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A karyological analysis of the artificial hybridization between *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840 (Pisces; Clariidae)

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A karyological analysis of an artificial hybridization (reciprocal crosses) between two African clariid catfish, *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840, was performed. *C. gariepinus* has a standard karyotype of $2n=56$, while *H. longifilis* has $2n=52$. The hybrids revealed an intermediate karyotype ($2n=54$), and it appears as if they have totalized the haploid chromosome number of both parental species, excluding gynogenesis or androgenesis. The hybrid karyotype is considered as aneuploid, although the hybrids proved to be fertile. No variation was found in the hybrids karyotypes.

Key words: *Clarias gariepinus*; *Heterobranchus longifilis*; karyology.

I. INTRODUCTION

Hecht & Lublinkhof (1985) were the first to report the artificial hybridization between the clariid catfishes *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840. Subsequently, no other studies on hybridization of other African clariids have been published. Boonbrahm *et al.* (1977) discussed the hybridization of Asian *Clarias* species; other papers have examined the hybridization between clariids and other catfish such as pangasiids (Tarnchalanukit, 1986) and heteropneustids (Mukhopadathy & Dehadrai, 1987). None of these papers, however, presented a karyological analysis of the hybridization.

Since 1987, one of us (M.L.) has successfully performed several artificial hybridizations between *C. gariepinus* and *H. longifilis*. Originally, these experiments were performed to examine hybrid characters (morphology, growth rate, sex ratio, fertility, etc.) and to compare them to those of the parental strains. These results are discussed in detail in Legendre *et al.* (1992).

In this paper, a karyological analysis of this intergeneric hybridization is given in order to obtain a better understanding of its mechanism.

II. MATERIALS AND METHODS

The following specimens were examined in this hybridization experiment:

C. gariepinus: Four males, 460 to 585 mm T.L. (weight 700–1041 g); five females, 394 to 440 mm T.L. (weight 415–572 g). All specimens from the experimental fish culture station of

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the Institut des Savannes (IDESSA), [formerly the Centre Technique Forestier Tropical (CTFT)], at Bouake, Ivory Coast. Specimens deposited in the Muséum National d'Histoire Naturelle (MNHN) Paris (registered MNHN 1989.492-500).

H. longifilis: Five males, 702 to 895 mm T.L. (weight 2963–5275 g); six females, 710–865 mm T.L. (weight 2813–4950 g). All specimens from the experimental fish culture station of the Centre de Recherche Océanographique at Layo, Ivory Coast (MNHN 1989.698-690; 1989.700-702; 1989.709-713).

Female *H. longifilis* × male *C. gariepinus*: One female, 190 mm T.L. (weight 41·5 g) (MNHN 1990.59).

Female *C. gariepinus* × male *H. longifilis*: Two males, 120 to 145 mm T.L. (weight 11·8–21·6 g); two females, 120 to 210 mm T.L. (weight 12·1–71·1 g); one specimen of undetermined sex, 110 mm T.L. (weight 9·3 g) (MNHN 1990.59).

Specimens of *Clarias* and *Heterobranchus* were identified following Teugels (1986) and Teugels *et al.* (1990), respectively.

The hybridization was performed at the experimental fish culture station of the Centre de Recherche Océanographique at Layo, Ivory Coast in April 1988. Details on the hybridization technique are given in Legendre *et al.* (1992).

Immediately after the hybridization, the specimens of *C. gariepinus* and *H. longifilis* were karyotyped, following the technique described by Ozouf-Costaz *et al.* (1990). Several newly hatched hybrid larvae were karyotyped, but the results were unsuccessful. This might well be related to Bolla's conclusion (1987) that the cellular division rate remains very low until the complete resorption of the yolk sac. Therefore 10 hybrid specimens were reared in a tank at the Muséum National d'Histoire Naturelle in Paris, where the six surviving specimens were karyotyped in January 1989, using the technique of Ozouf-Costaz *et al.* (1990), with the following modifications: 48 h prior to colchicine treatment, 0·5 g baker's yeast and 0·5 g glucose were suspended in 7 ml distilled water and activated at 40° C during 20 min. This suspension was then injected s.c. to each fish (1 ml per 100 g weight) in order to increase the mitotic index. Fish were kept in a well oxygenated aquarium (30° C) until colchicine treatment.

Staining of the nucleolus organizer regions followed the technique of Howell & Black (1980). C and G banding were unsuccessful in *C. gariepinus* chromosomes (see Ozouf-Costaz *et al.*, 1990); these techniques have not been applied to *H. longifilis* nor to the hybrids.

Nomenclature used is that followed by Ozouf-Costaz *et al.* (1990). Abbreviations: a, acrocentric; m, metacentric; NORs, nucleolus organizer regions; SAT, satellite; sm, submetacentric.

