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A STUDY OF BIOCHEMICAL POLYMORPHISM IN CARP (*Cyprinus carpio*): DETECT NEW ALLELES IN TRANSFERRIN

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Abstract: This study was carried out at the college of agriculture and marine science centre, Basrah university. The present study was conducted to investigate the existence of polymorphism at transferrin (**Tf**) locus in the Carp (*Cyprinus carpio*). A polyacrylamide gel electrophoresis (PAGE) under alkaline condition method was used to distinguish Carp **Tf** alleles. Use the gel documentation program in this study. Analysis of **116** animals revealed that all animals were polymorphic, showing many genotypes. There was very clear biodiversity in the **Tf** gene. Seven **Tf** genotypes consisting of 4 homozygote types (**CC, DD, FF** and **GG**) and two heterozygote types (CD, DG and FG) were detected. These fractions are controlled by co-dominant autosomal genes according to the Mendelian laws of inheritance. The highest gene frequencies were calculated 0.50 for **Tf** D, 0.26 for **Tf** F and 0.12 for C and G. thus, carp (*Cyprinus carpio*) assemblages consistently tended to be more predominant to D allele. Differences between expected number and observed number for transferrin genotypes were no significant. This is useful in genetic improvement process through the selection. As far as we know, this is the first large-scale analysis on the genetic polymorphism in carp (*Cyprinus carpio*). Polyacrylamide electrophoresis, the technique employed in this study, allows rapid and efficient screening for the presence of polymorphism in **Tf**.

Key words: Carp, Genetic Polymorphism, Transferrin, biodiversity

Introduction

Transferrin, one of class I genetic markers, is the most heterogeneous polymorphic blood protein in Carp *(Valenta et al., 1976 and Csizmadia et al., 1995)*, in goose *(Valenta and Stratil, 1978)*, in chicken *(Vyshinsky and Muravjev, 1970)* and sheep *(Jaayid et al., 2011)*, a total of 7 co-dominant alleles have been

found in its locus. Transferrin polymorphism was demonstrated in different breeds in fish. Since then, several reports have been published concerning the gene frequencies in this systems and about the possible influence of these polymorphism on disease resistance *(Jurecka et al., 2009)*. This protein, belonging to the group of beta-globulins, is found not only in blood serum, but also milk and semen. The main function of transferrin in the organism is to participate in iron metabolism and in immune responses. Conservation of genetic variety of strains maintained in live gene banks is a high-priority task. Description of the genetic structure should be the first step in this work. By applying different biochemical-genetic markers such as transferrin and isoenzymes, the individuals and the populations could be well characterised genetically. Based on this, breeding programs as well as conservation of races can be carried out without disappearance of genes from the pool. The conservation of genetic resources is based on two different concepts, namely in situ and ex situ conservation methods. For actual implementation of these conservation methods a sound knowledge of the genetic structure. This knowledge will guarantee that the applied conservation measures will cover the genetic variation of that particular species.

The term "genetic polymorphism" defines the fact that each protein presents two or more forms genetically determined by autosomal and co dominant alleles. The study of polymorphism has many uses in medicine, biological research, and law enforcement. Over the last 10-20 years considerable interest has developed in blood protein polymorphism as well as increasing basic knowledge on protein fraction. A related use of polymorphism is widely employed in agriculture. Electrophoretical techniques have been used extensively as a method to analyse the biochemical, systematic and ecological characteristics of marine and freshwater fishes *(Wiegertjes et al., 1995; Ford, 2001; Kohlmann et al., 2003)*. The aim of these survey was to describe the polymorphism of transferrins of carp strains in the live gene bank. Many gaps still exist in the understanding of identification and conservation of breeds as well as the genes controlling these traits in Iraqi fish. Identification and conservation are not sufficiently characterized, they are underutilized in conventional breeding programmes, and there is insufficient research on the ways to select breeds or individuals carrying the most advantageous traits. Transferrin gene frequencies have not been studied in Iraqi Carp populations only one paper *(Jaayid and Aziz, 2009)*, This paper (1) describes transferrin polymorphisms in Carp (*Cyprinus carpio*), (2) presents evidence of multiple a phenotypes in Carp (*Cyprinus carpio*) and (3) investigate and propose management and utilisation strategies for fish resources in Iraq.

