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Alleles and genotype frequencies of transferrin in common carp (*Cyprinus carpio*) in Southern Iraq

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Abstract

Common carp (*Cyprinus carpio*) is widely found across Asia and Europe. The species is popular for its tolerance to physiological and environmental stresses. Alleles encoding transferrin (Tf) in common carp have been studied for their role in identification of tolerant type and to investigate breeding pattern. However, the data about allelomorphs of common carp Tf from Iraq has been described less frequently. Therefore, this study aimed to determine the frequencies of various Tf alleles in 142 common carp specimens collected from 32 different locations. The allele forms were detected through SDS PAGE and their abundance was correlated with physicochemical parameters of the sampled water. Our results indicated a degree of genetic polymorphism in common carp Tf as presence of C, D, F and G was detected. The allele DD was found to be the most prevalent followed by FF while other alleles were present with reduced frequencies. Hardy-Weinberg equilibrium showed a marked difference from observed values with a high x^2 value (251.57; $P< 0.001$), suggesting that selective forces, population structuring or environmental factors may have influenced Tf genotype distribution within the study population. The low observed heterozygosity than the expected heterozygosity and Shannon diversity index reflected substantial allelic variation. The allelic dominance was also correlated with the water temperature and iron concentration. Hence, population structuring of common carp may be linked more to physicochemical variability than to random mating. The role of Tf in iron metabolism and immune system necessitates further investigations to support aquafarming and to maintain a sustainable supply of animal protein.

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Introduction

The common carp (*Cyprinus carpio*) is one of the economically and culturally significant freshwater fish species found in various countries. Its aqua-culturing has been dated back to more than two thousand years in East Asia from where it was spread to Middle East and Europe (1). The aquafarming of common carp is favored due to its adaptation, high growth rate, tolerance to environmental stresses and omnivorous feeding habits (2,3). The species can be cultivated in cold temperate lakes to warm irrigation ponds rendering it an important source of food for rural livelihoods, particularly in developing regions (4,5).

In global fish production and local economies, common carp aquaculture contributes significantly along with tilapia, and grass carp. Indeed, it can be cultured with these species and helps in optimizing resource utilization and maintaining ecological balance (6,7). Common carp farming also provides sustainable source of income to small farmers, affordable animal protein to local communities and support ancillary industries such as feed processing and marketing (8). In some regions, such as in South and West Asia, common carp has been used as a model fish for selective breeding programs, aimed at enhancing disease resistance, feed conversion efficiency, and tolerance to the environmental stresses (9,10). Hence, understanding its

genetic diversity will help to expand its practical utility and aquaculture sustainability.

Genetic studies on common carp mainly focused on identification of molecular markers through isozyme electrophoresis and DNA-based techniques (11,12). In this context transferrin (Tf) appeared as a relevant marker (13). Tf is a serum glycoprotein which is involved in iron homeostasis and has a role in innate immunity (14). Generally, Tf captures iron from serum thereby making this mineral unavailable to pathogen and hence provides protection to the fish (13). In common carp and other fish, Tf modulates oxidative stress, maintains plasma iron balance and participates in immune signaling cascades during infection (15).

Polymorphism in fish Tf has been long recognized through electrophoresis revealing co-dominant alleles of A, B, C, D and so on (16,17). Based on amino acid substitution, isoelectric point and conformation, these alleles exhibit variations. In addition to variations in physicochemical properties, these alleles also had varying iron binding-capacity, and they differ in their interaction with pathogen and ability to resist environmental stress (18,19).

It has been established that certain Tf phenotypes render fish resistant to pathogens such as resistance to *Trypanoplasma borreli* while some phenotypes render the animal immunocompromised and can delay the post-infection recovery (18,19). These functions have been attributed to alteration in glycosylation patterns that impacts protein stability and plasma turnover (20,21). Moreover, Tf expression is influenced by environmental conditions such as temperature, pH and presence of iron that also help the fish to adapt to ecological changes (21,22). The allelic variation is also considered as an indicator of genetic structure, migration and adaption of the fish species. The easy detection of allelic differentiation has rendered it as a good marker to infer population differences among carp stocks (23,24). The distribution of Tf alleles across carp population also reflects both historic introductions and local selection pressures. Hence in aquacultures, propagation of particular strains or hybrids can be traced by looking at Tf alleles (19,21).

In population genetics of fish, Tf served as a valuable indicator of genetic structure, migration and adaptation (12). Tracing Tf alleles helps population geneticists to understand historic introduction of a particular type and local selection pressure. This selection pressure may have arisen due to high pathogen load, fluctuating temperatures and inclusion of waste water contaminated with heavy metals (15). However, hatchery practices can lead to reduced allelic diversity thereby reduce adaptive potential leaving the fish vulnerable to pathogens (22).

