SHORT REPORTS

KARYOTYPE OF HYBRID CATFISH (*CLARIAS MACROCEPHALUS* X *CLARIAS GARIEPINUS*)

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(Received November 1, 1997)

ABSTRACT

The F1 hybrid catfish, resulted from artificial insemination between males of Clarias gariepinus *and females of* Clarias macrocephalus*, was studied for the karyotype. The diploid chromosome number was 54 and consisted of 18 metacentric chromosomes, 18 submetacentric chromosomes and 18 telo- to acrocentric chromosomes.*

INTRODUCTION

Within the last few years, the study of fish karyotypes has stimulated the interest of many researchers. However, the small size and large number of chromosomes in fish, and the lack of a standard technique for fish chromosomal preparations make this study difficult. About 20,000 species of fishes are living today, among which the chromosome number of about 2000 has been determined. Of these, approximately 1320 have been karyotyped (AL-Sabti, 1991). Karyotypes of fishes have received special attention in recent years because of the interest in fish cytogenetics and the fact that fish are used for monitoring environmental carcinogens and cytotoxic chemicals (Kligerman, 1979; Landolt and Kocan, 1983; AL-Sabti *et al.*, 1984; AL-Sabti, 1985, 1986). Toxicologists have become interested in chromosomes of fishes within the last few years as indicators of water pollutants.

The hybrid catfish is an interspecific hybrid of Thai catfish (*Clarias macrocephalus*) and African catfish (*Clarias gariepinus*) by artificial insemination technique. The hybrid catfish possesses better characteristics than the parental species, including fast growth rate, more tolerance to adverse environmental conditions and diseases, a richer and better flavour and greater nutritional value.

Because of these characteristics, the hybrid catfish has become an economically important food in Thailand and some other Asian countries. The artificial insemination of the hybrid catfish is done in the rainy season when the parental catfishes reach maturity. Hormone extract, either human chorionic gonadotropin (HCG) or luteinizing hormone releasing hormone (LHRH) or analogue, is injected into the parental fishes. The hormone activates semination in male and stimulates egg maturity in female. Usually successful artificial insemination occurs between males of *C. macrocephalus* and females of *C. gariepinus*. The percentage of live embryos produced varies at each time of fertilization. The hybrids are generally sterile.

The karyotypes of African catfish (*C. gariepinus*) and Thai catfish (*C. macrocephalus*) have been studied by some investigators (Sittikraiwong, 1987; Ozouf-Costaz *et al.*, 1990). This paper presents the results of detailed studies of chromosome complements of the hybrid catfish (*C. macrocephalus* x *C. gariepinus*).

MATERIALS AND METHOD

Chemicals

All chemicals (AR grade) were purchased from Sigma Co. They were used without further purification.

Fishes

The hybrid catfish, aged 3 months, were purchased from Ayudthaya province. They were then reared in a one ton fiber glass tank at the National Inland Fisheries Institute until used. The fishes were fed twice a day with catfish's meal.

Method for chromosome study

Each fish was injected intraperitoneally with a dose of 0.1 ml of 0.1% colchicine per 20 grams body weight and placed in a well aerated container for 6 hours. After that, kidney tissue was removed from the fish and immersed in distilled water for 10 minutes before being fixed with Carnoy's fixative. The staining procedure was modified from Kligerman and Bloom (1977). The fixed tissue was cut into very small pieces (1-2 mm), placed in a well, minced with 1 drop of 60% acetic acid, and left for 15 minutes. After that the cells were drawn from the well with hematocrit tubes and dropped off onto clean glass slides at the height of 12-24 inches above the slide. The slides were air-dried before staining with 10% Giemsa solution in phosphate buffer pH 7.4, for 25 minutes.

Microscopy, photography and karyotyping.

