Genetic variation in plasma transferrins of tambaqui, *Colossoma macropomum* (CUVIER 1818)

by

A.S. Teixeira & A. Jamieson

Aylton Saturnino Teixeira, Ph. D., Instituto Nacional de Pesquisas da Amazônia (INPA), Caixa Postal 478, 69.000 Manaus-Am., Brasil.
Alan Jamieson, Ph. D., MAFF Fisheries Laboratory, Lowestoft, Suffolk NR 33 OHT, England.
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Abstract
Blood specimens of tambaqui, *Colossoma macropomum,* an Amazonian fish of commercial importance, were examined for transferrin variants by treating blood plasma specimens with rivanol before electrophoretic analyses in starch gel media. A total of 6 co-dominant alleles at the transferrin locus in tambaqui was postulated to account for the 6 varieties of molecular components in 10 observed phenotypes. The population sample showed good genetic balance, characteristic of a unit stock.

Keywords: *Colossoma macropomum,* Amazon Region, Electrophoresis, Transferrin Polymorphism, Allele Frequency.

Resumo
Amostras de plasma sanguíneo de tambaqui, *Colossoma macropomum*, um peixe amazônico comercialmente importante, foram testadas para se investigar variantes de transferrinas. Os especimens do plasma foram tratados com rivanol, antes das análises eletroforéticas em gel de amido. Foram postulados um total de 6 alelos co-dominantes no locus das transferrinas de tambaqui, perfazendo 6 formas moleculares em 10 fenótipos observados. A amostra populacional mostrou um bom balanço genético, característico de uma unidade de estoque.

Introduction

Transferrin is an iron binding protein in the blood plasma of all vertebrates. Variant forms of this molecule occur in different species, and polymorphic forms occur within many species. An autosomal gene, homologous throughout the Vertebrata, is responsible for these co-dominant variants. The early description of transferrin polymorphism was shown in human blood sera by starch gel electrophoresis (SMITHIES 1957). Since Smithies' first report, at least 22 different alleles segregating at the human transferrin locus have been reported by conventional electrophoresis. By using advanced electrophoretic methods, e. g. isoelectric focusing, it is possible to resolve greater numbers of transferrin alleles in human blood sera (KÜHNL et al. 1981).

Following the discovery of transferrin variants in man, several examples of transferrin polymorphism were reported in cattle (HICKMAN & SMITHIES 1957; ASTHON & McDOUGALL 1958), sheep and goat (ASTHON & McDOUGALL 1958), pig (KRISTJANS-SON 1960) and chicken (OGDEN et al. 1962). Transferrin variants in fishes was first shown in *Anguilla anguilla* L. (DRILHON & FINE 1960), later in carp, *Cyprinus carpio* (CREYS-SEL et al. 1964) and in gadoids (MØLLER & NAEVDAL 1966). The present report is the first description of transferrin polymorphism in an Amazonian fish species.

Tambaqui is one of the most important species of fish caught in the Amazon commercial fisheries (GOULDING 1981), and one of the most appreciated food species in Manaus, where an estimated 8,217 tonnes were landed in 1978 (PETRERE 1982). The taxonomy of tambaqui at the specific level was a controversial topic among classical taxonomists. For instance, BRITSKI (1977) clarified the taxonomy of the two congeneric species of Amazon fishes which have two vernacular names, tambaqui and pirapitinga. Up to 1977, both fishes were reported as *Colossoma bidens* (SPIX 1829). BRITSKI, however, using morphological and meristic criteria, decided that tambaqui must be classified as *C. macropomum* (CUVIER 1818) and pirapitinga as *C. bidens.*

At the family level the phylogenetic position of the genus *Colossoma* requires critical revision. For instance, the genus *Colossoma* has been placed within Characidae (GREEN-WOOD et al. 1966), whereas, another classification places this genus within a new family called Serrasalmidae (GÉRY 1977). The family Serrasalmidae has also been considered as a subfamily within the family Characidae (GREENWOOD et al. 1966; BRITSKI 1977; MACHADO-ALLISON 1982). The present note attempts to describe protein types which should contribute to the species definition of the taxon.

