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Karyotypes of the Wild And Hatchery-Bred Parentals and the Intraspecific Hybrids of Clarias Gariepinus

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ABSTRACT

This study presents the diploid chromosome number of Clarias gariepinus parentals from the wild and hatchery and their intraspecific hybrids. The kidney cells were used for the preparation of metaphase chromosome spreads. The diploid chromosome number for both parentals and hybrids was found to be 2n=56. The karyotypes for the wild parental revealed were 21metacentric, 1submetacentric, 2subtelocentric and 4telocentric and that of hatchery-bred parental revealed 23metacentric, 1submetacentric, 2 submetacentric and 2telocentric. The hybrids karyotype was 21metacentric, 2 subtelocentric and 5 telocentric for WH while HW had 19metacentric, 1subtelocentric and 8telocentric. The cytogenetic characteristics of both parentals and hybrids varied morphologically. This study provides information for hybridizations and possible manipulations of chromosomes in C. gariepinus, evolutionary study, classification and taxonomy and in monitoring aquatic toxicity.

Keywords: *Chromosome, Clarias Gariepinus, Karyotype, Hybrids, Mitotic Metaphase*

INTRODUCTION

The understanding of chromosomal analysis in animal breeding is germane for the purpose of classification, genetic control and evolutionary study, this might be reason Kligerman and Bloom (1977) and Amemiya, (1986) reported that fish karyological studies is a useful tool in acquiring knowledge in the fields of toxicology, mutagenesis, systematic, aquaculture and evolution. Much work has been done on chromosomal study of vertebrate groups but fish chromosomal studies which have not been wide spread or obtained much interest among Researchers. Gold *et al.* (1990) reported that standard karyotypes of less than 10% of more than 20,000 discovered species of fish have been reported. *Clarias gariepinus* is very common, most cultured and well accepted among farmers and consumers in Nigeria. *C.gariepinus* has increasing commercial importance in fisheries and aquaculture. *C. gariepinus* is indigenous to the inland waters of much of Africa and they are also endemic in Asia minor in countries such as Israel, Syria and the South of Turkey. *C. gariepinus* has been widely introduced to other parts of the World including the Netherlands, Hungary, much of East Asia (CABI, 2017).

Cytogenetic study of fish in Nigeria water has not been extensive, it is in line with this that this study was carried out to re-evaluate the chromosome number and morphological characteristics of chromosome of the parentals and hybrids of *Clarias gariepinus* from wild and hatchery environment. This was also with the aim to revealing karyotypic formulars of both parentals and intraspecific hybrids of *C. gariepinus.*

MATERIALS AND METHODS

Samples of both the wild (WW) and hatchery-bred (HH) of *C. gariepinus* were collected with trap net from Igun abandoned gold mine reservoir, Igun, Atakumosa Local Government area, Osun State, Nigeria and Leventis Foundation, Ilesa East Local Government, Osun State, Nigeria. Samples were transported to the Biology Department laboratory of Osun State College of Education, Ilesa for acclimatization and the practicals. The samples were later separated into males and females and kept separately. Four mating groups which are WW and HH for parentals, WH and HW for hybrids

were obtained from mating combinations of the wild and hatchery-bred samples of *Clarias gariepinus*. Artificial fertilization was done using eggs and milts harvested from gravid females and males induced with synthetic hormone (ovaprim at 0.5ml/kg of body weight). Hatchlings obtained from each of the mating groups were stocked in to different labeled plastic tanks for four days when the yolk sac was fully absorbed, then the fry were restocked at the rate of 100 fry per tank in triplicates for each mating combinations. The fish were then fed and maintained till maturity stage..

CHROMOSOME PREPARATIONS

Samples of growing *C. gariepinus* were selected from each mating combinations for chromosome analysis. Fish for the chromosomal studies were injected intraperitioneally with 0.05% of freshly prepared Colchicine solution per gram of body weight. The injected fish were left in separate holding tank for 3-4hours. The head were covered with hand towel to hold and immobilize it, the fish were dissected to harvest the kidney.

