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Loss of genetic variability at the transferrin locus in five hatchery stocks of tambaqui (*Colossoma macropomum*)

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Abstract

Knowledge and conservation of the genetic variability in stocks maintained as live gene banks have become a high priority task for Brazilian fish culture. The aim of the present survey was to assess the transferrin allelic diversity of five hatchery stocks of tambaqui (*Colossoma macropomum*). The tambaqui stock from Pentecoste, the oldest maintained in Brazilian hatchery stations, retained three of the six alleles detected in wild populations of tambaqui from the Amazon River. Other hatchery stocks, directly or indirectly derived from the Pentecoste stock, did not show transferrin allelic variability. Insufficient number of founders and genetic drift due to sampling errors seem to be the main causes leading to loss of genetic diversity in tambaqui hatchery stocks. Appropriate management strategies are required in order to improve the genetic potential of tambaqui stocks in Brazil.

INTRODUCTION

The knowledge of genetic variability is of major importance for the successful conservation and management of fish species. Fishery biologists have long been using allozyme and isoproteins to electrophoretically characterize stocks and populations (Allendorf and Phelps, 1980; Sánchez *et al*., 1996). Transferrin (TF) is one of the isoproteins widely used in this characterization (Calcagnotto *et al.*, 1999). This protein participates in the transport of iron needed for the biosynthesis of hemoglobin; in most fish species the transferrin locus is polymorphic, presenting 2 to 13 alleles (Kirpichnikov, 1981).

Tambaqui (*Colossoma macropomum*), the second largest scaled fish of the Amazon basin ictiofauna, is of great economic importance in commercial fishing and fish farming programs in Brazil (Goulding, 1981). This species has been submitted to intensive capture since the early 60s; catching or selling tambaqui specimens less than 55 cm long has become illegal (Goulding and Carvalho, 1982). While fish culture programs involving tambaqui are being carried out in Brazil, most of the hatchery tambaqui stocks are derived from the stock of the Centro de Pesquisas Ictiológicas Rodolpho von Ihering, DNOCS, Pentecoste, State of Ceará, developed from 74 fingerlings obtained from the upper Amazon at Iquitos, Peru, in 1972 (Bezerra e Silva and Gurgel, 1987).

The aim of the present study was to assess the allelic variation at the transferrin locus of tambaqui obtained from five different hatchery stocks, as a first step towards the mappping of its living banks in Brazil.

MATERIAL AND METHODS

The 437 tambaquis assayed were obtained from five different hatchery stocks (Table I): Pentecoste stock (PE) - cultivated at the Centro de Pesquisas Ictiológicas Rodolpho von Ihering (DNOCS, Pentecoste, State of Ceará); Pirassununga stock (PI) - reared in the Centro Nacional de Pesquisa de Peixes Neotropicais (CEPTA/IBAMA, Pirassununga, State of São Paulo); Betume stock (BE) - reared in the Companhia de Desenvolvimento do Vale do São Francisco (CODEVASF) hatchery of Betume, State of Sergipe;. Pacatuba stock (PA) - cultivated in the IBAMA hatchery (Pacatuba, State of Sergipe); Jundiaí stock (JU) - developed in the Jundiaí hatchery (Propriá, State of Sergipe). The last four stocks were established in the late 1970s using fish derived from the PE stock; in 1986, the PI stock received additional 2,000 fingerlings from the PA stock.

Blood samples from adult and juvenile fish were taken from the caudal vein using heparinized syringes and centrifuged to produce plasma. Prior to electrophoresis, plasma samples were subjected to treatment with 0.4% rivanol (Teixeira and Jamieson, 1985). Electrophoretic separation of transferrin was carried out in vertical polyacrylamide gels (Davis, 1964), stained according to the histochemical procedures described by Mueller *et al.* (1962). Nomenclature assigned to transferrin loci and alleles followed the convention proposed by Shaklee *et al.* (1990). After identification of transferrin alleles, the following parameters were determined: allele frequency, the Hardy-Weinberg distribution and the proportion of homozygous and heterozygous individuals. Allelic diversity was estimated according to the expression $A = n' - 1/n - 1$ (Allendorf and Ryman, 1987) the inbreeding coefficient was $F = 1 - (H_o/H_e)$ (Li and Horvitz, 1953).

RESULTS

Three codominant transferrin alleles were present in the Pentecoste stock (PE), namely TF-$^{*}b$, TF-$^{*}c$ and TF-$^{*}d$

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(Figure 1, Table II). A significant deviation from the Hardy-Weinberg expectations ($\chi^2 = 66.54$; $P < 0.001$) was detected in this stock (Table II). The heterozygous genotype TF-b/TF-c presented the highest frequency, but the homozygotes predominated in the PE stock (Table II), the inbreeding coefficient was 0.28.