Most of the information available on the migration of adult tambaqui is derived from commercial fisheries and from observations of fishermen (GOULDING 1981; MACHADO-ALLISON 1982). Some information about spatial distribution and migration patterns of larval, juvenile and adult tambaqui, has already been presented for the mid-western and central Amazon Basin (GOULDING & CARVALHO 1982) and for the Orinoco Basin (MACHADO-ALLISON 1982). The population structure of tambaqui continues unknown. The use of numbered tags attached to fish to trace stock movements in the Amazon Basin has proved inadequate because it is so difficult to retrieve information about marked fish recaptured in inacessible areas. The development of genetic tags offers an alternative approach (JAMIESON 1974).

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Electrophoretic methods provide protein-gene data which can be used to examine taxonomic groups, species, sub populations and individuals (AYALA 1975; SARICH 1977). Use of genetic tags to screen the composition of commercial fish stocks shows great potential for their management.

This preliminary report describes the use of a differential precipitation method to isolate transferrin molecules in tambaqui blood plasma, followed by an electrophoretic testing method which differentiates transferrin molecules according to their net electrostatic charge differences. Variable transferrin results demonstrate the potential use of natural molecular markers in the genetic analyses of stocks among Amazonian fishes.

Material and Methods

Sample Collection

One hundred and three tambaqui specimens were caught from Manaquiri Lake, 03° 30'S and 60° 30'W (Figure 1). This lake is situated in the lower Solimões river, approximately 1230 km from its delta, as the crow flies. The Manaquiri Lake is about 60 km from Manaus. It is connected to the Solimões river by a long and narrow channel of approximately 35 km.
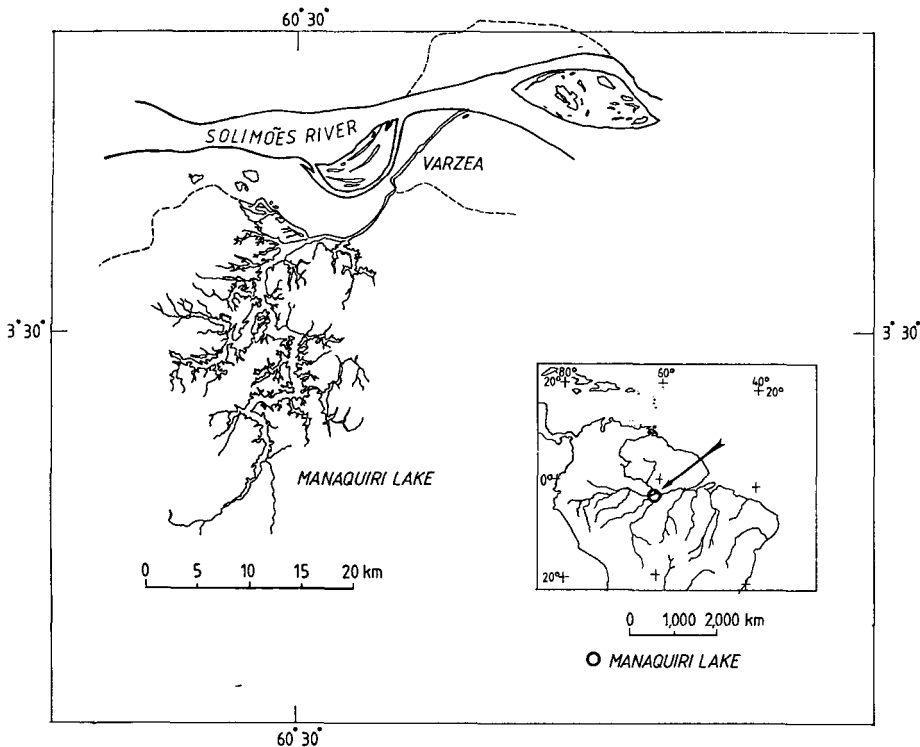

Fig. 1:
The geographic source of the fish sample in the Manaquiri Lake is shown in relation to the main river.

Live tambaqui were caught in gill nets between May 1979 and March 1980. When the captures were carried out nocturnally, the individuals were maintained in fibreglass tanks with oxygen supply, until blood sampling on the next day. The overall lengths of the tambaqui ranged from 16 - 50 cm.

