RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
PRINT ISSN 2221-1896, ONLINE ISSN 2223-0343
www.roavs.com

A study of biochemical polymorphism in Carp (*Cyprinus carpio*): new alleles in transferring

Talib A. Jaayid[1], Muntaha Y. Yousief[1], Jaafer M. Owaid[1] and Najim M. Aziz[2]

[1]Animal Production Department, College of Agriculture, [2]Marine Science Center, Basrah University, IRAQ

Abstract

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. Analysis of 116 samples revealed that all animals were polymorphic, showing many genotypes with 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 such as 0.50 for Tf D, 0.26 for Tf F and 0.12 for C and G. Thus, carp assemblages consistently tended to be more predominant to D allele. Differences between expected number and observed number for transferrin genotypes were no significant. 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) goose (Valenta and Stratil, 1978) chicken (Vyshinsky and Muravjev, 1970) and sheep (Jaayid et al., 2011). Transferrin polymorphism was demonstrated in different breeds in fish. Since then, several reports have been published concerning the gene frequencies in these systems and about the possible influence of this 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 in 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. By applying different biochemical-genetic markers such as transferrin and isoenzymes, the individuals and the populations could be genetically well characterised. 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 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 this 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 except only one report (Jaayid and Aziz, 2009) is available. This paper describes (1) transferrin polymorphisms in Carp (*Cyprinus carpio*), (2) presents evidence of multiple phenotypes in Carp and (3) investigate and propose management and utilisation strategies for fish resources in Iraq.

Materials and Methods

A polyacrylamide gel electrophoresis (PAGE) of transferrin protein fractions was carried out in 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 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 analysis was performed on the basis of the Hardy-Weinberg law.

Results and Discussion

Figure 1 shows the electrophoretical patterns of some individual carp 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 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 show variation in the sample of Carp (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 of co-dominant alleles at an autosomal locus (Khaertdinov, 2000).

The Carp 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 found seven transferrin variants (A,B,C,D,E,F, and G) in 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 that reporter by 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 & G).

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

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

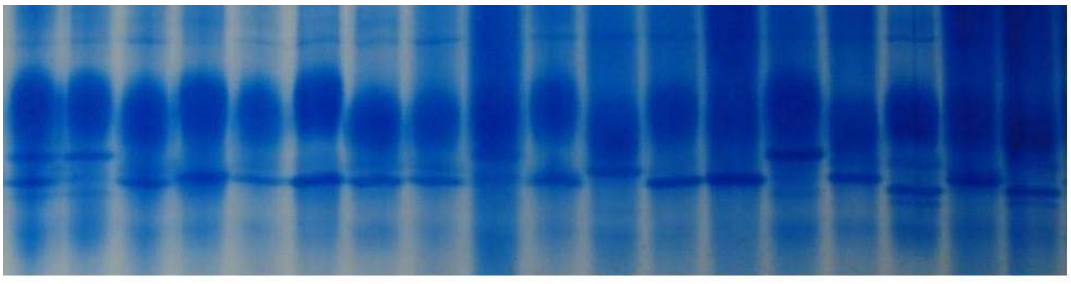

Fig. 1: Different transferrin genotypes as detected by polyacrylamide gel disc electrophoresis patterns at 8.6 in Iraqi Carp: 1-DG, 2-GG, 3-8-DD, 9-DG, 10-DD, 11-FF, 12-13-DD, 14-GG, 15-FF, 16-CD, 17-FF, 18-CD.

