CHROMOSOMAL AND HISTOLOGICAL EVIDENCES OF INFERTILITY IN F2 AND F2 BACKCROSS. HYBRID GGENERATIONS OF *CLARIAS ANGUILIARIS AND HETEROBRANCHUS LONGIFILIS.*

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ABSTRACT

Male meiosis was studied in nine different mating combinations in parental, first, second and backcross generation hybrids of *Clarias anguillaris* and *Heterobranchus longifilis*. 27 bivalents were recorded in metaphase I for seven mating combinations. The number of bivalents in F_1 hybrid male x *C. Anguillairs* female could not be determined due to a high degree of clumping of the chromosomes. All metaphase I cells observed in female F_1 hybrid x male *H. longifilis* had three complex bivalents consisting of 43.3% giant ring and 56.7% giant rod chromosomes.

The number of ring bivalents per cell was higher in parental *H. longifilis* than parental *C. anguillaris*. The number of ring bivalents per cell increased from F_1 (6.7 and 8.2) to F_2 backcross (13.5) hybrid generations indicating increasing chromosomal instability of backcross hybrids over F_1 and F_2 hybrids.

INTRODUCTION

The intergeneric hybrid between the African Catfish, *Heterobranchus* species (male) and *Clarias* species (female) commonly known as `Heteroclarias' is widely used in aquaculture in Nigeria (Madu *et al*, 1992; Salami *et al, 1993;* Adeyemo *et al*, 1994; Aluko, 1995 and Nwadukwe, 1995) and in other countries (Legendre *et al,* 1992). The F1 hybrid obtained from the cross between these two clariid species is fertile (Aluko, 1995 and Nwadukwe 1995). The presence of natural bybridization in any two species is an indication that the two species are genetically very close and interfertile. However, there is no evidence in the literature that the F1 hybrids of these two species could hybridize naturally in the wild.

Inspite of the widespread culture and commercial importance of *Clarias, Heterobranchus* and their hybrids in African aquaculture, virtually nothing is known about the underlying genetic basis of these species and their hybrids. A clear understanding of the underlying genetic mechanisms would help in overcoming existing taxonomic problems and thus lead to rapid progress in the development of new genetically improved hybrids. According to Srivastav and Raina (1986), the degree of pairing (homology) of meiotic chromosomes is important in elucidating innate cytogenetic mechanisms, which is an index of the hybridization potential of the product of a cross betweeen two species.

This study was therefore designed to investigate the possibility and implications of meiotic chromosomal differences of the parents, F_1, F_2 and backcross hybrid generations of *Heterobranchus longifilis* and *Clarias anguillaris.*

Materials and Methods:

Parental *H. longifilis and C. anguillaris* as well as breeders of F_1 hybrids of both sexes utilized for the investigation were obtained from the concrete tanks at the Hatchery Complex of National Institute for Fisheries Researh (NIFFR), New Bussa. nigeria. Al beeders were injected with 4mg/kg dry carp pituitary hrimone for a latency period of 10 hours.

Eggs and sperm of the parents and the F1 hybrids were mixed together to generate nine different combinations as follows;-
H. longifilis (males x females), C anguillaries (male x female) h. logifilis female x male C anguilaris, F1 hybrid male x F1 hybrid female, F1 Hybrid male x female parental H. longifilis, F1 hybrid male x female C anguilaries, F1 hybrid female x H logifilis male, and F1 hybrid female x parental C. anguillaris male.

Fertilized incubated at 25 until hatching under aquarial conditions. Fry were fed with live zooplankon ad libitum for about four weeks. Fingerlings were fed with 49% CP exotic feed adlibitum in concrete tanks rich in zooplankton until maturity.

Testes were obtained by sacrificing mature male fish from each mating cpmbination for reparation of meiotic chromosomes. The testes were fixed directly in freshly prepared fixative (one part of acetic acid to three parts of ethanol) Small pieces of the testes were chopped using fine scissors or razor blade and macrated in two or three drops of 50% glacial acid solution unil cell suspension was obtained.

Chromosome slides were prepared by placing a few drops of the cell suspension on a clean pre-warmed slide on a slide dryer. Chromosomes were stained with F.L.p.orcein consisting of equal volumes of formic cid, lactic acid, propionic acid and distiled water mixed with 2g of orcein power for about 30 minutes.