Blood specimens were drawn from the heart into 3 ml glass test tubes containing dry heparin as anticoagulant. Some small fishes were bled by cutting their caudal penducles. Plasma specimens were separated by centrifugation in a microhaematocrit centrifuge (FA Haeracus Christ No. 912) for 20 minutes at 12,000 rpm at room temperature and stored at 5 °C. The material was transported by river to INPA, Manaus, AM, Brazil, maintained at 5 °C, then by air to the Fisheries Laboratory, Lowestoft, England where it was stored at -25 °C until testing.

Rivanol precipitation method
The application of a rivanol precipitation method for isolating ß$_1$ (metal combining) globulins was initially shown by BOETTCHER et al. (1958). Rivanol has now been successfully used to fractionate several other proteins from human blood plasma (STEINBUCH 1980 (review)). This method has also been applied to isolate bovine serum transferrins (EFREMOV et al. 1971) and has been useful for separating out several transferrin types in the blood plasma of teleost fishes (PICHÔT 1971; JAMIESON & TURNER 1978; MANGALY & JAMIESON 1979).

The rivanol treatment of tambaqui plasma specimens prior to electrophoresis was carried out according to JAMIESON & TURNER (1978) except that plasma specimens of tambaqui were treated with rivanol solution at a ration of 1 : 2.

Electrophoresis
The electrophoresis procedures concerning preparation of starch gel medium, preparation of buffers for electrode tanks (0.06 M lithium hydroxide; 0.03 M boric acid) and for the gel (0.03 M tris (hidroxymethyl) aminomethane; 0.005 M citric acid), sample application, staining (1 % amido black for 5 minutes) and destaining techniques, followed those described by JAMIESON & TURNER (1978).

Notation
The transferrin bands were classified according to their relative electrophoretic mobilities toward the anode and measured relative to the advance of a borate line reaching 10.5 cm from the sample insert slot in the gels. Six different transferrin molecules were called C, E, F, G, H, and J in order to their mobilities. C being fastest and J slowest. Future analyses using more tambaqui from different geographic areas should test whether this tentative notation gives an adequate description of this locus in this species.

Statistical test
The observed numbers of tambaqui phenotypes were tested for genetic equilibrium. The expected numbers were calculated as per Hardy-Weinberg assuming random mating and no differential selection between transferrin genotypes. Chi-square was used to determine probability. The degrees of freedom were calculated according to CRISP et al. (1978) in which $d_f = \frac{1}{2} (m^2 - m)$ where m = no. of isoalleles. Rare alleles with frequencies less than 0.05 have been pooled for statistical purposes where one degree of freedom was lost per phenotype.

Results

The one hundred and three tambaqui blood plasma specimens treated with rivanol and analysed by starch gel electrophoresis, revealed 10 of 21 theoretically possible phenotypes, presumably due to 6 co-dominant alleles segregating at the transferrin locus (Table 1). The electromorph bands termed C, E, F, G, H, and J showed distinct relative anodic mobilities (Figure 2). Treated and tested plasma specimens from homozygote fishes appeared as single band patterns (Figure 3). The alleles E and G were the most common showing frequencies of 0.388 and 0.515 respectively. The allele H showed a frequency of 0.053 while

the remainder C, F and J revealed frequencies less than 0.050. The population sample revealed good agreement with Hardy-Weinberg expectations (Table 2). A contingency test applied to inspect the distribution of the T_f alleles against overall lengths of the specimens examined, showed no significant relationship (Table 3).

Table 1: Observed numbers of tambaqui transferrin phenotypes compared with expected numbers assuming Hardy-Weinberg equilibrium. Expected numbers of phenotypes are shown in parentheses below observed numbers.

TAMBAQUI TRANSFERRIN PHENOTYPES						PAIRED ALLELES	ALLELE FREQUENCY
C	E	F	G	H	J		
0 (0.01)	0 (0.80)	0 (0.04)	0 (1.06)	0 (0.11)	0 (0.03)	C	0.010
	14 (15.51)	1 (1.52)	44 (41.16)	5 (4.24)	2 (1.20)	E	0.388
		0 (0.04)	3 (2.02)	0 (0.21)	0 (0.06)	F	0.019
			25 (27.32)	6 (5.62)	1 (1.59)	G	0.515
				0 (0.29)	0 (0.16)	H	0.053
					0 (0.02)	J	0.015
							1.000

Table 2: Summary presentation of data shown in Table 1. Chi-square test for genetic balance showed no significant difference in the sample population ($\chi^2_4 = 2.128; 0.80 > P > 0.70$).