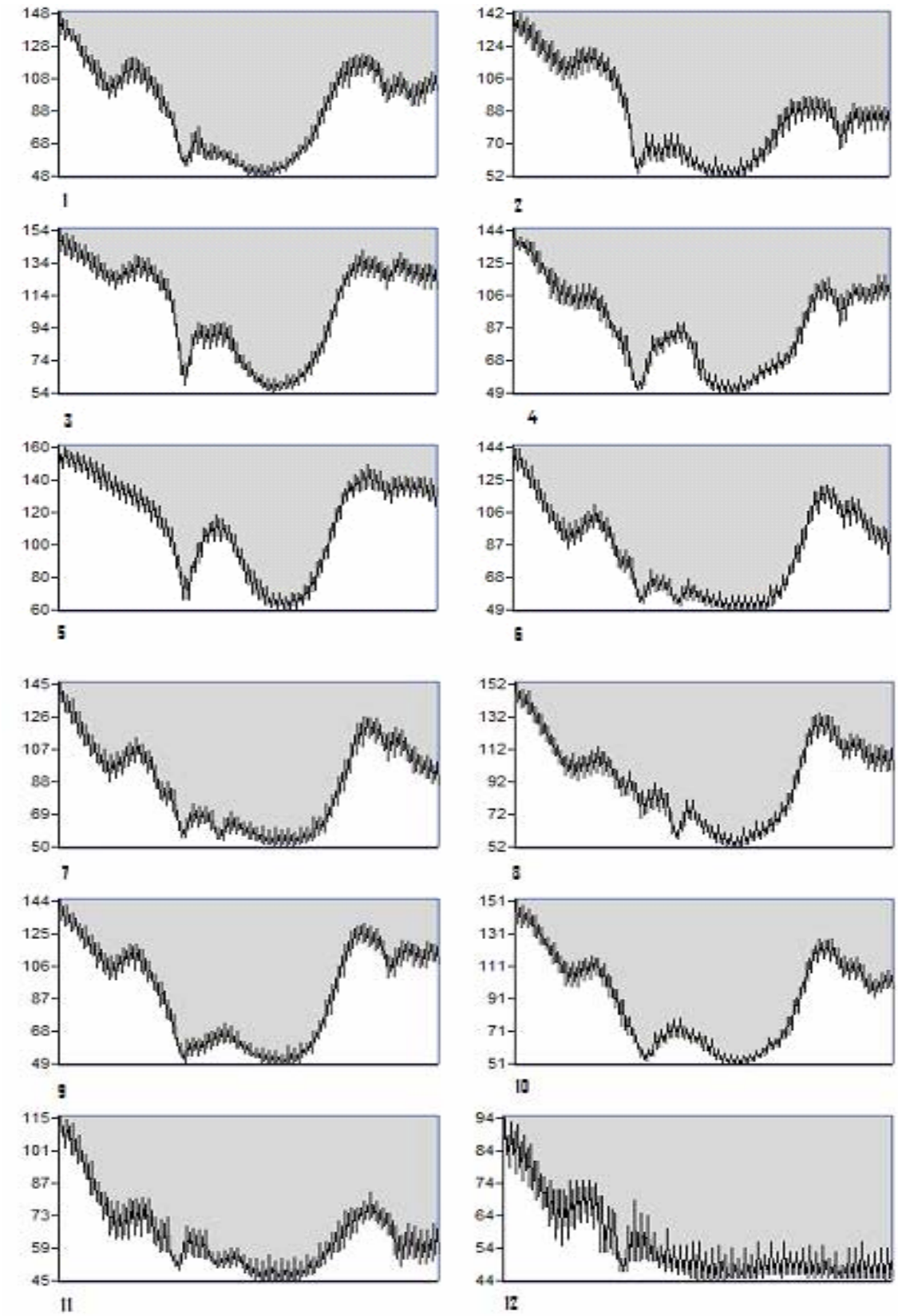

Fig. 2: Some of pictures for Fig. no. 1 showed the density of transferrin for lanes no. 1-18 in Iraqi Carp.

References

Baker, E.N. 1994. Structure and reactivity of transferrins. *Advances in Inorganic Chemistry*, 41: 389–463.

Csizmadia, C.S., Jeney, Z.S., Szerencses, I. and Gorda, S. 1995. Transferrin polymorphism of some races in a live gene bank of common carp. *Aquaculture*, 129: 193-198.

Ford, M.J. 2001. Molecular evolution of transferrin: evidence for positive selection in salmonids. *Molecular, Biology and Evolution*, 18: 639–647.

Irnazarow, I. and Bialowas, S.H. 1994. Genetic characteristics of carp breeding lines at the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences Golysz. 1. Polish lines. *Acta Hydrobiology*, 36: 125–142.

Jaayid, T.A. and Aziz, N.M. 2009. Study of transferrin polymorphism in a population of Carp (*Cyprinus carpio*). *Marsh Bulletin*, 2: 162-168.

Jaayid, T.A.. Yousief, M.Y Zaqeer, B.F. and Owaid, J.M. 2011. Genetic polymorphism of transferrin in Arabi sheep breed. Dayala international conference.

Jurecka, P., Geert, F., Wiegertjes, B., Krzysztof, L., Rakus, A., Andrzej Pilarczyk, A. and Ilgiz, I.A. 2009. Genetic resistance of carp (Cyprinus carpio L.) to Trypanoplasma borreli: Influence of transferrin polymorphisms. *Veterinary Immunology and Immunopathology*, 127: 19–25.

Jurecka, P., Ilgiz Irnazarowa, A., Adrie, H. Westphal, B. Maria Forlenza, C, Joop, A.J. Arts, C., Huub, F.J. Savelkoul, C., Geert, F. and Wiegertjes, C. 2008. Allelic discrimination, three-dimensional analysis and gene expression of multiple transferrin alleles of common carp (Cyprinus carpio L.). *Fish & Shellfish Immunology*, 30: 1–9.

Khaertdinov, R.A. and Gataulin, A.M. 2000. The selection to increase protein and improvement of technological properties of milk-Kazan, Russia. P: 164.

Kohlmann, K. Gross, R., Murakaeva, A. and Kersten, P. 2003. Genetic variability and structure of common carp (Cyprinus carpio) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. *Aquatic Living Resources*, 16: 421–431.

Valenta, M. and Stratil, A. 1978. Polymorphism of transferrin and conalbumin in the domestic goose (Anser anser). *Animal Blood Groups and Biochemical Genetics*, 2: 129–132.

Valenta, M., Stratil, A., Slechtova, V., Kalal, L. and Slechta, V. 1976. Polymorphism of transferrin in carp (Cyprinus carpio L.): Genetic determination, isolation, and partial characterization. *Biochemical Genetics*, 14: 27-45.

Vyshinsky, F.S. and Muravjev, V.I. 1970. Polymorphism of chicken serum transferrin. Xlth European Conference on Animal Blood Groups and Biochemical Polymorph. Pp: 425-428.

Wiegertjes, G.F., Groeneveld, A. and Muiswinkel, W.B. 1995. Genetic variation in susceptibility to Trypanoplasma borreli infections in common carp (Cyprinus carpio L.). *Veterinary Immunology and Immunopathology*, 47: 153–161.

Wojtczak, M., Dietrich, G.J., Irnazarow, I., Jurecka, P., Slowinska, M. and Ciereszko, A. 2007. Polymorphism of transferrin of carp seminal plasma: Relationship to blood transferrin and sperm motility characteristics. Comparative Biochemistry and Physiology: Molecular Biology, 148: 426-431.