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Study of transferrin polymorphism in a population of Carp (*Cyprinus carpio*)

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Abstract

This study was carried out at the college of agriculture, Basrah university. Fifty blood samples taken from carp **(*Cyprinus carpio*)** analyzed by Polyacrylamide gel electrophoresis (PAGE) under alkaline conditions at pH 9.8. The present study is aimed to provide the first insight into the genetic constitution of carp based upon analysis of Transferrin. Five genetic types of transferrin were revealed (Tf1Tf1, Tf2Tf2, Tf5Tf5, Tf3Tf2 and Tf1Tf5) in Carp controlled by four allelic genes of that locus (Tf1, Tf2, Tf3 and Tf5). These fractions are controlled by co dominant autosomal genes according to the Mendelian laws of inheritance. Differences in gene frequencies between alleles were observed. The gene frequencies of Tf1, Tf2, Tf3 and Tf5 were 0.15, 0.51, 0.29 and 0.05, respectively. The predominant genotype was Tf2Tf2 (44%) then Tf3Tf3 (22%). Transferrin was identified as highly polymorphic protein markers which is a result of importance for future genetic characterization of the carp .

Key words: Carp, Genetic Polymorphism, Transferrin

1- Introduction

Transferrin is β-globulin which is characterized by its specific ability to reversibly bind iron and various other metal ions. Generally, it exhibits a high degree of polymorphism and belongs to the well-studied systems in man and different animal species (Valenta et al., 1976). Genetic studies on Transferrin polymorphism has been demonstrated by gel electrophoresis in many vertebrates. These include men (Smithies and Hiller 1959), Monkeys

(Goodman and Poulik 1961), cattle (Ashton 1958; Smithies and Hickman 1958), sheep and goats (Ashton and Ferguson 1963; Efremov and Braend 1964), horses (Braend and Stormont 1964), Burros (Niece and Kracht 1967), pigs (Ashton 1960a), mice (Ashton and Braden 196l), chickens (Ogden et al., 1962), and gadoid fishes (Moller and Naevdal 1966).

In serum most of the iron molecules are bound to transferrin (Tf), which is a highly polymorphic protein in fish ((Patrycja et al., 2008).

Serum transferrin (Tf) is a single monomeric glycoprotein of 80-kDa belonging to the transferrin family that also includes lactoferrin in milk, ovotransferrin in avian egg white and melanotransferrin in melanoma cells. The major role of Tf is transport of iron that participates in a wide variety of metabolic processes, including regulation of the immune system, DNA synthesis and oxygen and electron transport. Serum Tf is synthesised in the liver and secreted into the blood. It exists as a mixture of iron-free (apo), one iron (monomeric) and two iron (holo) forms. The relative percentage of each form depends on the concentration of iron and Tf in plasma Tf also is important for the transport of metals. Other than iron. Since the metal binding sites of Tf are occupied by iron only for approximately 30%, other metals can be bound without requiring the displacement of the more tightly bound iron (Patrycja et al., 2008).

Material and methods

Electrophoresis

Genetic types of transferrin in blood were analyzed by Vertical discontinuous polyacrylamide gel electrophoresis (PAGE) under alkali conditions. The PAGE of whey blood fractions was carried out on 13-cm x 22-cm x 4-mm with 20 wells according to the method described by Khaertdinov and Jaayid (2002). The separating gel (T= 7.5; C = 2. (8M urea) contained 0.06 M HCL pH 8.3; the stacking gel (T = 3; C = 20; 8M urea) O.O6,M H3PO4 pH 6.7. After applying an output voltage of 200 volts for 10 minutes, the inserts were removed and the same voltage continued for a further 15 minutes. The output voltage was then increased to 250 volts and continued until the brown line had migrated 9 cm beyond the insert line. The gel was then removed, sliced and stained for 10 min. with 0.1 % (w/v) Amido Black in methanol-acetic acid-water (50/7/43 by vol.). The gel was destained with a solution containing methanol.-acetic acid-water (40/10/50 by vol.).

Statistical analysis

The allele frequencies in the transferrin fraction were estimated by direct counting of the phenotypes. To test differences

between observed and expected genotypes frequencies, a chi-square (χ^2) analysis was performed on the basis of the Hardy-Weinberg law.