TAMBAQUI TRANSFERRIN PHENOTYPES				PAIRED ALLELES	ALLELE FREQUENCY
E	G	H	RARE*		
14 (15.51)	44 (41.16)	5 (4.24)	3 (3.52)	E	0.388
	25 (27.32)	6 (5.62)	6 (4.67)	G	0.515
		0 (0.29)	0 (0.48)	H	0.053
			0 (0.20)	RARE	0.044
					1.000

*Rare alleles with frequencies less than 0.05 (C, F and J)

Table 3: A contingency test for the T_f alleles against overall lengths of tambaqui specimens. The rare alleles (C, F and J) have been grouped with the H allele for chi-square analysis. The expected numbers are presented in parentheses.

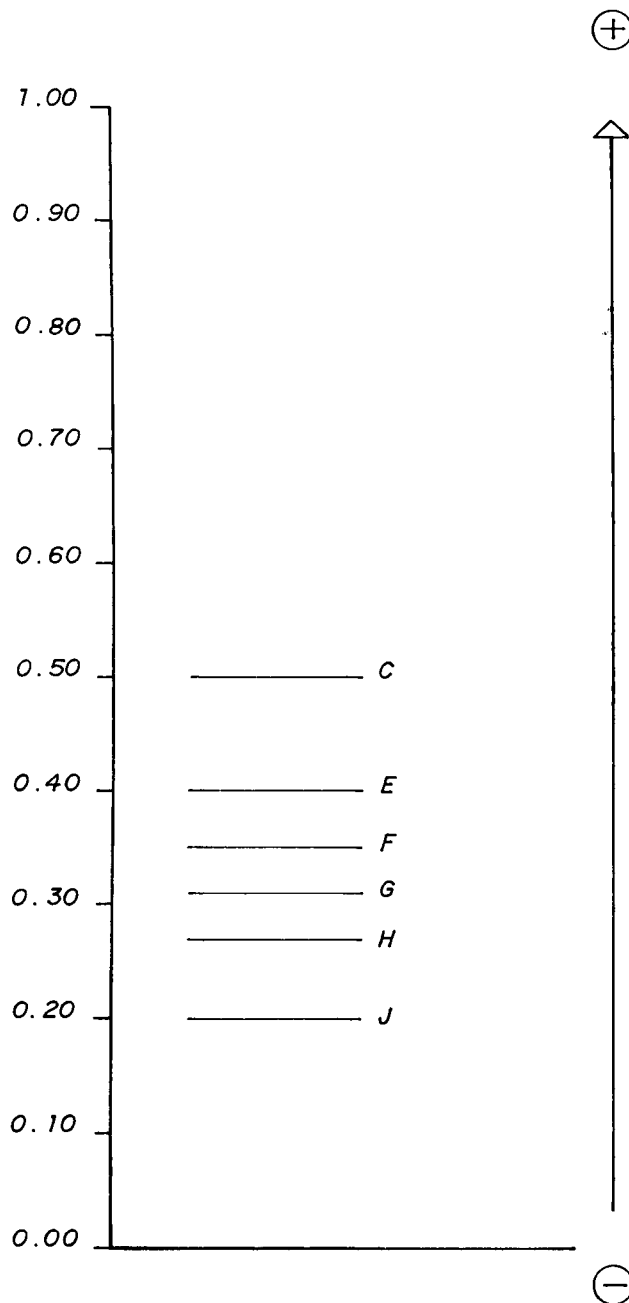| LENGTH (cm)	TRANSFERRIN ALLELES			NO. OF FISH TESTED	x^2	d.f.	PROBABILITY
	E	G	H				
10 – 20	7 (5.44)	5 (7.20)	2 (1.36)	7	1.420	2	0.50 – 0.30
21 – 30	32 (30.29)	37 (40.14)	9 (7.57)	39	0.613	2	0.80 – 0.70
31 – 40	36 (40.39)	60 (53.51)	8 (10.10)	52	1.701	2	0.50 – 0.30
41 – 50	5 (3.88)	4 (5.15)	1 (0.97)	5	0.581	2	0.80 – 0.70
TOTAL	80	106	20	103	4.315	6	0.70 – 0.50

Fig. 2:
The transferrin molecules migrated at different rates of migration towards the anode. Their rates were measured relative to a borate line at 10.5 cm from the inserts. After repeat tests, control tambaqui sera of known phenotypes were run parallel to unknown phenotypes to compare and identify each electromorph.