Materials and Methods

Electrophoresis. A polyacrylamide gel electrophoresis (PAGE) of transferrin protein fractions was carried out on 13-cm x 22-cm x 4-mm with 24 wells according to the method developed by *Khaertdinov and Gataulin (2000)*. 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 distained with a solution containing methanol-acetic acid-water (40/10/50 by vol.).

Statistical analysis. The allele frequencies in the transferrin 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

Figure 1 shows the electrophoretical patterns of some individual carp (*Cyprinus carpio*) protein samples. two bands were detected when transferrin was run and stained in Amido Black in methanol-acetic acid-water (50/7/43 by vol.). The Carp (*Cyprinus carpio*) transferrin types were named according to the nomenclature suggested by Irnazarow and *Bialowas (1994)* and *Jurecka et al. (2009)*. The results obtained for the transferrin that show variation in the sample of Carp (*Cyprinus carpio*) are presented in Table 1. Gene frequencies were calculated by the method of gene counting as the mode of inheritance of each of the systems that do show variation is that of codominant alleles at an autosomal locus *(Khaertdinov, 2000)*.

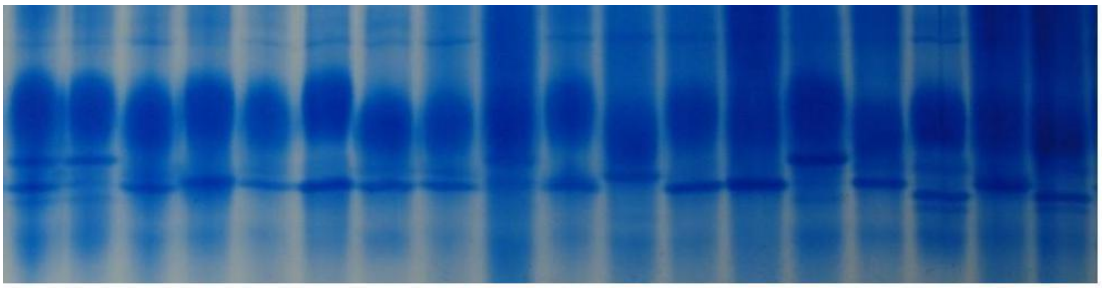
Table 1. Distribution of transferrin Frequency and gene frequency transferrin locus in Carp (*Cyprinus carpio*)

	Transferrin genotypes, n= 116							χ^2	**Gene frequency**			
	CC	**DD**	**GG**	**FF**	**CD**	**DG**	**FD**		**C**	**D**	**G**	**F**
Number	8	45	23	24	8	8	15	21.54	0.12	0.50	0.12	0.26
%	6.72	37.82	6.72	20.71	6.72	6.72	12.61					

The Carp (*Cyprinus carpio*) transferrin phenotypes are due to an autosomal locus with four co-dominant alleles, TfC , TfD, TfG and F. The D and F alleles were most frequent (0.5 and 0.26) respectively, while the C and G alleles were rare

