Aquaculture, 87 (1990) 271-277
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Karyological Analysis of Three Strains of the African Catfish, *Clarias gariepinus* (Clariidae), used in Aquaculture

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(Accepted 14 October 1989)

ABSTRACT

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: 271-277.

A karyological analysis of three strains of *Clarias gariepinus* used in fish culture, originating from different localities in Africa and Asia Minor, and thus representing populations from the distribution limits of this species, revealed an identical karyotype, 2n=56 with a pair of heteromorphic sex chromosomes (ZW) and a nucleolus organizer (NOR) location in a single pair of chromosomes, for all strains. These results confirm the synonymy between *C. gariepinus*, *C. lazera* and *C. mossambicus* as introduced by Teugels in 1982.

INTRODUCTION

From the start of experimental fish culture in Africa in the early fifties, species of the catfish genus *Clarias* were shown to be highly suitable for this purpose. Their size, fast growth, omnivoracity and resistance to extreme environmental conditions, together with the relatively simple techniques for their artificial reproduction and their almost Pan-African distribution, favoured their culture. *Clarias* culture has become popular and many African fish culture stations are presently working with these fishes.

The taxonomy of African *Clarias* species used in aquaculture has for a long time been extremely confusing. Papers and reports dealt mainly with three nominal species: *Clarias gariepinus* (Burchell 1822), *C. lazera* Valenciennes 1840 and *C. mossambicus* Peters 1852. Teugels (1982, 1984, 1986) introduced the synonymy of these three nominal species, whose identification used to be

0044-8486/90/$03.50

based merely on the geographic origin of the specimens. Teugels' synonymy relied for the most part on the results of a comparative morphological and osteological study of populations sampled in different localities all over tropical Africa but not South-East Asia, although data have also been obtained from a biological study of populations from Israel and southern Africa (Bruton et al., 1990). As a result of the synonymy, the name *Clarias gariepinus* is at present generally accepted.

Recently we were able to examine living specimens of *Clarias gariepinus* originating from three fish culture stations (Israel, Ivory Coast and Central African Republic). This paper deals with the karyological analysis of three strains of *C. gariepinus*, in order to double-check the synonymy and as part of a continuing programme on the cytogenetics of clariid catfishes used in fish culture (*Clarias gariepinus, Heterobranchus longifilis* and their hybrids).

MATERIALS AND METHODS

The following specimens, subsequently deposited in the Muséum National d'Histoire Naturelle (MNHN), Paris (France), and the Musée Royal de l'Afrique Centrale (MRAC), Tervuren (Belgium), were examined in this study:
- MRAC 88-08-p-1-15 from the experimental fish culture station of the Katholieke Universiteit Leuven (Belgium), originating from the Hulagh swamps above Lake Kinneret, Israel; 15 specimens (seven females and eight males), 240–465 mm standard length.
- MRAC 88-08-P-16-28 from the experimental fish culture station of the Katholieke Universiteit Leuven (Belgium), originating (via the Landbouwhogeschool, Wageningen, The Netherlands) from a fish culture station at Bangui, Central African Republic; 13 specimens (six females and seven males), 240–380 mm standard length.
- MNHN 1989-492 to 500 from the experimental fish culture station of the Institut des Savannes (IDESSA), formerly the Centre Technique Forestier Tropical (CTFT), at Bouaké, Ivory Coast; nine specimens (five females and four males), 412–585 mm standard length.

The specimens from IDESSA (Bouaké) were examined under field conditions in the experimental fish culture station of the Centre de Recherches Océanographiques at Layo (Ivory Coast). The others were studied under laboratory conditions at the Zoological Institute of the Katholieke Universiteit Leuven (Belgium).

Chromosome preparations were obtained directly from the anterior kidney using the method of Doussau de Bazignan and Ozouf-Costaz (1985) with the following modifications: dorsal or intraperitoneal injection of 0.5% colchicine (0.3 ml per 100 g weight); hypotonic treatment: 25 min at 20°C or 20 min at 25°C in 0.56% KCl. For standard karyotypes, the air-dried preparations were stained with 4% Giemsa. Staining of the nucleolus organizer regions (NORs)

followed the technique of Howell and Black (1980). Some attempts with C-banding technique for the study of constitutive heterochromatin were carried out (following Sumner, 1972) but did not provide any positive results. Well spread metaphases were selected and photographed using Agfa-ortho professional film (ASA 25) and a Zeiss II photomicroscope. For establishing the karyotypes, the best photographs were used for cutting out, pairing and classifying chromosomes in decreasing size, so that the karyotypes could be compared with each other. According to the nomenclature of Levan et al. (1964), chromosomes were considered as metacentric (m) when centromere index (C) was 46–50, submetacentric (sm) when C was 35–45 and acrocentric (a) when C was less than 35.