The slides were viewed under a binocular research microscope .
Metaphase I chromosomes were phytographed using a phtomicroscope.
Negatives of the film were printed and photomicrographs of each mating combination analysed.

Results and Discussion

Parentals

In *H. longifilis* about 92.8% of the metaphase I examined had 27 bivalents (Plate 1 Ai&ii and Table 1). The remaining 7.2% were either less than or more than 27 bivalents. On the average, out of the 27 bivalents, 10.3 were ring and 16.7 were rod bivalents (Table 1).
About 90% of the metaphase I cells examined in *C. anguillaris* had 27 bivalents (Plate 1 Bi&ii) and Table 1). A few cells (10%) showed 23 and 29 bivalents. The mean values of ring and rod bivalents per cell were 5.0 and 22.0 respectively (Table 1). The report of Aluko (1996) on the chromosome complement of *C. anguillaris* support the present result on the 27 bivalents of the same species. There is no record in literature on the chromosome number of this species to date. Swanson (1957) reported that ring chromosomes originated from rod chromosomes. The presence of the ring bivalents in the parentals is an indication of high degree of homology in the chromosomes of the parentals. This homology of the chromosomes will give room for precise pairing of chromosomes of (diagrama) the F1 hybrids to form bivalents and consequently influence the viability of the F_1 crosses.

Table 1: Mean number of associations, ring and rod bivalents pe. involving parental *H. longifilis, C. anguillaris* and their F1 hybrid.

{PRIVATE } Genetic Group	n	Association at metaphase I	Mean number of ring bivalents	Mean number of rod bivalents
H. longifilis	27	27 II	10.3	16.7
C. anguillaris	27	27 II	5.0	22.0
Hetcla	27	27 II	6.7	20.3
Clahet	27	27 II	8.2	18.8

F_1 x F_1	27	27 II	10.6	16.4
F_1 x H. long	27	27 II	11.0	16.0
F_1 x C. ang	C	C	C	C
F_1 x H. long	3	Rg & Rd Compl.	1.3	1.7
F_1 x C. ang	27	27 II	13.5	13.5

27II = 27 bivalents
Rg = Ring complex
Rd = Rod complex
H. long = Heterobranchus longifilis
C.and = Clarias anguillaris
C = Clumping of bivalents
Hetcla = H. Longifilis x C. anguillaris
Clahet = H. longifilis x C. anguillaris

F1 Hybrids:

27 bivalents were recorded in about 80% metaphase cells examined in *H. longifilis* male x *C. anguillaris* female hybrid (Plate 1 Ci & ii, Table 1) out of which a mean of 6.7 were ring and 20.3 rod bivalents (Table 1).

Over 90% of the metaphase 1 cells viewed had 27 bivalents in *H. longifilis* female x *C. anguillaris* male reciprocal hybrid cross (Plate 1 Di & ii and Table 1). A mean of 8.2 and 18.8 ring and rod bivalents respectively were observed (Table 1). The level of ring chromosomes (7.7) in *H. longifilis* female x *C. anguillaris* male and less than parental average in *H. longifilis* male x *C. anguillaris* female. These hybrids were fertile.

F_2 Hybrid:

Plate 1 Ci & ii shows the selfing of the cross involving male *H. longifilis* x *C. anguillaris* female, that is F_1 male x F_1 female. About 87.5% of the cells identified as metaphase 1 had 27 bivalents and mean values for ring and rod bivalents were 10.6 and 16.4 respectively (Table 1). This result show an increase in the number of bivalents that were ring and a decrease in the number of rod bivalents compared to the parental F_1.

Backcross Hybrids:

About 87.5% of the metaphase I cells examined in the cross involving male F1 and female *H. longifilis* contained 27 bivalents and 12.5% with 26 bivalents. (Plate 1 Fi & ii and Table 1). On the average, 11.0 of the 27 bivalents were ring and 16.0 rod bivalents. The level of ring bivalents is an indication of increasing chromosomal instability of the backcross hybrid over the F_1 and F_2 hybrids.

All the cells observed in the cross involving male F_1 and female *C. anguillaris* contained clumped bivalents. No count could be done on the metaphase I as a result of the clumping (Plate 1 Gi & ii). Clumping of chromosomes is an aberration that could have sterility consequences.