An Olympus microscope equipped with a built in automatic camera with magnification of x10, x40 and x100 was used. Identification of chromosomes followed the methods of Le Jeune (1965), Chen and Ruddle (1970) and Chen (1971) with some modification. The chromosomes were arranged into 3 groups according to the centromere position, i.e., metacentric, submetacentric and telo- to acrocentric groups. Karyotypes and idiograms of the hybrid catfish were prepared by using the centromeric index (C.I.) and relative length (R.L.) value of the chromosomes. The C.I. and R.L. value could be calculated from the formulas: C.I. = L_l/L_T; R.L. = $L_T/\Sigma L_T$ where $L_T = L_s + L_l$ (L_l = length of long chromosome arm; L_s = length of short chromosome arm; L_T = total length of each chromosome) and ΣL_T = sum of L_T values from each pair of chromosomes in that cell. The chromosomes could be put together as "homologous chromosomes" when the C.I. , L_T and R.L. values were equal to one another.

The chromosomes are grouped according to the C.I value as follows(Le Jeune, 1965; Chen and Ruddle, 1970; Chen, 1971):

Centrometric position	Chromosome type	C.I value
median	metacentric (m)	0.50-0.59
sub-median	submetacentric (sm)	0.60-0.75
subterminal or terminal	acrocentric (a)or telocentric (t)	0.76-1.0

RESULTS

Forty-eight fish were used for karyotype study. The diploid chromosome numbers were counted from 287 cells (or at the average of 6 cells per individual) at the metaphase stage with well spread chromosomes. The 2n chromosome numbers varied from 50 to 56 with 54 giving the highest percentage (31%) (Fig. 1). Karyotyping was done by grouping of homologous chromosomes based on centromeric index (C.I.), length of chromosome (L_T), and relative length (R.L.) value of chromosome arms. It was found that there were 3 types of karyotypes (Fig. 2-4). By using C.I., L_T and R.L. values, the idiogram of hybrid catfish chromosomes was obtained (Fig. 5) from the study of 6 well spread chromosomes from kidney cells. These cells possessed 54 chromosomes (or 27 pairs) which consisted of 18 metacentric chromosomes, 18 submetacentric chromosomes, and 18 telo- to acrocentric chromosomes (Fig. 5).

DISCUSSION

A karyotype is the characterization and analysis of chromosomes according to their sizes, shapes and morphology (Denton, 1973). The study of karyotypes allows us to define accurately the genetic structure of the individual (Al-Sabti, 1991). The hybrid is intermediate between the two parental species, thus it should present each haploid set of chromosomes of the parental species. *Clarias gariepinus* has a diploid chromosome number of 56 (Fig. 6), consisting of 16 metacentric chromosomes, 22 submetacentric chromosomes and 18 telo- to acrocentric chromosomes (Ozouf-Costaz *et al.*, 1990), while Thai catfish (*C. macrocephalus*) have a diploid chromosome number of 52 (Fig. 7), consisting of 18 metacentric chromosomes, 20 submetacentric chromosomes and 14 telo- to acrocentric chromosomes (Sittikraiwong, 1987). The hybrid of *C. gariepinus* x *C. macrocephalus* showed the highest frequency of 2n = 54 (Fig. 1). However, there were 2 chromosomes that were not homologous, so they were grouped separately (Figs. 2-4). From observation, the hybrid catfish were mostly sterile. This result corresponds with the study of Nygren and colleagues (1975) on the hybrid of *Salmo salar* and *S. trutta*. They found that there was a relationship between hybrid sterility and production of unbalanced gametes of the hybrids. In addition, 3 different karyotypes of the hybrid catfish could be obtained from this study (Figs. 2-4). This result corresponds with Terao *et al.* (1965) who found that the fertility among hybrids from crosses between species there were a variety of different karyotypes. In-addition Al-Sabti (1991) mentioned that fish karyotypes are not identical as in humans or in other animal species, so for fish there is no standard karyotype because differences not only exist between species, but polymorphism often occurs within one fish species. These changes in number/and or morphology of chromosomes could be caused by chemical or physical effects on mechanisms of chromosome formation. This may also explain why differences exist in the karyotypes of different individuals of the same species from different waters (localities). Changes may also occur as a result of the amplification of genes involved in detoxification which is most likely to occur in polluted environments.