The anterior portion of the headkidney was removed and placed inside a mortal containing hypotonic solution (0.56%KCl) for 50minutes, the content was then macerated with pestle to homogenize the solution and the tissue using modified method of Sofy *et. al* (2008). The supernatants were removed by pouring it into centrifuge tubes, the tubes were centrifuge for 7minutes at 1000rpm then the supernatant removed. Fixation was done by adding 6ml of mixture of absolute methanol and acetic acid (3:1) for about 30minutes and centrifuged for 7minutes at 1000rpm then the supernatant removed.Re-fixation was carried out two more times as earlier described. More fixative was added to the cell concentration at the bottom of the test tube, the cells were spread on pre-warmed slides with Pasteur pipette. Slides were allowed to dry on slide warmer at 60^0C for 24hrs. The slides were stained with 6% Giemsa stain (6ml Giemsa stock solution and 94 ml Sorensen's buffer pH= 6-8) for 20-25minutes thereafter slides were washed under gentle running water and the slides were dried on slide warmer at 60^0C for 24hrs before microscopic examination.

DETERMINATION OF CHROMOSOME NUMBER

Several fields of mitotic metaphase from slide preparations were examined; the photographs of good spreads were taken on light photomicroscope (Olympus) with oil immersion at 1000x magnification.

CHROMOSOMES CLASSIFICATION

The karyotyping of chromosomes was done according to length in pairs starting with the longest to the shortest and their length measured using GIMP corel draw professional XIII. Classifications of chromosome were done following Levan, *et al*., (1964).

RESULTS

Figures 1-4 show the metaphase spread of each of the parentals and the hybrids of *C. gariepinus*. A modal chromosome number of 2n=56 was established in the metaphase of cells from the kidney of each of the mating combinations. The frequency of diploid chromosome number varied between 53 and 58 per metaphase. Modal chromosome number of 2n=56 was established for over 90% of metaphase cells examined. All chromosomes in the karyotype had homologous pairs. Figures 5-8 show the karyotype of both parental and the hybrids of *C. gariepinus*. There were marked differences in the chromosome size, shape and type of each of the mating group examined and sex chromosome cannot be distinguished morphologically. The karyotype constitution of the parental of hatchery-bred included 23pairs of medial, 1pairs of submedial, 2pairs of subterminal and 2pairs terminal while that of wild parental included 21pairs of medial, 1pairs of submedial, 2pairs of subterminal and 4pair of terminal. The hybrid of WH karyotype constitution includes 21pairs of media, 2pairs of subterminal and 5pairs of terminal while HW karyotype constitution included 19pairs of medial, 1pairs of subterminal and 8pairs of terminal. Tables 1-4 described the chromosome length, long arms, short arms and arm ratio of the *C.gariepinus*. Table 5 represents summary of the morphological and numerical data of the four mating groups. The sums of mean total length of chromosomes of the hybrids were intermediate to the parentals.