In the cross between female F_1 and male *H. longifilis*, all the metaphase I cells examined had three complex bivalents (Plate 1 Hi& ii). Each complex probably contain an average of nine bivalents. In each set of the three complex bivalents, a mean value of 1.3 were ring and 1.7 rod bivalents (Table 1). These mean values translate to 11.7 (43.3%) ring and 15.3 (56.7%) rod bivalents assuming that the metaphase I contains 27 bivalents 27 bivalents as in the other mating combinations discussed above.

The likage of all the chromosomes to produce three translocation complexes consisting of 43.3% giant ring and 56.7% giant rod chromosomes could have arisen after a series of at least nine interchanges with non-homologous chromosomes. This phenomenon has been well documented in some plants like *Rhoeo discolor* in the family *Commelinaceae* where a ring of 12 chromosomes was found in *Oenothera* species where all 14 chromosomes were linked in a ring at meiosis (Swanson *et al*, 1967). According to Swanson *et al* (1967), as the translocation complex increases in size, the number of independent linkage groups decreases leading to an aggrregate effect of sharply reducing the component of variability. The overall effect could be sterility.

Over 90% of metaphase I cells examined in female F1 x male *C. anguillaris* had 27 bivalents (Plate 1 Ii & ii). The mean values of 13.5 ring and 13.5 rod bivalents were observed.

The level of chromosomal aberration at metaphase I is very high and significant in all the backcross hybrids examined. According to Swanson *et al* (1967) ring chromosomes are associated with phenotypic abnormalities.

The fertility of the sexes could be further confirmed by histological sectioning of the testes and ovaries?

Sterility of species could be of advantage in aquaculture in terms of better growth performance over non-sterile populations. However, the report of Aluko (1995b) indicated that the backcross hybrids were not as fast growing as the F_1 and F_2 hybrids. As a result of the present finding and others highlighted in Aluko (1995a), the propagation of backcross hybrids of *Heterobranchus* and *Clarias* species should not be encouraged.

REFERENCES

Adeyemo A.A., G.A. Oladosu and A.O. Ayinla (1994): Growth and survival of fry of African catfish species, *Clarias gariepinus* Burchell, *Heterobranchus bidorsalis* Geoffery and Heteroclarias reared on *Moina dubia* in comparison with other first feed sources. *Aquaculture* 119:41-45.

Aluko P.O. (1995a): Variation in Dorsal and Adipose fin length of the F_1 F_2 and Backcross Hybrids involving Parental *Heterobranchus longifilis* and *Clarias anguillaris*. 1995, *NIFFR Annual report.*

Aluko P.O. (1995b): Growth characteristics of first, second and backcross generations of the hybrids between *Heterobranchus longifilis* and *Clarias anguillaris*. 1995 *NIFFR Annual Report.*

Aluko P.O. (1996): Karyotypic Analysis of the African Catfish, *Clarias anguillaris*. 1996 *NIFFR Annual Report.*

Legendre M., G.G. Teugels, C. Cauty and B. Jalabert (1992): A Comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell 1822), *Heterobranchus longifilis* (Valenciennes, 1840), and their reciprocal hybrids (Pisces: Clariidae). *J. Fish Biol* 40:59-79.

Madu C.T., S. Mohammed, A. Mezie, J. Issa and E.O. Ita (1992). Comparative growth, survival and morphometric characteristic of *Clarias gariupinus, Heterobranchus bidorsalis* and their hybrid fingerlings. *NIFFR Annual Report* pp 56-61.

Nwadukwe F.O. (1995): Hatchery propagations of five hybrid Groups by Artificial Hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (Val) (Clariidae) using dry, powdered carp pituitary hormone. *J. Aqua. Trop* 10:1-11.

Salami, A.A., O.A. Fagbenro and D.H.J. Sydenham (1993): The production and Growth of Clariid Catfish Hybrids in concrete tanks. *The Isreali J Aqua-Bamidgeh* 45 (1): 18-25.

Srivastav, P.K. and S.N. Raina (1986): Cytogenetics of Tephrosia VI. Meiotic systems in some taxa. *Cytologia* 51:359-373.

Swanson, C.P. (1957): *Cytology and Cytogenetics.* Published by Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 596 pp.

Swanson, C.P., T. Merz and W.J. Young (1967): *Cytogenetics* Published by Prentice-Hall International Inc., London. 194 pp.