Results and discussion

Five genotypes ($Tf^1 Tf^1$, $Tf^2 Tf^2$, $Tf^5 Tf^5$, $Tf^2 Tf^3$ and $Tf^1 Tf^5$) of 15 theoretically possible ones, were detected, encoded by four co-dominant alleles (Tf^1, Tf^2, Tf^3 and Tf^5) (Table 1). Individual homozygote fish produced a single transferrin band, whereas individual heterozygote fish showed two transferrin bands (Figure 1, 2 and 3). This monomeric structure of transferrin molecules, with single-locus co-dominant inheritance, is homologous with the variation observed at the transferrin locus in all of the many vertebrate species tested.

Most common allele among the population samples belonging to the Carp was relatively homogeneous So the allele Tf^2 of transferrin occurred at a higher frequency than the allele 1, 3 and 5 in Carp breed (0.51, 0.15, 0.29 and 0.05) respectively. The genotypic frequencies of Tf1tf1, Tf2Tf2, and Tf5Tf5 were 10, 22 and 44, respectively.

To test for genetic balance in Carp **(*Cyprinus carpio*)**.population samples from each area, χ^2 tests for Hardy-Weinberg expectations were applied. The χ^2 values revealed that the Carp population was not in Hardy-Weinberg equilibrium as there was a continuous migration of animals in the herd studied.

Table 1: Gene frequencies of transferring in Carp **(*Cyprinus carpio*)**.

Genotypes	No. of animals	%	Gene frequency
Tf^1Tf^1	5	10	0.15 (Tf^1)
Tf^2Tf^2	22	44	0.51 (Tf^2)
Tf^5Tf^5	11	22	0.05 (Tf^5)
Tf^3Tf^2	7	14	0.29 (Tf^3)
Tf^1Tf^5	5	10	

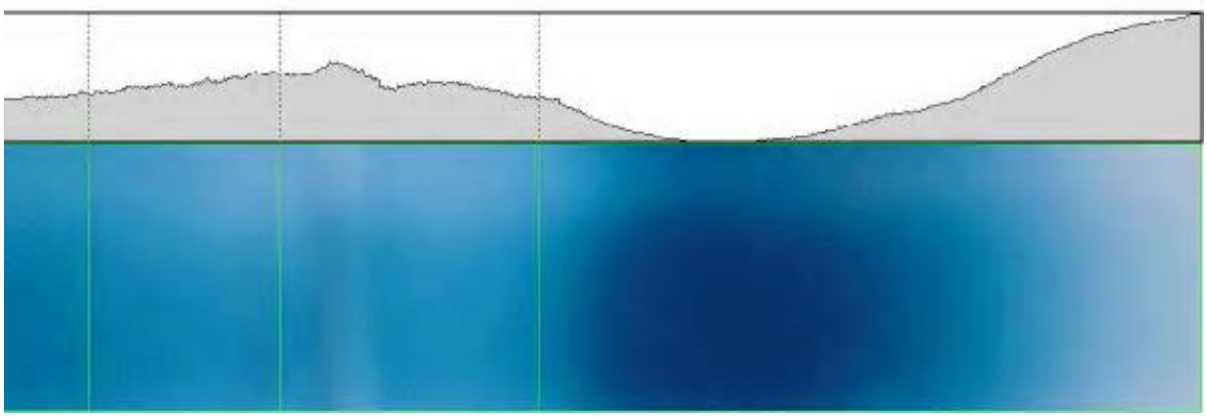

Fig. 1: Transferrin genotypes were identified by PAGE electrophoresis (Khaertdinov and Jaayid, 2002). Serum samples were taken from (n=50).