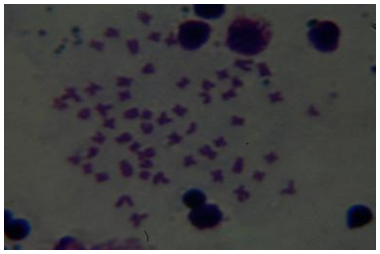

Fig1. *Mitotic metaphase chromosome of wild (WW) C. gariepinus.*

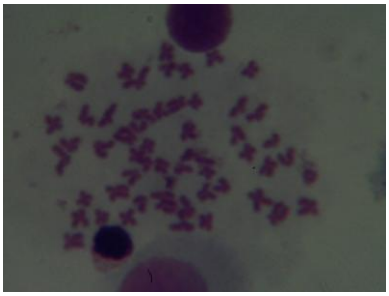

Fig2. *Mitotic metaphase chromosome of (HH) C. gariepinus hatchery-bred*

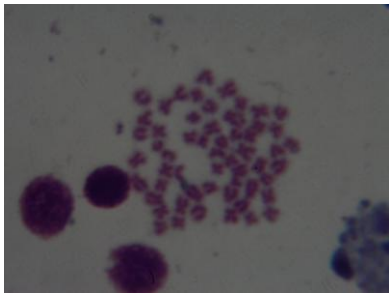

Fig3. *Mitotic metaphase chromosome of intraspecific hybrid (WH) C. gariepinus.*

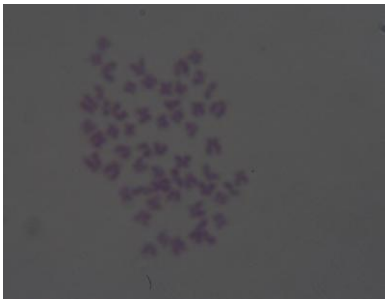

Fig4. *Mitotic metaphase chromosome of intraspecific hybrid (HW) C. gariepinus.*

Fig5. *Karyotype of diploid chromosome of C. gariepinus for WW mating.*

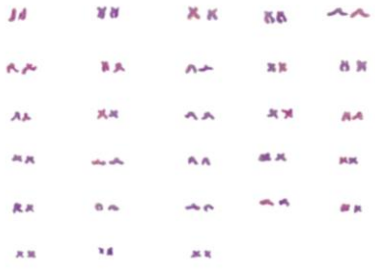

Fig6. *Karyotype of diploid chromosome of C. gariepinus for HH mating.*

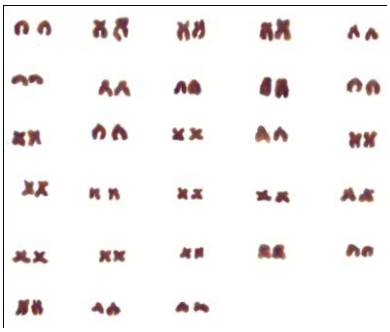

Fig7. *Karyotype of diploid chromosome of C. gariepinus for WH mating.*

Fig8. *Karyotype of diploid chromosome of C. gariepinus for HW mating.*

DISCUSSION

A modal diploid chromosome number of 2n=56 was observed in the cells of all the mating combinations. This is in line with the records of Awodiran *et. al.*, (2014), Ifeoluwa *et. al.*, (2011), Karahan and Ergene (2011) and Okonkwo and Obiakor (2010). However the modal no of 2n=56 recorded in this study differs from report of Fagbuaro (2012) and Richter *et. al.*, (1987). They reported modal diploid chromosome number of 2n = 54 for *C. gariepinus.* The differences could be as a result of variation in their strains, mosaicism or chromosome polymorphism which has been reported in several families of Siluriformes or technical problems during preparations. Fagbuaro (2012) reported that variable chromosome number is a common phenomenon in some fish species. The karyotypic formular reported for *C. gariepinus* in this study varied among the mating combinations the wild parental had 21metacentric, 1submetacentric, 2 subtelocentric and 4telocentric, while the hatchery-bred (HH) had 23metacentric, 1sumetacentric, 2 subtelocentric and 2 telocentric. Among the hybrids, the WH had 21metacentric, 2 subtelocentric and 5 telocentric while HW had 19metacentric, 1subtelocentric and 8 telocentric. Karyotypic formulars reported in this study differ from the reports of some Researchers such as Fagbuaro (2012) who

reported 24 metacentric, 10 submetacentric and 10 subtelocentric and Ifeoluwa *et al.*, (2011) reported 25 metacentric, 14 submetacentric, 14 subtelocentric and 3 telocentric. Awodiran *et. al.*, (2014) in their own reported 3metacentric, 6 submetacentric, 14 subtelocentric and 5 acrocentric.

Table1. *Mean Length, Long and Short Arms Ratio of Mitotic Chromosome Of C.Gariepinus (Ww)*

No of chromosome of WW	Length of long arm (mm)	Length of short arm (mm)	Long arm : short arm ratio	Total length (mm)	Total length %	Description of chromosome
1	4.155	1.667	2.491	5.822	4.906	m
2	3.860	1.928	2.002	5.780	4.871	m
3	4.359	1.402	3.109	5.761	4.855	st
4	3.972	1.739	2.285	5.711	4.813	m
5	4.058	1.651	2.457	5.709	4.811	m
6	3.550	1.536	2.310	5.087	4.287	m
7	3.648	1.302	2.802	4.950	4.171	m
8	3.186	1.763	1.806	4.949	4.171	m
9	3.339	1.091	3.059	4.431	3.734	st
10	2.522	1.908	1.322	4.430	3.733	m
11	2.704	1.785	1.514	4.490	3.784	m
12	2.552	1.936	1.318	4.489	3.783	m
13	4.480	0.000	0.000	4.480	3.775	t
14	3.001	1.469	2.043	4.470	3.767	m
15	2.593	1.450	1.788	4.043	3.407	m
16	2.258	1.377	1.639	3.635	3.063	m
17	2.226	1.309	1.705	3.535	2.979	m
18	2.420	1.109	2.181	3.530	2.975	m
19	3.523	0.000	0.000	3.523	2.969	t
20	2.219	1.301	1.705	3.520	2.966	m
21	1.993	1.507	1.323	3.500	2.950	m
22	3.500	0.000	0.000	3.500	2.950	t
23	1.870	1.612	1.160	3.482	2.934	m
24	2.249	1.085	2.071	3.334	2.810	m
25	1.661	1.570	1.057	3.231	2.723	m
26	1.831	1.291	1.419	3.122	2.631	m
27	3.120	0.000	0.000	3.120	2.629	t
28	2.282	0.748	3.048	3.030	2.553	sm