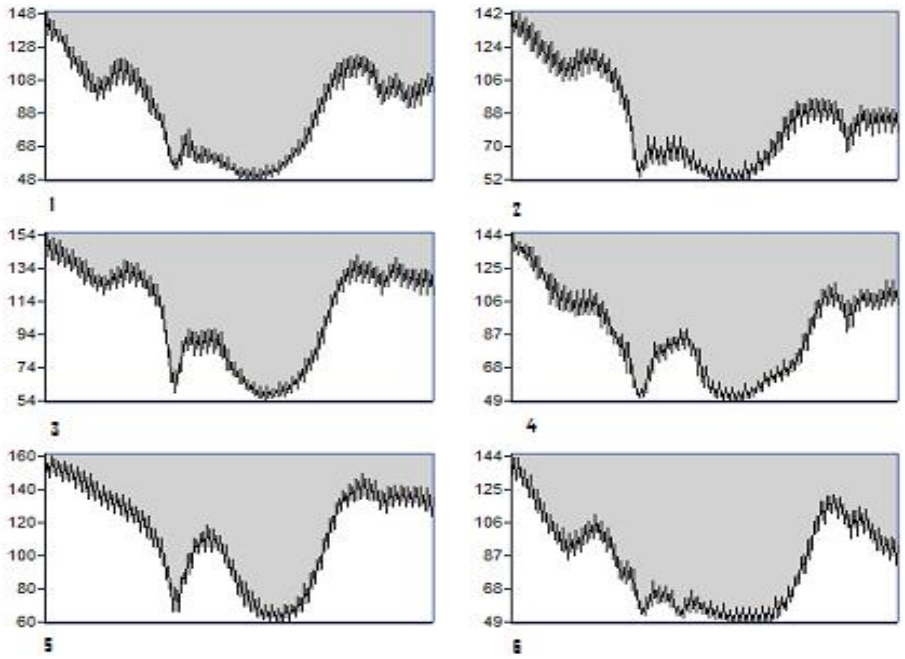
alleles (0.12) (Figure 2). The gene frequency for D allele obtained in the sample is within the range of those observed in *Jurecka et al. (2008), Csizmadia et al. (1995)* and *Wojtczak et al. (2007)* while *Valenta et al. (1976)* have been found Seven transferrin variants (A,B,C,D,E,F, and G) in Carp.

Tf



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 1. Different transferrin genotypes as detected by polyacrylamide gel disc electrophoresis patterns at 8.6 in Iraqi Carp: 1-DG, 2-GG, 3-8-DD, 9-DG,10-DD, 11-FF, 12-13-DD, 14-GG, 15-FF, 16-CD, 17-FF, 18-CD.