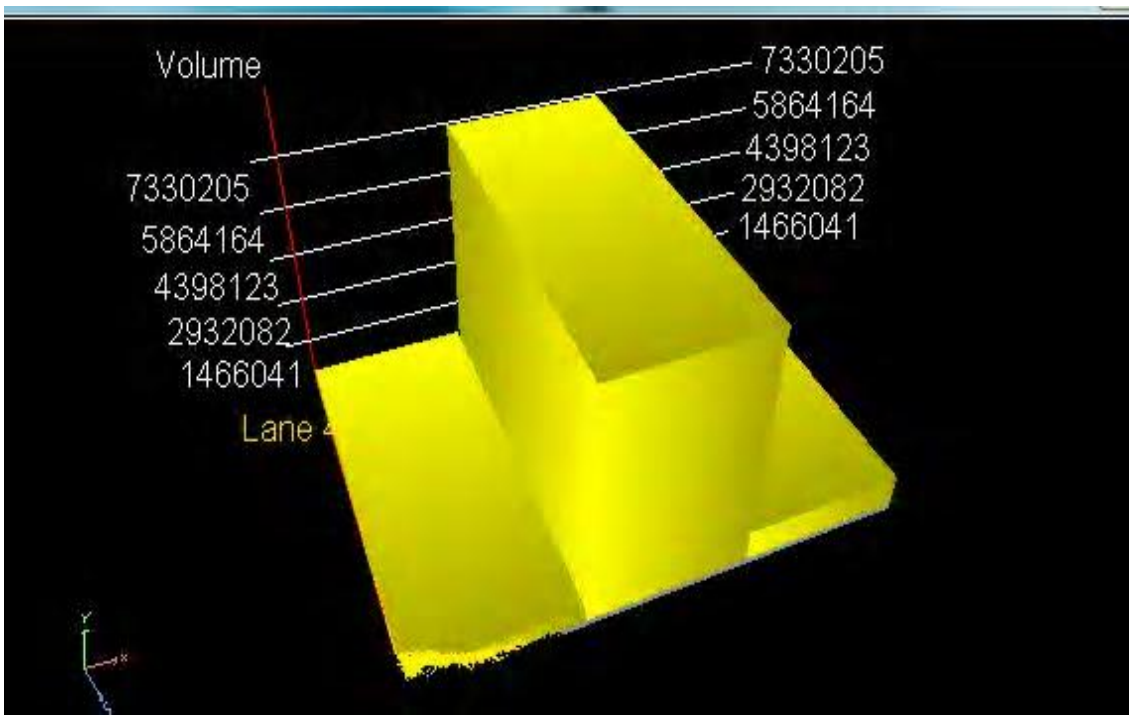

Fig. 2: Polyacrylamide gel electrophoresis profile of Carp serum sample containing the reference transferrin genotype.