Table2. *Mean Length, Long And Short Arms Ratio Of Mitotic Chromosome Of C.Gariepinus (Hh)*

No of chromosome of HH	Length of long arm (mm)	Length of short arm (mm)	Long arm : short arm ratio	Total length (mm)	Total length %	Description of chromosome
1	4.583	2.782	1.647	7.365	4.678	M
2	4.818	2.485	1.938	7.303	4.639	m
3	4.449	2.939	1.513	7.388	4.693	M
4	5.303	1.769	2.997	7.072	4.492	sm
5	5.252	1.639	3.203	6.892	4.378	st
6	4.713	2.022	2.331	6.735	4.278	m
7	3.844	2.314	1.661	6.158	3.911	m
8	4.319	0.882	4.894	5.202	3.304	st
9	3.446	2.643	1.303	6.090	3.868	M
10	4.668	2.471	1.888	7.139	4.535	m
11	2.980	2.222	1.341	5.202	3.304	M
12	3.269	2.035	1.606	5.304	3.369	M
13	3.520	1.680	2.095	5.200	3.303	m
14	2.963	2.337	1.268	5.300	3.366	M
15	3.586	1.699	2.111	5.285	3.357	m
16	3.124	2.154	1.450	5.278	3.352	M

17	3.790	1.432	2.653	5.230	3.322	m
18	5.230	0.000	-	5.230	3.322	t
19	3.251	1.952	1.665	5.203	3.305	M
20	3.057	2.143	1.427	5.200	3.303	M
21	3.071	2.137	1.437	5.208	3.308	M
22	4.932	0.000	-	4.932	3.133	t
23	3.428	1.383	2.477	4.812	3.056	m
24	3.233	1.419	2.277	4.653	2.955	m
25	2.789	1.861	1.498	4.650	2.954	M
26	2.513	2.124	1.183	4.637	2.945	M
27	3.222	1.167	2.760	4.389	2.788	m
28	2.602	1.778	1.464	4.380	2.782	M

Table3. *Mean Length, Long And Short Arms Ratio Of Mitotic Chromosome Of C.Gariepinus (Wh)*

No of chromosome of WH	Length of long arm (mm)	Length of short arm (mm)	Long arm : short arm ratio	Total length (mm)	Total length %	Description of chromosome
1	6.996	0.000	-	6.996	4.862	t
2	4.689	2.084	2.249	6.773	4.707	m
3	4.296	1.731	2.482	6.027	4.189	m
4	4.295	1.636	2.625	5.931	4.122	m
5	4.231	1.658	2.552	5.889	4.093	m
6	5.877	0.000	-	5.877	4.084	t
7	4.158	1.711	2.430	5.869	4.079	m
8	5.854	0.000	-	5.854	4.069	t
9	3.977	1.863	2.135	5.840	4.059	m
10	5.780	0.000	-	5.780	4.017	t
11	3.278	2.184	1.501	5.462	3.796	m
12	3.878	1.251	3.099	5.129	3.565	st
13	3.226	1.802	1.790	5.028	3.495	m
14	4.049	0.951	4.257	5.000	3.475	st
15	3.180	2.138	1.487	5.318	3.696	m
16	2.955	2.166	1.363	5.121	3.559	m
17	3.441	1.427	2.410	4.869	3.384	m
18	2.717	2.023	1.343	4.740	3.294	m
19	2.848	1.888	1.508	4.736	3.292	m
20	3.354	1.346	2.492	4.700	3.267	m
21	2.694	2.006	1.343	4.700	3.267	m
22	2.828	1.723	1.641	4.551	3.163	m
23	3.138	1.319	2.379	4.457	3.098	m
24	2.673	1.328	2.012	4.001	2.781	m
25	3.966	0.000	-	3.966	2.756	t
26	2.219	1.648	1.346	3.868	2.688	m
27	2.645	1.202	2.199	3.847	2.674	m
28	2.055	1.499	1.370	3.554	2.470	m