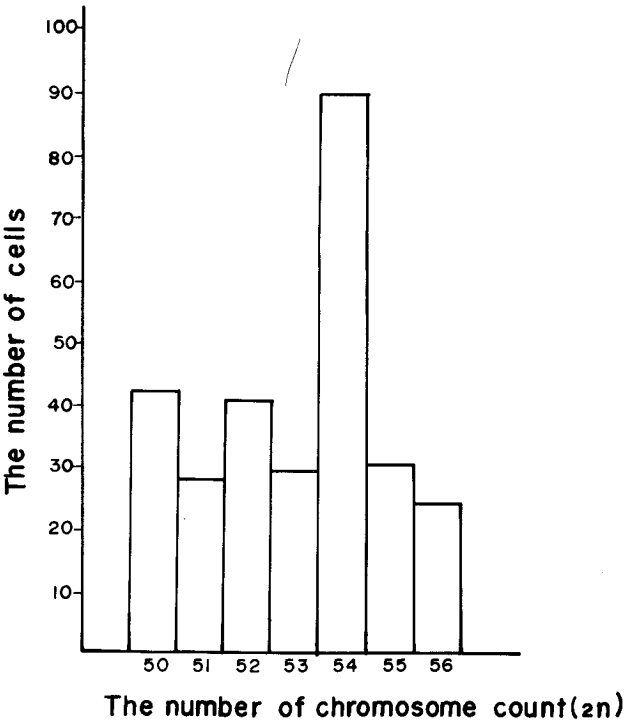

Fig. 1. Frequency distribution of the chromosome number of hybrid catfish.

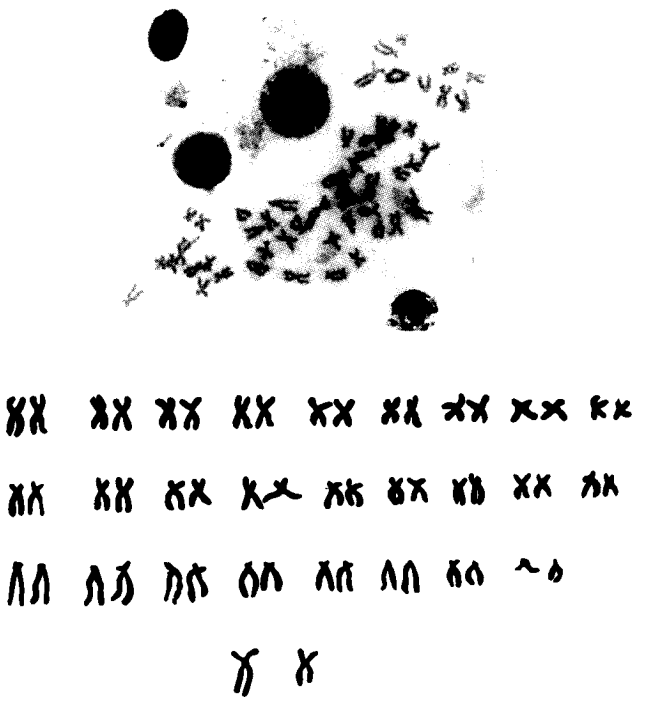

Fig. 2. A karyotype of somatic chromosomes of hybrid catfish. (2n = 54)

Fig. 3. A karyotype of somatic chromosomes of hybrid catfish. (2n = 54)

Fig. 4. A karyotype of somatic chromosomes of hybrid catfish. (2n = 54)