Prints of representative standard karyotypes of males and females of the parental species and the hybrids are available from the author's address.

III. RESULTS

Species of the genus *Heterobranchus* differ morphologically from those of the genus *Clarias*, mainly in the presence of a large adipose fin, originating immediately behind the rayed dorsal fin and reaching the caudal fin base; the adipose fin is supported by extended neural spines (Teugels, 1983). The hybrids of *H. longifilis* and *C. gariepinus* have an intermediate external morphology and also show extended neural spines in the adipose fin.

STANDARD KARYOTYPES

1. C. gariepinus

This species shows a modal chromosome number of $2n = 56$ (Table I), including one heteromorphic pair ZW in females and one pair of chromosomes with satellites (SAT). The chromosome formula is as follows: males: 8m + 24sm + 24a, with the arm number (NF) = 88; females: 8m + 25sm + 23a, NF = 89. A detailed description

TABLE I. Frequency distribution of the diploid chromosome number of *Clarias gariepinus*, *Heterobranchus longifilis* and *Clarias gariepinus* × *Heterobranchus longifilis*, examined in this study

Species	No. of specimens			Diploid chromosome no. (*2n*)													No. of metaphases plates			
	Male	Female	Total	45	46	47	48	49	50	51	52	53	54	55	56	57	Counted	Karyotyped		
																		Male	Female	Total
C. gariepinus (*CGA*)	4	5	9	—	—	—	—	—	2	2	1	3	5	8	24	1	46	4	4	8
H. longifilis (*HLO*)	4	7	11	3	4	8	12	17	20	32	160	4	1	—	—	—	261	11	12	23
Female *HLO* × male *CGA*	—	1	1	3	—	3	—	2	10	15	18	39	230	8	1	2	331	9	11	20
Female *CGA* × male *HLO*	2	3	5																	

of the karyotype has been given by Ozouf-Costaz *et al.* (1990); the reader is referred to this paper for further details.

2. H. longifilis

Due to field conditions, the quality of the chromosome preparations of this species was somewhat inferior to that for *C. gariepinus*: chromatids were generally more condensed.

The modal chromosome number of $2n=52$ has been observed (Table I). The chromosome formula is as follows: males: 6m+24sm+22a, NF=82; females: 6m+25sm+21a, NF=83.

Despite the difference in diploid chromosome number between *H. longifilis* and *C. gariepinus*, the general morphological appearance of the chromosomes in both species is very similar. The diversity in size and shape facilitates pair–group comparisons between the two species. It should be noted that two teams have worked independently on the analysis of the metaphases; both tried to reproduce the morphological classification of the chromosomes as obtained for *C. gariepinus*. Identical results of pair classification for *H. longifilis* were obtained by both teams.

Pair number 2 (large acrocentrics) and pair number 23 (small metacentrics) as found in *C. gariepinus* are lacking in *H. longifilis*.

A pair of heteromorphic sex chromosomes ZW was found in all females (a large submetacentric and a small acrocentric, as in female *C. gariepinus*). The difference in size of the chromosomes of this pair is less pronounced than in *C. gariepinus*. The W-chromosome seems smaller. This, however, may be related to their more contracted arms.

The ZZ pair in *Heterobranchus* has a similar appearance as that found in *C. gariepinus*.

Using the conventional Giemsa staining, about half of the metaphases used to study the karyotype revealed the presence of one pair of chromosomes with satellites. They show a similar morphology as that found in *C. gariepinus*, though they appear to be slightly smaller. They also turned out to be the NOR-bearing chromosomes. The scarcity of early metaphase stages obtained in this material together with the linked contraction of the arms might explain why a pair of NOR-bearing chromosomes was found only once (in a male specimen). Such associations are generally observed in prometaphases.

3. C. gariepinus × H. longifilis

The mitotic index was very significant in the hybrids, due to the injection of baker's yeast.

Identical karyotypes were obtained for both female *H. longifilis* × male *C. gariepinus* and female *C. gariepinus* × male *H. longifilis* hybrids.

The modal chromosome number is $2n=54$ (Table I). The chromosome formula is as follows: males: 7m+24sm+23a, NF=85; females: 7m+25sm+22a, NF=86.

The hybrid karyotype contains all the chromosome pairs common to both parental species. In addition, it has two unpaired chromosomes, one large acrocentric and one small metacentric.

Using only the Giemsa staining, one pair of chromosomes with satellites is generally found. NORs are also present in the hybrids. The size of one of the NOR-bearing chromosomes is distinctly smaller than that of the other one.

One ZW heteromorphic pair is found in all female hybrid karyotypes.

The ZZ chromosome pair in male hybrids is similar to that found in male *C. gariepinus* and in male *H. longifilis* specimens.

IV. DISCUSSION

C. gariepinus and *H. longifilis* have a similar morphology and differ mainly in the adipose fin complex present in the latter. Striking similarities are also found in the karyotypes of both species and most pairs appear homologous. Obviously this has favoured the hybridization.