Table4. *Mean Length, Long And Short Arms Ratio Of Mitotic Chromosome Of C.Gariepinus (Hw)*

No of chromosome of HW	Length of long arm (mm)	Length of short arm (mm)	Long arm : short arm ratio	Total length (mm)	Total length %	Description of chromosome
1	7.158	0.000	-	7.158	5.460	t
2	5.724	0.000	-	5.724	4.366	t
3	5.710	0.000	-	5.710	4.355	t
4	5.625	0.000	-	5.625	4.290	t
5	3.976	1.459	2.725	5.435	4.146	m
6	3.198	2.230	1.434	5.428	4.140	m

7	3.570	1.753	2.036	5.323	4.060	m
8	5.273	0.000	-	5.273	4.022	t
9	3.295	1.923	1.713	5.219	3.981	m
10	3.930	0.766	5.127	4.696	3.582	st
11	3.124	1.477	2.114	4.601	3.509	m
12	4.419	0.000	-	4.419	3.371	t
13	2.481	1.929	1.286	4.410	3.364	m
14	2.936	1.601	1.833	4.537	3.461	m
15	4.400	0.000	-	4.400	3.356	t
16	2.559	1.822	1.404	4.381	3.342	m
17	4.380	0.000	-	4.380	3.341	t
18	2.903	1.471	1.973	4.374	3.336	m
19	2.672	1.700	1.574	4.370	3.333	m
20	2.709	1.610	1.682	4.319	3.294	m
21	2.537	1.571	1.614	4.109	3.134	m
22	2.618	1.682	1.556	4.301	3.281	m
23	2.965	1.135	2.611	4.100	3.127	m
24	2.367	1.633	1.450	4.000	3.051	m
25	2.134	1.866	1.144	4.000	3.051	m
26	1.971	1.766	1.116	3.737	2.850	m
27	2.255	1.400	1.610	3.655	2.788	m
28	2.385	1.034	2.306	3.419	2.608	m

Table5. *Summary of the morphological and numerical data of the mating groups.*

Mating groups	Total length of haploid chromosome (mm)	Total length long arm (mm)	Total length short arm (mm)	2n
WW	118.664	83.131	35.536	56
HH	157.437	105.955	51.469	56
WH	143.883	105.297	38.584	56
HW	131.103	99.274	31.828	56

The differences could be as a result of their different population, geographical location and genetic divergence due to dissimilar selective pressures. It had been reported that in Siluroid families, chromosomes arms and numbers varied greatly and can be used as tools for classifying and delineate the species of a typical fish. Karyotypic formular within the groups in this study varied. The parentals and the hybrids with varied karyotypic forms may possibly lead to mosaic condition in fish within a population.

This view is supported by the report of Eyo in 2005 who stated that variation among the chromosomal karyotype of fish species in a population is possible. It may possibly be that karyotypic forms may not only differ with population but also with the capability contain by fish populations to interbreed thereby resulting in genetic diversity associated with speciation.

The occurrence of chromosome number around modal values among the Clariids may suggest chromosomal changes associated with the process of speciation within the group, possibly through high rate of hybridization (Awodiran *et al* 2014 and Eyo, 2005).

The pattern of inheritance of total length of haploid chromosome and total length of long arm by hybrids appeared to be intermediate to that of the parentatals. The range of the total length of haploid chromosome and total length of long arm could serve as a means of identifying hybrids of *C. gariepinus* and it could also be used as means to solving problems relating to phyletic relationship, taxonomic status and speciation studies among *Clarias* species. In this work, there was no chromosomal aberration of any sort in any of the group despite their background which shows proper pairing of the genes during fertilization.

There is need for more genetic studies on other Siluriform family and other species in Nigeria for proper pairing of fishes to improve aquacultural practices in this part of the world.

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- Staff of Laboratory Department , Osun State Management Board, Ilesa, Osun State

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