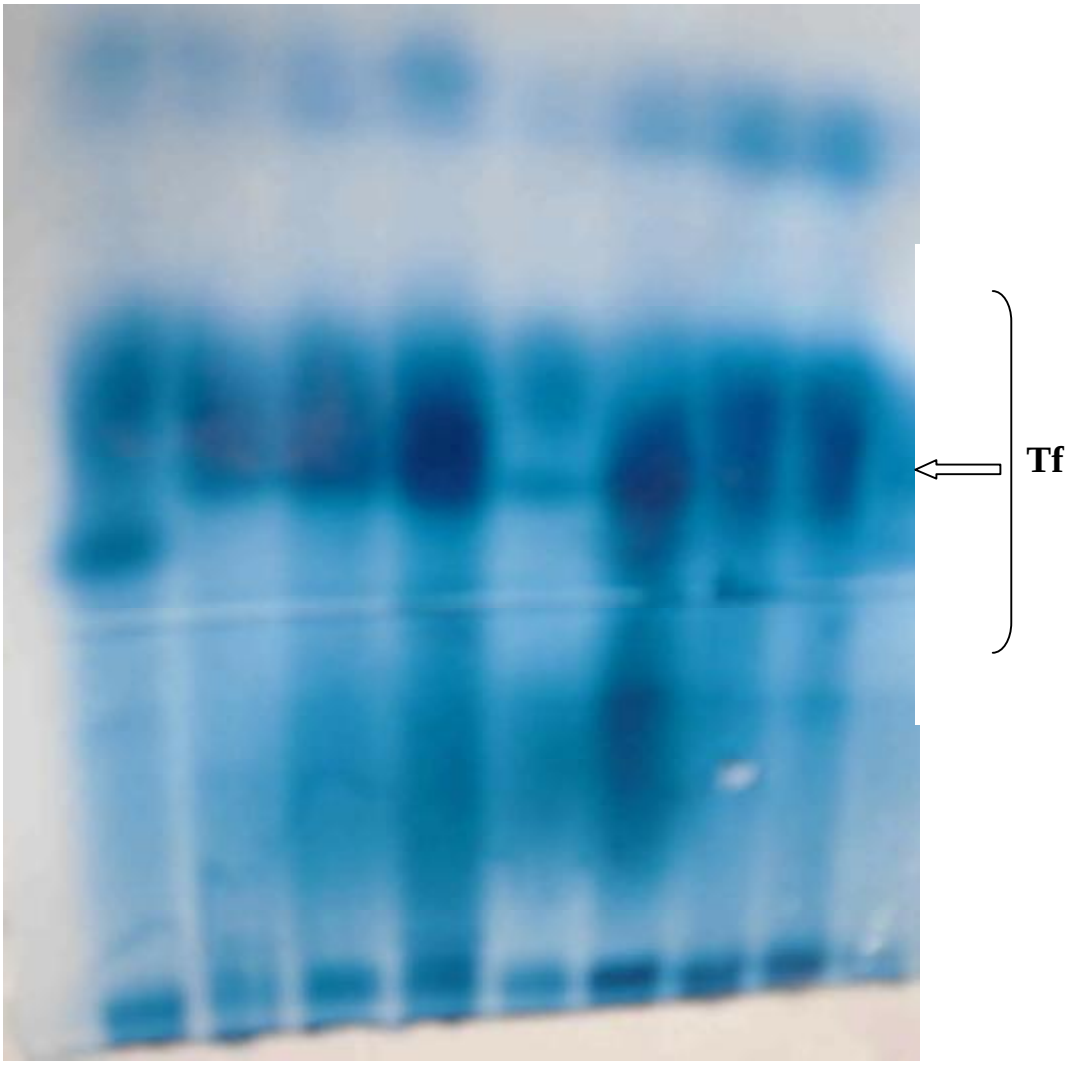

Fig. 3: Different transferrin genotypes as detected by non-reducing polyacrylamide gel electrophoresis (PAGE) in common carp **(*Cyprinus carpio*)**.

Conclusion

The frequency of Tf^5 allele was found to be lower than that of Tf^2 allele in the studied breed. These results further confirm that carp **(*Cyprinus carpio*)** is predominantly of transferrin 2 type. We present new data for transferrin alleles frequencies for 50 samples of Carp **(*Cyprinus carpio*)**. Genetic polymorphism is widespread, and provides opportunities to test the genetic stability of species.

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دراسة التشكل الوراثي للترانسفيرين في مجتمعات اسماك الكارب (*Cyprinus carpio*)

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الخلاصة

اجريت هذه الدراسة في كلية الزراعة-جامعة البصرة على اسماك الكارب الاعتيادي، تم اخذ خمسين عينة دم وتم تحليل العينات في جهاز الترحيل الكهربائي العمودي بوجود ممادة متعدد الاكريل امايد وبوسط قاعدي (PH9-8). هدفت االدراسة لاعطاء نظرة اولية حول التكوين الوراثي لاسماك الكارب الاعتيادي اعتماداً على تحليل جين الترانسفيرين. اوضحت النتائج وجود خمسة تراكيب وراثية للترانسفيرين في الكارب الاعتيادي (Tf1Tf1, Tf2Tf2, Tf5Tf5, Tf3Tf2 and Tf1Tf5F) يسيطر عليها اربعة اليلات لذلك الموقع ((Tf1، Tf2، Tf3 و Tf5) تقع هذه الاليلات تحت السيادة المشتركة وحسب قوانين مندل في الوراثة.

لوحظ اختلافات في تكرارات جين الترانسفيرين، حيث كانت في كل من الاليل (Tf1، Tf2، Tf3 و Tf5) 0.15، 0.51، 0.29 و 0.05 على التوالي. تفوق التركيب الوراثي Tf2Tf2 (44%) ثم Tf3Tf3 (22%). نستنتج من هذه الدراسة بان جين الترانسفيرين ذو تعدد اشكال وراثية عالية وبتالي تعتبر هذه النتيجة مهمة للدراسات المستقبلية في وصف اسماك الكارب الاعتيادي.