RESULTS

The mitotic index and chromosome quality obtained for specimens from Israel and the Central African Republic were generally good. On the contrary, the proportion of metaphases was much lower for specimens from the Ivory Coast and the chromosome arms were more condensed, possibly due to difficulties in the field conditions. We counted at least five metaphase plates per specimen and examined at least 20 photographs per strain (Table 1).

Standard karyotypes

The three strains showed a modal chromosome number of 2n=56 (Table 1). Comparison of their karyotypes did not reveal any numerical or morphological variability. Representative male and female karyotypes from two different strains (Israel and Central Africa, respectively) are shown in Fig. 1. They

TABLE 1

Frequency distribution of diploid chromosome numbers in three strains of *Clarias gariepinus*

Strain	Number of specimens			2n								Total number of metaphase plates			
	♂	♀	Total	50	51	52	53	54	55	56	57	Counted visually[a]	Karyotyped		
													♂	♀	Total
Israel	8	7	15	–	1	2	5	11	14	103	3	139	29	15	44
Africa (Bangui)	7	6	13	2	–	1	5	4	12	40	1	65	14	9	23
Africa (Bouaké)	4	5	9	2	2	1	3	5	8	24	1	46	4	4	8
Total	19	18	37	4	3	4	13	20	34	167	5	250	47	28	75

[a]Either through the microscope or via photographs.

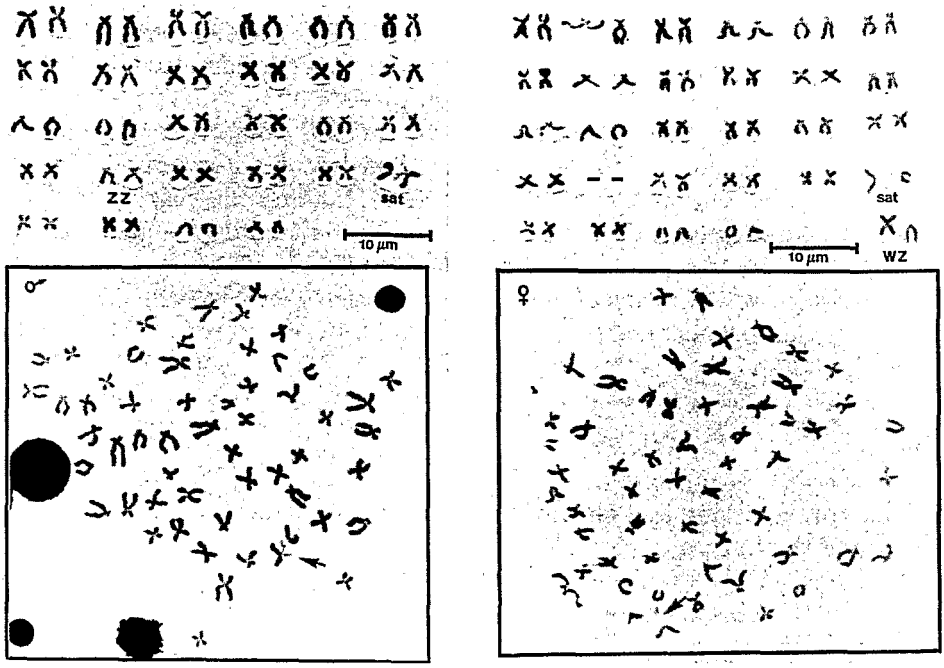

Fig. 1. Representative standard karyotypes of a male (Israel strain) and a female (Central Africa strain) of *Clarias gariepinus*. ZZ and WZ, sex chromosomes; arrows show the associated satellites (sat).

are characterized by a high proportion of meta–submetacentric chromosomes. Some short chromosomes have very similar sizes, making pairing difficult. However, the low proportion of acrocentrics allows a more accurate definition, some of them being easily identified (pairs 2, 27 and 28). All female karyotypes exhibited a heteromorphic pair (a large submetacentric and a small acrocentric) corresponding to a pair of small acrocentrics in males. These chromosomes may be of the sex complex ZZ–ZW and thus are indicated with these symbols on Fig. 1. In both male and female, the sm chromosomes of pair 24 (arrow) are linked together by their short arms, which appear as satellites (sat). This association was found in about 75% of the metaphase plates studied, regardless of the strain used.

The following chromosome formulae were established: males: 8m+24sm+24a, NF (arm number)=88; females: 8m+25sm+23a, NF=89.

