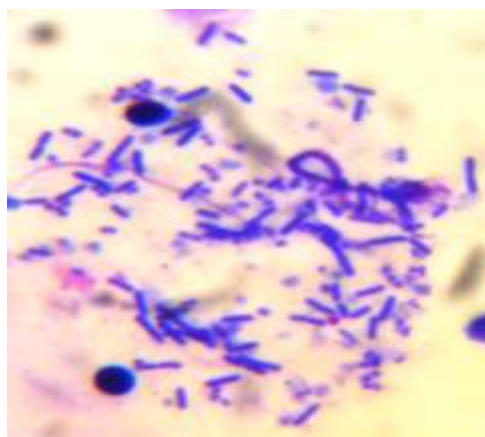
- Henegariu, O., Heerema, N.A., Wright, L.L., Bray-Ward, P., Ward, D.C., and Vance, G.H. 2001. Improvements in cytogenetic slide preparation: controlled chromosome spreading, chemical aging and gradual denaturing. *Cytometry* 43: 101–109.
- Hoornbeek, F. K., and Burke, P. M. 1981. Induced chromosome member variation in the winter flounder. *J. Hered.* 72: 189-192
- Kligerman, A.D. and Bloom, S.E. 1977. Rapid chromosome preparations from solid tissues of fishes. *J Fish Res Board of Canada*, 34: 249-261.
- Olaniyi, W.A. Aniyi, J.O., Adefolaju, J.A. and Olusegun, O. 2018. Evaluation of catfish species of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their Hybrids Ploidy. 43rd Annual conference of Nigerian Society of Animal Production, on “Exploring Science and Technology Innovations for Sustainable Livestock Development”, Okoli, I.C., Ogbuewu, I.P., Emanalome, O.O., and Esonu, B.O. (eds). New SAAT Auditorium, Federal University of Technology, Owerri, Imo State, Nigeria. March 18-22, 2018, pp 1103-1106.
- Olaniyi, W.A., and Omitogun, O.G. 2012. Induction of diploid gynogenetic larvae of African catfish, *Clarias gariepinus* Burchell (1822). *Ife Journal Agriculture*, 25: 73-82.
- Olaniyi, W.A., and Omitogun, O.G. 2014. Induction of triploidy and erythrocyte cell size analysis of triploid African catfish, *Clarias gariepinus* (Burchell, 1822). *Animal Research International*, 11(3): 2079 – 2086.

¹ The female parent species are firstly mentioned in the breeding program

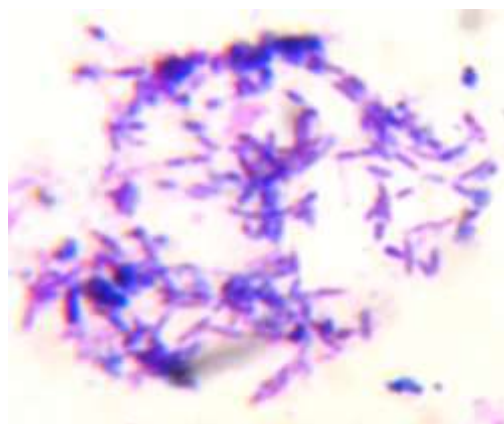
- Olaniyi, W.A., and Omitogun, O.G. 2020. Hybridization between *Clarias gariepinus* (Burchell 1822) and *Heterobranchus bidorsalis* (Geoffroy Saint Hilaire, 1809). 45th Annual conference of Nigerian Society of Animal Production, on “Exploring The Goldmine of Nigerian’s Agro-Revolution”, Mancha, Y.P., Kalla, D.J.U., Bello, K.M., Mbap, S.T., AbdulKarim, M., Igila, T., and Danbrini, S. (eds). Abubakar Tafawa Balewa University, Bauchi and Federal Polytechnic Bauchi, Bauchi State, Nigeria. March 15-19, 2020, pp 270 – 273.
- 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*, 87:271-7.
- Thorgaard, G.H., Jazwin, M.E., and Stier, A.R. 1981. Polyploidy induced by heat shock in rainbow trout. *Trans. Am. Fish Soc.*, 110: 546-550
- Yamazaki, F., Onozato, H., and Arai, K. 1981. The chopping method for obtaining permanent chromosome preparation from embryos of teleost fishes. *Bull. Jpn. Soc. Sci. Fish*, 47: 963.

Table 1. Frequency distribution of the triploid hybrid chromosome number of catfish species of *Clarias gariepinus*, *Heterobranchus bidorsalis*

Sample Species	No of Samples	Triploid chromosome number (3n)							
		<80	80	81	82	83	84	85	>85
<i>C. gariepinus</i> × <i>H. bidorsalis</i>	5	-	1	5	9	2	1	-	4
<i>H. bidorsalis</i> × <i>C. gariepinus</i>	5	2	5	1	1	-	-	1	1



a



b

Plate 1(a and b): Representative chromosome spread from triploid: (a) hybrid ♀ *C. gariepinus* × ♂ *H. bidorsalis*, 3n=84±2; (b) reciprocal hybrid ♀ *H. bidorsalis* × ♂ *C. gariepinus*, 3n=80±2.