The hybrid karyotype is completely intermediate between that of the two parental species. It appears that the hybrid has totalized the haploid chromosome number of both *C. gariepinus* and of *H. longifilis*: the homologous pairs are all present and in addition, two unpaired chromosomes, one large acrocentric and one small metacentric can be discerned. The latter two obviously originate from the *C. gariepinus* karyotype. The composition of the hybrid karyotype therefore definitely excludes gynogenesis or androgenesis, processes in which the embryo contains respectively maternal or paternal chromosomes only, due to a failure of the nucleus of the male or the female gamete respectively, to participate in fertilization.

Due to the presence of the two unpaired chromosomes, the hybrid can be considered as aneuploid. Generally, aneuploids are sterile (Gorenflot & Raicu, 1980) and their gonads are abnormally developed. Legendre *et al.* (1991) however, reported that the hybrids are not entirely sterile. They develop fertile gonads although the numbers of spermatozoa and oocytes produced are considerably lower than in both parental species. Reproduction was thus possible; also a backcross with the parental species was successfully performed. This might be related to the presence of the complete haploid set of *H. longifilis* combined with the homologous chromosomes of *C. gariepinus*.

As an alternative to the suggested aneuploidy, however, it is also possible that the chromosome number differences in *C. gariepinus* and *H. longifilis* are due to translocations or Robertsonian fusions (Ferguson, 1980; Gorenflot & Raicu, 1980) and that the hybrid has the complete diploid set albeit not in strict chromosome pairs. Analysis of pairing in meiotic metaphases might reveal the chromosome homology. This is the subject of further research. In view of the difficulties in reproducing the hybrids however, we presently are tempted to accept the aneuploidy hypothesis.

No variation was found in the hybrid karyotype. This homogeneity could be an argument in favour of the propagation of their use in aquaculture. But at the present stage, no clear advantage in using the hybrid instead of *H. longifilis*, has been demonstrated (Legendre *et al.*, 1992).

Determination of the hybrid sex on the basis of their gonads, revealed a direct relation with the presence of the ZZ or ZW chromosomes. Therefore, we were able to confirm recent assumptions, discussed by Ozouf-Costaz *et al.* (1990), on the role of these chromosomes in sex determination.

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References

Bolla, S. (1987). Cytogenetic studies in Atlantic salmon and rainbow trout embryos. *Hereditas* **106,** 11–17.

Boonbrahm, M., Tarnchalanukit, W. & Suraniranat, P. (1977). Experiments on hybridization of fresh-water catfish, *Clarias macrocephalus* Günther and *Clarias batrachus*. Research Report of the Kasetsart University, 143.

Ferguson, A. (1980). *Biochemical Systematics and Evolution*. Glasgow: Blackie.

Gorenflot, R. & Raicu, P. (1980). *Cytogénétique et Évolution*. Paris: Masson.

Hecht, T & Lublinkhof, W. (1985). *Clarias gariepinus* × *Heterobranchus longifilis* (Clariidae: Pisces): a new hybrid for aquaculture. *South African Journal of Science* **81,** 620–621.

Howell, W. M. & Black, B. A. (1980). Controlled silver staining of nucleolus organizer regions with a prospective colloidal developer: a one step method. *Experientia* **36,** 1014–1015.

Legendre, M., Teugels, G. G., Cauty, C. & Jalabert, B. (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). *Journal of Fish Biology* **40,** 59–79.

Mukhopadathy, S. M. & Dehadrai, P. V. (1987). Survival of hybrids between air-breathing catfishes *Heteropneustes fossilis* (Bloch) and *Clarias batrachus* (Linn.). *Matsya* **12–13,** 162–164.

Ozouf-Costaz, C., Teugels, G. G. & Legendre, M. (1990). Karyological analysis of three strains of the African Clariidae catfish *Clarias gariepinus* used in aquaculture. *Aquaculture* **88,** 271–277.

Tarnchalanukit, W. (1986). Experimental hybridization between catfishes of the families Clariidae and Pangasiidae in Thailand. *Environmental Biology of Fishes* **16,** 317–320.

Teugels, G. G. (1983). La structure de la nageoire adipeuse dans les genres de poissons-chats *Dinotopterus*, *Heterobranchus* et *Clarias* (Pisces; Clariidae). *Cybium* **7,** 11–14.

Teugels, G. G. (1986). A systematic revision of the African species of the genus *Clarias* (Pisces, Clariidae). *Annales du Musée royal de l'Afrique Centrale* **247,** 1–199.

Teugels G. G., Denayer, B. & Legendre, M. (1990). A systematic revision of the African catfish genus *Heterobranchus* (Pisces; Clariidae). *Zoological Journal of the Linnean Society* **98,** 237–257.