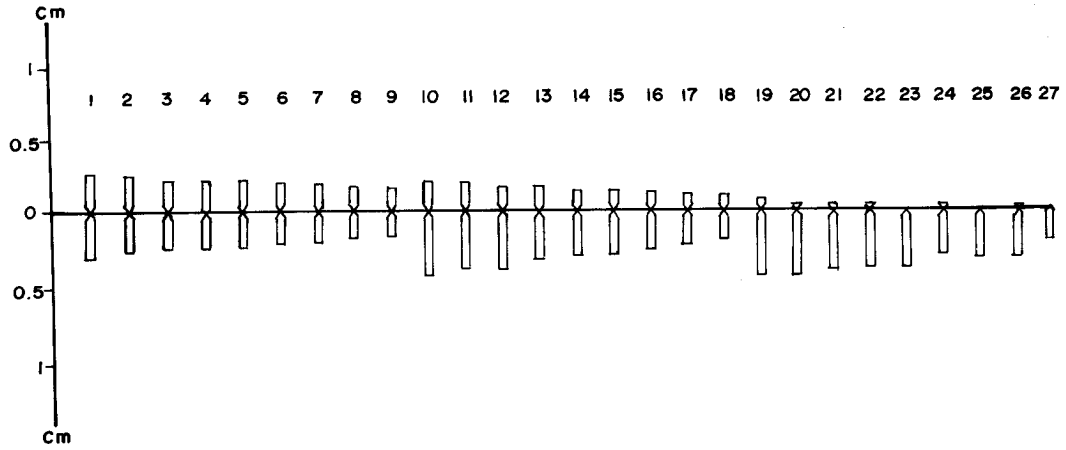

Fig. 5. Idiogram of hybrid catfish.

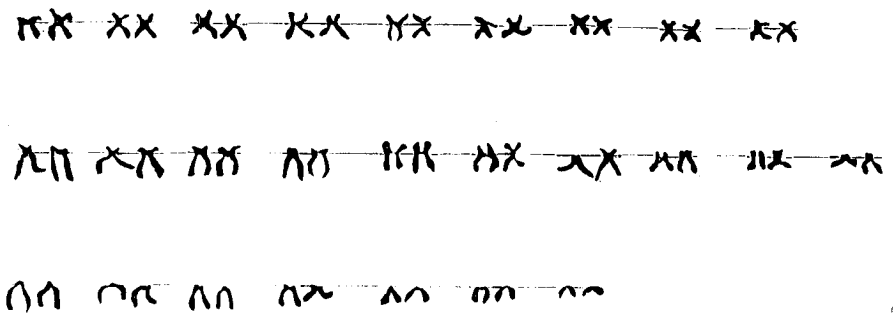

Fig. 6. The karyotype of *Clarias macrocephalus*. (2n = 52).
From: Sittikraiwong, 1987.

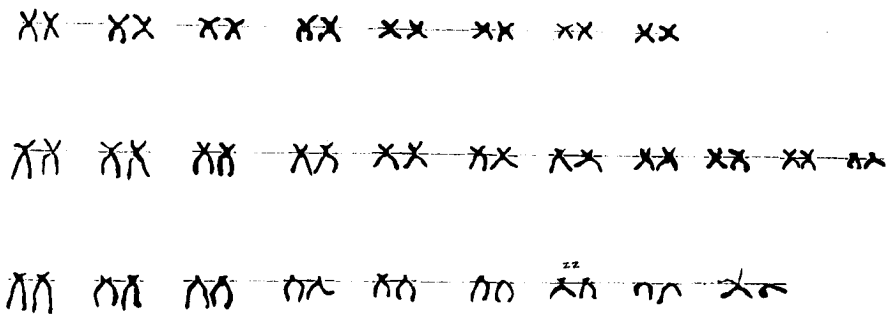

Fig. 7. The karyotype of *Clarias gariepinus*. (2n = 56).
From: Ozouf-Costaz *et al.*, 1990.

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บทคัดย่อ

ในการศึกษาคาริโอไทป์ของปลาดุกบิ๊กอุยซึ่งได้จากการผสมเทียมระหว่างปลาดุกรัสเซียเพศผู้และปลาดุกอุยเพศเมียพบว่าจำนวนดิปพลอยด์โครโมโซม มีค่าเท่ากับ 54 และประกอบด้วยโครโมโซมชนิดต่างๆ ได้แก่ metacentric 18 คู่, submetacentric 18 คู่ และ telocentric (acrocentric) อีก 18 คู่