Nucleolus organizer regions (NORs)

Fig. 2 compares the same metaphase plate after Giemsa staining and after silver staining. Interestingly, we noticed that the silver stain often provided better results, as the chromosome morphology was more clearly and accurately revealed. In all the silver-stained metaphases from the three strains, the chro-

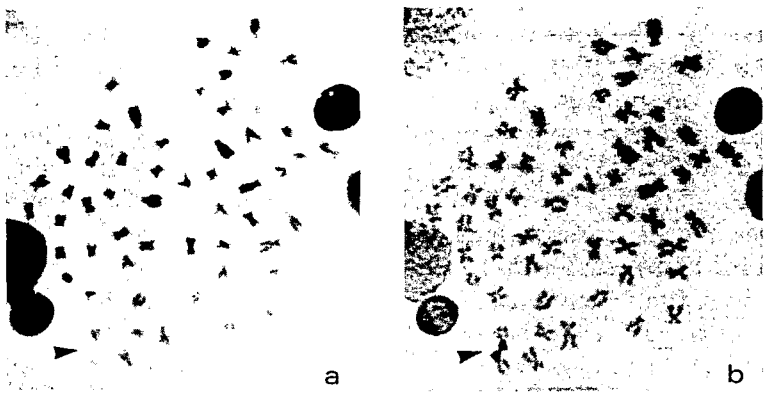

Fig. 2. Conventional Giemsa staining (a) and silver staining (b) demonstrating the corresponding position of NORs with the associated satellites (arrows).

mosomes bearing NORs (arrows) correspond to the pair of homologs linked together, and the NORs are located on their whole satellite short arm.

DISCUSSION

The most recent and complete data summarizing siluroid karyotypes have been produced by Rab (1981) and Vasiliev (1985). In siluroid families, chromosome and/or chromosome arm numbers exhibit a great variability, and we can assume that the karyotype is specific and that this criterion can be used for the species characterization. Moreover, in fish, the NOR-bearing chromosomes, as well as the position of the NORs on the chromosomes, are remarkably diverse among the species and no intraspecific variation is recorded except for species having multiple NOR sites or very large NORs (Takai and Ojima, 1986). We tried to test for diversity among the three strains by comparing their NOR-bearing chromosomes. As no significant differences between the three *Clarias* strains were found in the chromosome number, formula and the position of the NORs, this analysis clearly corroborates the results obtained by morphological and osteological studies and thus supports the synonymy introduced by Teugels (1982, 1984, 1986). It also seems to indicate that in this species, the karyotype is very stable as we did not detect any variability among the 37 specimens from three different geographic areas.

The diversity of the NORs and their relationship with species differentiation and evolution could be used in further studies on species of Clariidae. The location of the NORs in a single chromosome pair and their terminal position are generally considered as plesiomorphic characters. NOR-bearing chromosomes can also be used as markers for the recognition of parental chromosome sets in the chromosome complements of the hybrids. The mechanism of their association remains difficult to interpret, although such associations have been

frequently observed in fish (Foresti et al., 1981; Thode et al., 1983; Bolla, 1987; Feldberg et al., 1987). Rab (1981) assumes that the separation or association of satellites depends on the degree of chromatid spiralization.

Our analysis also revealed the presence of differentiated sex chromosomes that may belong to the ZZ–ZW system. Many cases of heterogamety, involving different mechanisms (see Bertollo et al., 1983), have already been observed in fish. Moreover, recent investigations on fish species (Ojima et al., 1984; Galetti and Foresti, 1986) assume that, as in other vertebrates, there is a close relationship between the sex chromosome differentiation and heterochromatinization of one of the previous homologs. However, since the ZZ–ZW chromosome system was C-band negative, this hypothesis could not be confirmed. In catfishes, the presence of differentiated sex chromosomes has not been recorded (see Vasiliev, 1985) except male heterogamety for one species of north American catfish *Noturus taylori* (Le Grande, 1981). Nevertheless, some of our investigations concerning other families of African siluriforms (unpublished) often revealed the existence of heterochromosomes, and we believe that with the development of in vivo techniques, they should be more frequently detected.

The first successful hybridization between the two species of Clariidae, *Clarias gariepinus* and *Heterobranchus longifilis*, was done in 1985 by Hecht and Lublinkhof. However, karyotypes of the parental species and of their hybrids remained unknown. It is of interest to know the karyotype of the species used for this hybridization in order to estimate their genetic purity. Furthermore, a study of the phylogenetic relationships between these species by karyological and biochemical methods would allow a prediction of the possible genomes obtainable in the hybrids. Finally, the analysis of their karyotypes would clarify the mechanism involved in karyogamy, as well as why these hybrids are viable and relatively fertile. This study is the first in a series to characterize the genomes of clariid catfishes used for fish culture and will be followed by definition of the genetic structure of hybrids. Although banding techniques and studying chromosomes at meiosis are particularly difficult to undertake in the field, the marker chromosomes detected in the standard karyotypes of *Clarias gariepinus* appear to be useful in comparing different species. Furthermore, these markers may also be useful in identifying parental genetic input in hybrid karyotypes.

ACKNOWLEDGEMENTS

We are indebted to Professor F. Ollevier (Katholieke Universiteit Leuven, Belgium) for granting facilities to work in his laboratories and to Miss L. Noterdaeme, Mr. P. Van Damme from Leuven and Mr. J. Slembrouck from Abidjan for their technical assistance. This paper forms part of the PEDALO Pro-

gramme (Poissons d'Eaux Douces de l'Afrique de l'Ouest), financed by ORSTOM and PIREN.

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