The difference in terms of allelic variability between the PA, BE, PI and JU stocks and the PE stock, from which they were derived, is striking. Tambaqui from the PI, PA, BE and JU stocks were all monomorphic, fixed for a single allele TF*-*d* (Figure 1B, Table II).

To estimate allelic diversity (A) we assumed that the Iquitos population, which originated the PE stock, presented the same six alleles detected in the tambaqui from the Manaquiri Lake (Teixeira and Jamieson, 1985). It was shown that, the PE stock retained only 40% of the genetic variation originally present in the wild Iquitos population of the upper Amazon. In the other four stocks A = 0, since all but one allele, were lost.

Table I - Frequency of transferrin alleles in five Brazilian tambaqui stocks.

Hatchery	Frequency of TF alleles			Sample size
	b	*c*	*d*	
Pentecoste (PE)	0.50	0.35	0.15	95
Pacatuba (PA)	0	0	1.0	100
Betume (BE)	0	0	1.0	80
Jundiaí (JU)	0	0	1.0	80
Pirassununga (PI)	0	0	1.0	82

DISCUSSION

Considerable loss of transferrin genetic variability was detected in stocks of tambaqui cultivated in five different hatcheries from the northeastern (Pentecoste, Pacatuba, Betume and Jundiaí) and the southeastern (Pirassununga) regions of Brazil.

The number of alleles present at the transferrin locus is an important measure of genetic variation in tambaqui.

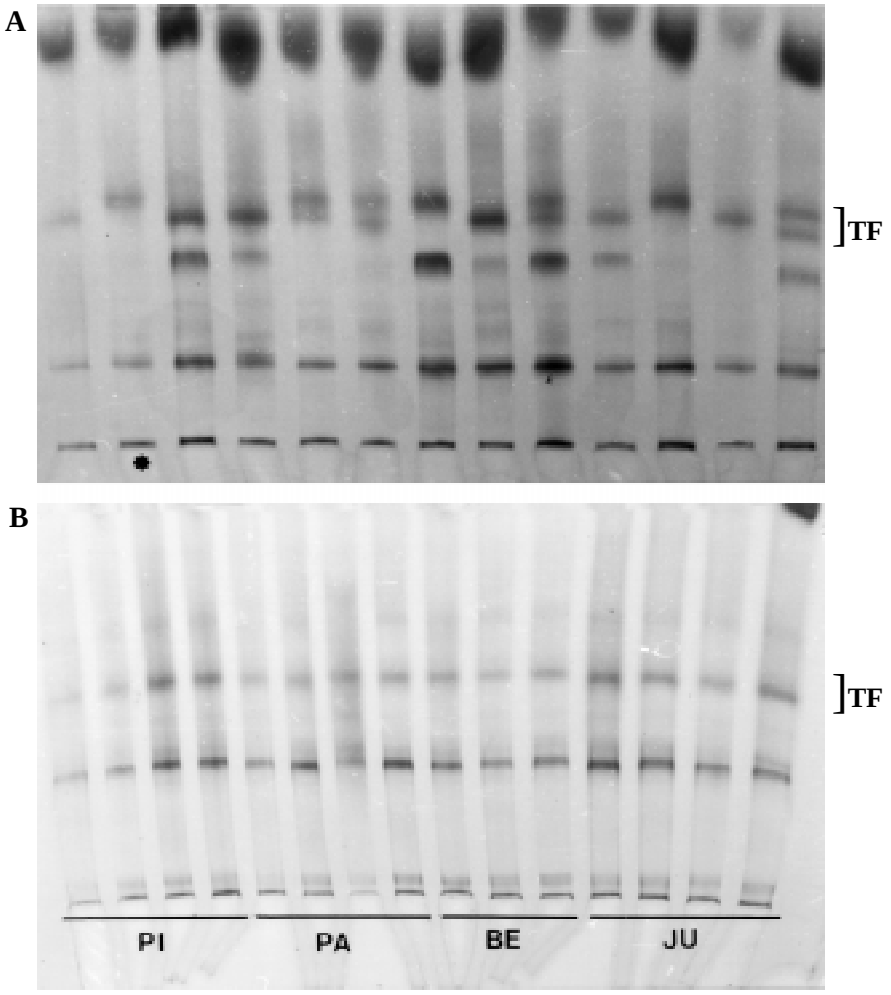

Figure 1 - Electrophoretic patterns of transferrin (TF). **A**, Allelic variability detected in the Pentecoste stock; *specimen from the Pirassununga stock. **B**, Monomorphism detected at Pirassununga (PI), Pacatuba (PA), Betume (BE) and Jundiaí (JU) stocks.

