

GYNOGENETIC FISH PRODUCED BY A BACKCROSS INVOLVING A MALE HYBRID (Female *Colossoma macropomum* x Male *Piaractus mesopotamicus*) AND A FEMALE *Piaractus mesopotamicus*

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ABSTRACT

The cytogenetic analysis of the B1 progeny obtained in a backcross involving a male F1 hybrid (produced by parental female *Colossoma macropomum* x parental male *Piaractus mesopotamicus*) and a P1 female *P. mesopotamicus* showed that individuals of B1 generation presented karyotypes identical to the parental female lineage, suggesting that they were produced by gynogenesis. The production of gynogenetic fish by controlled crosses and the application of cytogenetic markers to identify the parental contributions are important in programs of interspecific hybridization in fish culture.

Key words: Gynogenesis, neotropical fish, *Colossoma macropomum*, *Piaractus mesopotamicus*

RESUMO

Peixes ginogenéticos produzidos pelo retrocruzamento envolvendo macho híbrido (Fêmea de *Colossoma macropomum* x macho de *Piaractus mesopotamicus*) e fêmea de *P. mesopotamicus*

A análise citogenética da progênie B1 ("backcrossed") obtida através de retrocruzamento envolvendo um macho híbrido F1 (produzido por parental fêmea *Colossoma macropomum* x parental macho *Piaractus mesopotamicus*) e uma fêmea P1 de *P. mesopotamicus*, mostrou que os peixes da geração B1 apresentaram cariótipos idênticos ao parental da linhagem feminina, sugerindo que estes animais tenham sido produzidos por ginogênese. A produção de peixes ginogenéticos através de cruzamentos controlados e o emprego de marcadores citogenéticos com a finalidade de identificar as contribuições dos parentais são bastante úteis em programas de hibridações interespecíficas na área de piscicultura.

Palavras-chave: Ginogênese, peixes, *Colossoma macropomum*, *Piaractus mesopotamicus*.

INTRODUCTION

The Neotropical characid fish species tambaqui (*Colossoma macropomum*) and pacu (*Piaractus mesopotamicus*), are extensively used in Central and South American fish culture programs (Saint-Paul, 1985; Senhorini *et al.*, 1988; Hernandez, 1989), and in the last years have been introduced in different countries (e.g. Melard *et al.* 1993). Programs involving interspecific hybridization between *C. macropomum* and *P. mesopotamicus* are being carried on in Brazil, since 1985 (Bernardino *et al.*, 1986), in order to put together some valuable zootechnical features of tambaqui and pacu (Senhorini *et al.*, 1988). F1 hybrids originated from crosses in which the parental female is *C. macropomum* are designated as "tambacu", and the ones originated from crosses in which the parental female is *P. mesopotamicus*, are designated as "paqui" (Bernardino *et al.* 1990; Senhorini *et al.* 1988).

F1 females "tambacu" and "paqui" present in general a severe reduction in gonadal development and abnormal quantitative and qualitative gametogenesis, suggesting the occurrence of gonadic sterility (Foresti *et al.* unpublished data; Toledo-Filho *et al.* 1994). About 96% of the F1 male "tambacu" present also gonadic sterility and about 4,0% show zygotic sterility and/or very low fertility (Toledo-Filho *et al.* 1994). An experimental backcross between a F1 male hybrid "tambacu" and a P1 female of *P. mesopotamicus*, was held during the summer of 1990 and produced a small number of B1 offspring that was named "pacucu" (Bernardino *et al.* 1990).

In the present paper, the results of the chromosome analysis of two "pacucu" specimens are described and discussed under the perspectives of cytogenetics and aquaculture.

MATERIALS AND METHODS

Artificial interspecific crosses involving P1 females of *C. macropomum* and P1 males of *P. mesopotamicus* were performed during the summer of 1985, resulting in a lineage of "tambacu" F1 hybrids (Bernardino *et al.* 1986). Three and five year old specimens of these "tambacu" male hybrids, were backcrossed with females of *P. mesopotamicus*, respectively in 1988 and 1990 (Bernardino *et al.* 1990). Fertility rates reached 80% in both experimental crossings and hatching rates were zero in 1988 and between 3-6% in 1990, but were followed by high levels of larval mortality and deformities. All the experimental crosses were performed at the Centro de Pesquisa e Treinamento em Aquicultura, CEPTA/IBAMA, Pirassununga, SP., Brazil.

In the present study, six "tambacu" F1 hybrid males and five *P. mesopotamicus* P1 females from the parental stocks and two B1 "pacucu" were analyzed cytogenetically. Mitotic chromosome preparations were made from gill and kidney tissues according to Almeida-Toledo *et al.* (1987). The C-banding technique was performed according to Sumner (1972).

RESULTS AND DISCUSSION

Cytogenetical analysis

The karyotypic constitution and C-banding pattern of the "tambacu" F1 male hybrid and of the P1 female *P. mesopotamicus* analyzed in the present study, were identical to those described by Almeida-Toledo *et al* (1987). Both parental lineages presented $2n=54$ chromosomes and identical karyotypes, indistinguishable by conventional Giemsa staining (Table I). The use of the C-banding technique permitted the identification of the P1 female *P. mesopotamicus* karyotype which presented two chromosome markers (in chromosomes 5 and 6); also, it were detected five chromosome markers (in chromosomes 3, 14, 20 and 5,6) in the F1 "tambacu" hybrid (three marker chromosomes 3,14 and 20 from the parental *C. macropomum* and two other marker chromosomes 5 and 6 from the parental *P. mesopotamicus*) (Table I). The karyotypes and C-banding patterns of the two B1 "pacucu" (Fig. 1), that resulted from the present backcross experiment between male "tambacu" F1 hybrid and P1 female pacu, were identical to those of the parental female *P. mesopotamicus*, suggesting that the "pacucu" were probably gynogenetic.