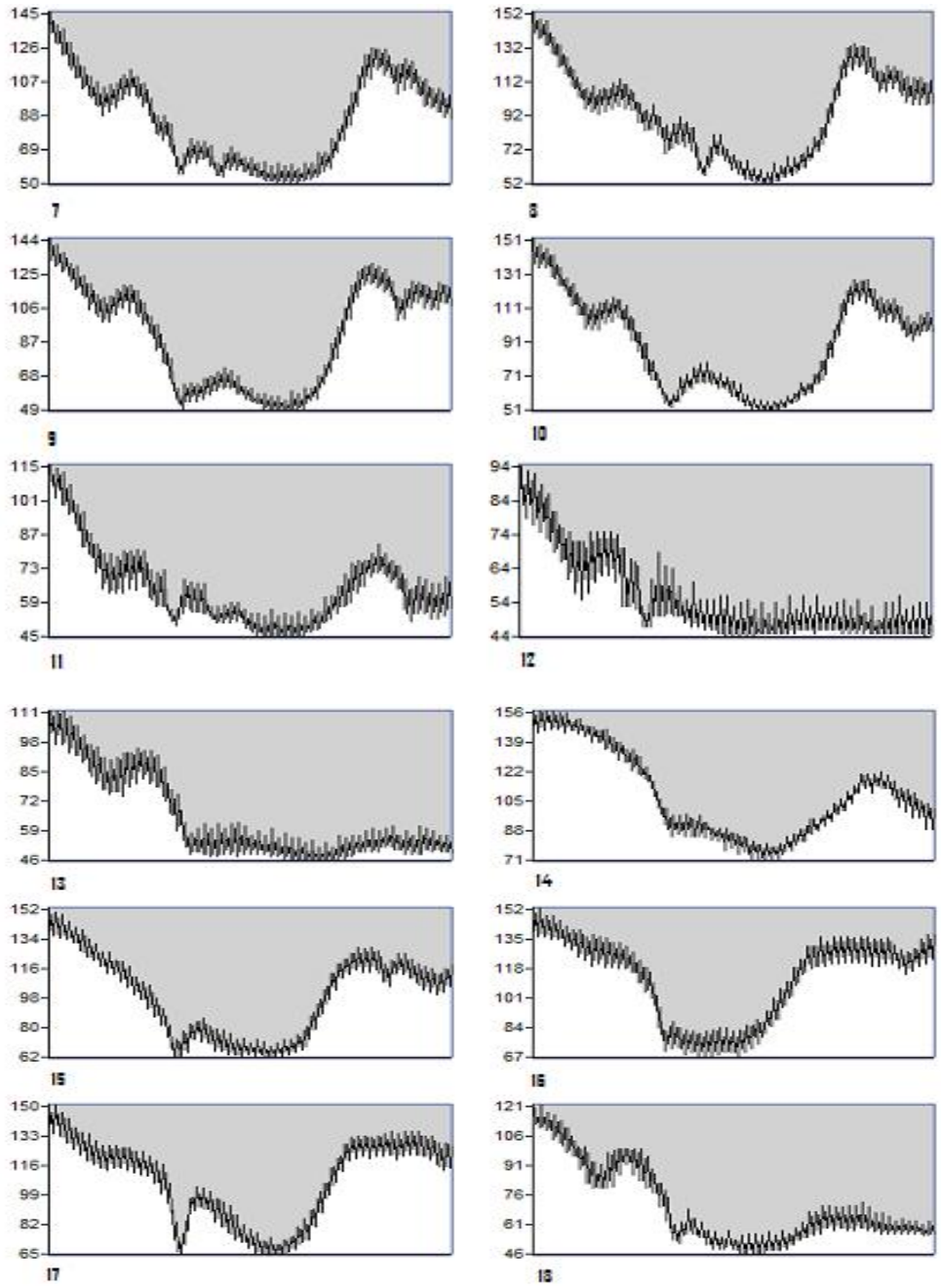


Figure 2. Some of pictures for Fig. no. 1 showed the density of transferrin for lanes no. 1-18 in Iraqi Carp.

Seven genotypes were identified for the transferrin (CC, DD, GG, FF, DC, DG and FD). The genotypes of transferrin alleles obtain in this study are similar to those reporter in *Csizmadia et al. (1995)*. They have found 20 transferrin genotypes (AA, BB, DD, EE, FF, GG, AB, AD, AF, AG, BD, BE, BG, DE, DF, DG, EF, EG, FG, and FH) caused by 7 alleles (A, B, D, E, F, H and G).

Conclusion

As far as we know, this is the first large-scale analysis on the genetic polymorphism in carp (*Cyprinus carpio*). Seven **Tf** genotypes consisting of 4 homozygote types (**CC, DD, FF** and **GG**) and two heterozygote types (CD, DG and FG) were detected

Acknowledgment

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Ispitivanje biohemijskog polimorfizma šarana (*Cyprinus carpio*): detekcija novih alela u transferinu

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Rezime

Ipsitivanje je izvedeno na Univerzitetu u Basri, Poljoprivrednom koledžu i Centru za morska istraživanja. Studija je urađena u cilju ispitivanja postojanja polimorfizma na transferin (**Tf**) lokusu kod šarana (*Cyprinus carpio*). Korišćena je metoda poli-akrilamid-gel elektroforeze (PAGE) u alkalnim uslovima za određivanje **Tf** alela šarana. Analiza **116** životinja je pokazala da su sve životinje bile polimorfne, i pokazivale više genotipova. Postojala je jasna biološka raznolikost (bio-diverzitet) kod **Tf** gena. Sedam **Tf** genotipova koji su se sastojali od 4 tipa homozigota (**CC, DD, FF** i **GG**) i dva tipa heterozigota (CD, DG i FG) je otkriveno. Ove frakcije kontorlišu ko-dominantni autozomni geni prema Mendeljejevom zakonu nasledstva. Najveća učestalost/frekvencija gena je utvrđena za **Tf** D - 0.50, **Tf** F - 0.26, i za C i G - 0.12. Prema tome, asamblaži šarana

(*Cyprinus carpio*) su dosledno pokazivali tendenciju da budu više dominantni na D alelu. Razlike između očekivanih brojeva i brojeva utvrđenih za transferin genotipove nisu bile signifikantne. Ovo je korisno za proces genetskog poboljšanja kroz selekciju. Koliko je poznato, ovo je prva velika analiza genetskog polimorfizma šarana (*Cyprinus carpio*). Poli-akrilamid elektroforeza, metoda koja je primenjena u ovoj studiji, omogućava brz i efikasan skrining prisustva polimorfizma na **Tf**.

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