Fig. 3:
The tambaqui plasma specimens analysed on starch gel electrophoresis with prior treatment by rivanol, produced electromorph bands which were assumed to be coded by 6 co-dominant alleles at the transferrin locus. The phenotypes from left to right are: CG, EG, FG, GH and GJ.

Discussion

The transferrin variants examined in a local population sample of tambaqui in an Amazon lake, Lago Manaquiri, indicated a series of co-dominant alleles. The genotypes of the tested specimens showed genetic equilibrium and are thought to represent some part of a racial unit of stock. Serum proteins, including transferrin, have been classified as rapidly envolving loci (SARICH 1977). In racial analyses, it is therefore advisable to look at these loci, since they show higher genetic variation within and between populations. Hence, the transferrin locus is highly suitable for characterizing populations of vertebrates. Some useful genetic description of vertebrate species have used information about the distribution of transferrin alleles. Examples include cattle races (JAMIESON 1966) and cod stocks (JAMIESON 1970, 1975).

The future research prospect is to extend the present description on transferrin locus to additional population samples of tambaqui from other geographical areas, trying to find out if this species shows sub-populational differences in the allele frequencies of this locus. The goal is to identify each fish stock according to its assortment of genes.

In the tropics, e.g. Amazon region, progress in fisheries science is still hampered by the lack of fundamental research concerning the correct scientific identification of their component units of stock. Biochemical-genetic methods can help to resolve the taxonomic complexity of tropical fishes by supplementing the traditional methods. As the biochemical-genetic methods are also capable of demonstrating variability below the species level, they identify those genetic resources which should be conserved for posterity. This applies to any biological species, but because fish form a considerable source of dietary protein in rainforest regions, the intelligent appreciation of fish stocks as a genetic resource is fundamental to their present practical management and future conservation.

References

ASHTON, G.C. & E.I. McDOUGALL (1958): Beta-globulin polymorphism in cattle, sheep and goats.- Nature **188**: 945 - 946.

AYALA, F.J. (1975): Genetic differentiation during the speciation process.- In: DOBZHANSKY, T., HECHT, M.K. & W.C. STEERE (eds.): Evolutionary Biology 8. Plenum Press, New York. pp. 1 - 78.

BOETTCHER, E.W., KISTLER, P. & H.S. NITSCHMANN (1958): Method of isolating the β_1 metal-combining globulin from human blood plasma.- Nature **181**: 490 - 491.

BRITSKI, H. (1977): Sobre o gênero *Colossoma* (Pisces, Characidae).- Supl. Ciên. Cult. **29** (7): 810.

CREYSSEL, R., SILBERZAHN, P., RICHARD, G. & Y. MANUEL (1964): Étude du sérum de carpe (*Cyprinus carpio*) par électrophorése en gel d'amidon.- Bull. Soc. Chim. biol. 46: 149 - 159.

CRISP, D.J., BEAUMONT, A.R., FLOWERDEW, M.W. & A. VARDY (1978): The Hardy Weinberg test – A correction.- Mar. Biol. 46: 181 - 183.

DRILHON, A. & J.M. FINE (1960): Différences individuelles dans le comportement électrophorétique des protéines et des lipoprotéines chez l'Anguille.- C. r. hebd. Séanc. Acad. Sci. Paris 250: 4044 - 4045.

EFREMOV, G.D., SMITH, L.L., BARTON, B.P. & T.H.J. HUISMAN (1971): Studies on bovine transferrin; isolation and partial characterization.- Anim. Blood Grps. Biochem. Genetic. 2: 159 - 177.