From a theoretical viewpoint, at least five possible hypothesis could be considered to explain the cytogenetic results obtained in the present paper with "pacucu": 1) the occurrence of premeiotic endomitosis, 2) ameiotic egg maturation, 3) hybridogenesis, 4) misidentification of the individuals involved in the experiment, and 5) retention of the second polar body during oogenesis. The first three possibilities could be discarded, since these three overmentioned phenomena have been reported to occur only in female lines produced by interspecific hybridization (Chevassus, 1979, 1983), and this is not the present case, where a male "tambacu" hybrid was used. The fourth possibility, a misidentification of specimens, can also be rejected, since after cytogenetic analysis, the "tambacu" hybrid stocks from CEPTA/IBAMA (Pirassununga) used in present paper, were identified by diagnostic C-band markers (Almeida-Toledo *et al.* 1987) and by electrophoretic species-specific patterns of esterases, hemoglobins, superoxidodismutase and transferrins (Calcagnotto, 1993). Another possibility, the occurrence of meiotic misdivision during oogenesis, could also produce diploid eggs if normal chromosome pairing is followed by a repression of either the first or the second meiotic division. Considering that the retention of the second polar body has been suggested as the most common mechanism responsible for the occurrence of occasional or artificially reduced eggs in fishes (Chourrout, 1987), the fifth hypothesis appears to be the most likely to explain the cytogenetic results already obtained with the "pacucu". According to this hypothesis, the diploid gynogenetic "pacucu" could be a B1 product of the backcrossing between male "tambacu" and female pacu that resulted from the diploidization process of the eggs due to the retention of the second polar body, without participation of the male genome of "tambacu".

Perspectives in aquaculture

The cytogenetic data described in the present paper offer new perspectives for the Brazilian programs of hybridization involving pacu and

tambaqui. The efforts to join desirable zootechnical traits in the reciprocal F1 hybrids normally named as "tambacu" and "paqui", have already produced good results in fishculture programs (Bernardino *et al.* 1986, 1990; Senhorini *et al.* 1988). From the cytogenetic point of view, the parental species pacu and tambaqui, as well as the F1 reversal hybrids present similar karyotypes and identical chromosome numbers, but a very different constitutive heterochromatin pattern (Almeida-Toledo *et al.* 1987, 1988). In addition, the good viability of F1 reciprocal hybrids and the codominant expression of four groups of electrophoretic markers (Calcagnotto, 1993), suggest that no major regulatory incompatibility should exist between pacu and tambaqui; cladistic analysis also indicates that pacu and tambaqui belong to two closely related genera (Machado-Allison, 1983). From the conservation genetics point of view, "tambacu" and "paqui" females, as well as the great majority of F1 hybrid males present gonadic sterility, and only about 4% of "tambacu" males present zygotic sterility and/ or very low fertility (Toledo-Filho *et al.* 1994). The production, however, of a few low fertile and / or zygotic sterile F1 progeny of "tambacu" males is not indicative that further hybridization or sexual competition with wild males are unlikely. This way, intensive care should be taken with "tambacu" F1 hybrid males, in order to prevent potential genetic introgression with wild populations of *P. mesopotamicus*, from the Parana-Paraguai hydrographic system in South America. The same type of prevention is also valid in relation to the "pacucu", specially because residual chromosome fragments of "tambacu" may be present in gynogenetic offsprings. From the fish culture perspective, on the other hand, if backcross between F1 hybrid males "tambacu" and P1 females *P. mesopotamicus* consistently produce gynogenetic animals, this could be a potentially useful tool for a number of aquaculture programs focused on the rapid generation of monosex all-female stocks and/or highly consanguineous lineages of *P. mesopotamicus*.

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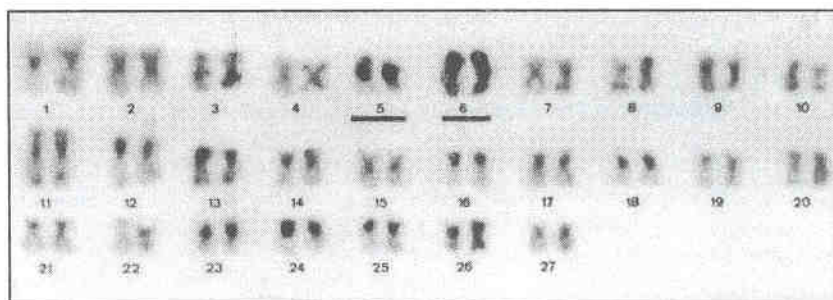


Fig. 1. C-banded karyotype of a "pacucu". The chromosome markers (pairs number 5 and 6) are indicated with bars.

TABLE I - Cytogenetic markers of P1 lineages of pacu and tambaqui, F1 reciprocal hybrids "tambacu" and "paqui", and B1 progeny of "pacucu"

Fish Lineages	Diploid Chromosome number and formulae	Marker Chromosomes (C-band)	Ref.
Pacu	54 (20 m + 34 sm)	5 6	(1,2)
Tambaqui	54 (20 m + 34 sm)	3 14 20	(1,2)
Paqui	54 (20 m + 34 sm)	5 6 3 14 20	(2)
Tambacu	54 (20 m + 34 sm)	3 14 20 5 6	(1)
Pacucu	54 (20 m + 34 sm)	5 6	(3)

(1) Almeida-Toledo *et al*, 1987

(2) Almeida-Toledo *et al*, 1988

(3) Present paper