Table II - Observed and expected frequencies of transferrin genotypes in the tambaqui stock of Pentecoste (PE).

TF genotypes	Frequencies	
	Observed (%)	Expected
bb	27 (0.284)	23.75
cc	15 (0.158)	11.81
dd	12 (0.126)	2.06
bc	37 (0.389)	33.50
bd	4 (0.042)	14.0
cd	0 (0.0)	9.88

While in wild tambaqui from the Manaquiri Lake, six codominant alleles segregating at the transferrin locus were detected (Teixeira and Jamieson, 1985), in the hatchery PE stock, founded by fingerlings from the upper Amazon basin (Iquitos), only three transferrin alleles were detected. The other four stocks PA, BE, PI and JU, established directly or indirectly by individuals from the PE stock, are monomorphic for the rarest allele present in PE, TF-**d*.

At least two events seem to be involved in the sudden decrease of transferrin allelic diversity, the first one taking place when the PE stock was founded, and the second one during the establishment of the other four stocks. Most probably, the PE stock was founded using a number of individuals insufficient to reflect the true genetic composition of the Iquitos population. To judge the potential importance of the loss of transferrin alleles we have assumed that the wild Amazonian population from Iquitos is similar to that from the Manaquiri Lake, analyzed by Teixeira and Jamieson (1985). The allelic transferrin frequencies (P) of the Manaquiri population were $P_1 = 0.010$; $P_2 = 0.388$; $P_3 = 0.019$; $P_4 = 0.515$; $P_5 = 0.053$ and $P_6 = 0.015$. Using the expression $\Sigma(n') = n - \Sigma(1-P_j)^{2N}$ (Allendorf and Ryman, 1987), where n' is the number of alleles of the derived stock, n the number of alleles of the donor population and P, the allelic frequencies in the donor population, it was possible to estimate the number of individuals that really contributed to the formation of the PE stock (N = 6). This small number of founder individuals is far from the reasonable absolute minimum of N = 50, suggested by several authors (Allendorf and Ryman, 1987).

The absence of transferrin allelic diversity could be due to genetic drift and to the use of a small number of founders during the establishment of the PA, BE and JU stocks, directly derived from PE (Bezerra e Silva and Gurgel, 1987). The transferrin monomorphism detected in PI could possibly be due to the 2,000 fingerlings that were brought from PA in 1986 (Geraldo Bernardino, personal communication) in which, most probably, the allele Tf-**d* was already fixed.

The loss of alleles can have noxious biological consequences. Firstly, it can make the stocks prone to disease, specially in the case of the transferrin loci, in which the allelic variability is somewhat related to bactericidal properties (Hegenauer and Saltman, 1975). Secondly, although only a few studies examining it are available (Quattro and Vrijenhoek, 1989), it is widely accepted that genetic diversity is positively correlated to the population's viability. Vrijenhoek *et al.* (1985), assuming that genetically depauperated populations would have reduced viability, rejected a population of *Poeciliopsis occidentalis* as a source of individuals for reintroductions. To avoid this situation, we suggest two strategies: firstly, continuous monitoring of Brazilian tambaqui stocks in order to detect possible harmful consequences of transferrin monomorphism and, secondly, the promotion of an adequate management program of tambaqui stocks aimed at increasing allelic diversity and the ensuing avoidance of the low levels of genetic variability detected in these five important Brazilian hatcheries.

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RESUMO

O conhecimento e a conservação da variabilidade genética mantida nos estoques de peixe são prioridades atuais da piscicultura brasileira. A utilização de marcadores genético-bioquímicos como a transferrina constitui uma eficiente ferramenta para a caracterização de estoques cultivados de peixes. O objetivo do presente trabalho foi analisar a diversidade alélica no loco da transferrina em tambaquis provenientes de cinco estações de piscicultura. Foram analisados 437 tambaquis através de eletroforese em gel de poliacrilamida. No estoque de Pentecoste, que é o mais antigo cultivado no Brasil, foram detectados três alelos enquanto que em outros quatro estoques, derivados de Pentecoste, só foi detectada a presença de um alelo fixado para a transferrina. O número insuficiente de fundadores e a deriva genética parecem ser os principais fatores envolvidos na perda de variabilidade genética nos estoques cultivados de tambaqui no Brasil. Estratégias de manejo apropriadas se fazem necessárias para tentar aumentar o potencial genético dos estoques de tambaqui utilizados na piscicultura nacional.

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