GÉRY, J. (1977): Characoids of the world.- T.F.H. Publications. New York. 672 pp.

GOULDING, M. (1981): Man and fisheries on an Amazon frontier.- Junk Publishers, The Hague. 137 pp.

GOULDING, M. & M.L. CARVALHO (1982): Life history and management of the tambaqui (*Colossoma macropomum*, Characidae): An important Amazonian food fish.- Rev. Brasil. Zool. Editada pela Soc. Brasil. Zool. 1 (2): 107 - 133.

GREENWOOD, P.H., ROSEN, D.E., WETZMAN, S.H. & G.S. MYERS (1966): Phyletic studies of tele-ostean fishes, with a provisional classification of living forms.- Bull. Am. Mus. Nat. Hist. 131: 33 - 455.

HICKMAN, C.G. & O. SMITHIES (1957): Evidence for inherited differences in the serum proteins of cattle.- Proc. Genet. Soc. Can. 2: 39.

JAMIESON, A. (1966): The distribution of transferrin genes in cattle.- Heredity, London 21 (2): 191 - 218.

JAMIESON, A. (1970): Cod transferrin and genetic isolates.- In: W. JUNK (ed.): 11th Eur. Conf. Anim. Blood Grps. Biochem. Polymorph. Warsaw, 1968. pp. 533 - 538.

JAMIESON, A. (1974): Genetic tags for marine fish stocks.- In: HARDEN JONES, F.R. (ed.): Sea Fisheries Research.- Elek Science, London. pp. 91 - 99.

JAMIESON, A. (1975): Enzyme types of Atlantic cod stocks on the North American banks.- In: C.L. MARKERT (ed.): Isozymes IV, Genetics and Evolution.- Academic Press, New York. pp. 491 - 515.

JAMIESON, A. & R.J. TURNER (1978): The extended series of T_f alleles in Atlantic cod, *Gadus morhua*.- In: B. BATTAGLIA & J. BEARDMORE (eds.): Marine Organisms.- Plenum Publishing Corporation. pp. 699 - 729.

KRISTJANSSON, F.K. (1960): Inheritance of a serum protein in swine.- Science 131: 1681.

KÜHNL, P., CONSTANS, J., VIAU, M. & W. SPIELMANN (1981): Isoeletric focusing of the common transferrin C allele subtypes.- In: R.C. ALLEN & P. ARNOUD (eds.): Electrophoresis '81.- Walter de Gruyter & Co., Berlin, New York. pp. 537 - 583.

MACHADO-ALLISON, A.J. (1982): Studies on the systematics of the subfamily Serrasalminae (pisces - Characidae).- Ph. D. thesis. The George Washington University.

MANGALY, G. & A. JAMIESON (1979): Genetic tags applied to the European hake, *Merluccius merluccius* L.- Anim. Blood Grps. Biochem. Genetic. 10: 39 - 48.

MØLLER, D. & G. NAEVDAL (1966): Transferrin polymorphism in fishes.- 10th Eur. Conf. Anim. Blood Grps. Biochem. Polymorph. Institut National de la Recherche Agronomique, Paris. pp. 367 - 372.

OGDEN, A.L., MORTON, J.R., GILMOUR, D.G. & E.M. McDERMID (1962): Inherited variants in the transferrins conalbumins of the chicken.- Nature 195: 1026 - 1028.

PETRERE, M., Jr. (1982): Ecology of the fisheries in the river Amazon and its tributaries in the Amazon state (Brazil).- Ph. D. thesis. University of East Anglia, U.K.

PICHÔT, P. (1971): Étude électrophorétique des protéines du serum et du cristallin des merlus de la cote nordouest Africaine.- Rapp. P.-v. Reun. Cons. perm. int. Explor. Mer. 161: 80 - 85.

SARICH, V.M. (1977): Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies.- Nature 265: 24 - 48.

SMITHIES, O. (1957): Variations in human serum ß-globulins.- Nature 180: 1482 - 1483.

STEINBUCH, M. (1980): Protein fractionation by ammonium sulfate, rivanol and caprylic acid precipi-tation.- In: J.M. CULLING (ed.): Methods of plasma protein fractionation.- Academic Press, London, New York, Sydney and San Francisco. pp. 33 - 56.