In Iraq, owing to extensive river systems of the Tigris and Euphrates along with their tributaries, common carp is prevalent naturally, as well as, farmed on large scale. Indeed, over the time, carp farming has become the backbone of the country's inland aquaculture (25,26). Studies have indicated presence of Tf alleles in Iraqi's carp similar to other regions (27). However, this extensive farming has brought the challenges of genetic homogenization and propensity to the exposure to new disease agents (28). It has also been indicated that exposure to diverse parasitic and bacterial infections rendered a constant selective pressure on the allelic prevalence of Tf (29). Yet there is scarcity of data highlighting the allelic diversity in Tf of carp across different regions in Iraq. Understanding prevalence of various Tf alleles will aid to explore the pattern of gene flow and genetic differentiation as shaped by hydrological connectivity and human mediated translocations. It will also render the farmers able to develop selective breeding protocols aimed at enhanced stock resilience. Moreover, Iraqi carp population occupy critical position in understanding the regional biogeography of carp, particularly in the context of migratory and trade corridor across middle east, Europe and Asia (23). Thus, characterizing Tf variation in Iraqi waters contributes to broader evolutionary and domestication history of this particular species across Asia and Europe.

Materials and Methods

Ethical approval

This study was conducted with the approval of Research Ethic Committee of the Marine Science Center, University of Basrah.

Study Area and Sampling

Specimens of the common carp were collected from different aquaculture and natural habitats representing major carp producing areas in Iraq. Sampling was carried out from study area comprising ponds and riverine stations along the Tigris and Euphrates basin and selected fish farms in southern Iraq including Basrah, Diwaniya, Hillah, and Kut. These sites represent variable ecological conditions including temperature, salinity, and water flow.

A total of 142 adult fish samples were collected during March to June 2023. Fish were netted manually or captured using seine nets with a minimum handling stress. Immediately, 2-3 mL of blood was withdrawn from the caudal vein using sterile syringes and was blood was transferred to heparinized tubes and kept on ice, until analyzed. The ethical standards recommended by the Basrah Local Government and the University of Basrah were followed in obtaining and handling the experimental animal (fish).

Plasma Preparation and Protein Extraction

Blood samples were centrifuged at 3,000 rpm for 10 min at 4^{o}C to separate plasma which was kept at -20 ^{o}C until electrophoretic analysis. Prior to electrophoresis, plasma samples were diluted (1:2 v/v) with saline. Total protein estimation was carried out using Bradford method

(Thermofisher Scientific, catalogue A55866) following the manufacturer's instructions. Various concentrations of bovine serum albumin (BSA) were used as standard (30).

SDS-PAGE Analysis of Transferrin

Tf polymorphism was analyzed by SDS PAGE following methods given earlier (18). Briefly, in a discontinuous system of 10% separating and 4% stacking gels, 20 µL of plasma samples diluted in SDS sample buffers were loaded. The samples were electrophoresed at a constant current of 20 mA using Mini-Protean (Biorad, USA). After electrophoresis, the gel was fixed in 10% trichloroacetic acid for 30 min followed by staining with Coomassie Brilliant Blue T-250 for 2 h. It was then destained using a mixture of methanol-acetic acid-water (40:10:50 v/v) until distinct protein bands appeared. The gels were air-dried and photographed.

Phenotype and Allele Identification

Tf phenotypes were determined by visual inspection of distinct banding patterns with each phenotype representing a combination of co-dominant alleles (such as A, B C). Homozygous individuals displayed single Tf bands whereas heterozygous exhibited two bands of intermediate mobility. Electrophoretic mobilities were compared with earlier reports. Consequently, genotype was assigned to the sample. The relative frequencies of alleles and genotypes were computed using standard population genetics formula. Observed (Ho) and expected (He) heterozygocities were calculated to assess genetic diversity within populations.

Allelic frequency (p_i) was calculated as:

$$p_i = \frac{2N_{ii} + N_{ij}}{2N}$$

Where Nii = Number of homozygotes for allele I, Nij = Number of heterozygote allele carrying I, N = Total number of individuals analyzed.

Chi-square test was performed to understand the deviation from Hardy-Weinberg equilibrium for each population using the formula:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where O and E denote observed and expected genotype counts, respectively. The data was analyzed at P<05.

Environmental and Ecological Correlations

Basic environmental parameters including temperature, pH and dissolved oxygen were recorded at each sampling site. In addition, total iron concentration was also analyzed using atomic absorption spectrophotometry. Briefly, water samples were filtered through 0.45 □m membrane and acidified to pH <2 with concentrated nitric acid. Measurements were taken at 248.3 nm using acetylene flame and compared with various concentrations of iron. The correlation between environmental factors and specific Tf was also evaluated to understand any adaptive response to ecological stressors, particularly given Tf's role and iron metabolism.

Statistical analysis

Descriptive statistics were computed for allele frequencies, genotype frequencies and environmental variables. Variable in allele distribution among populations were tested using analysis of molecular variance (AMOVA) and pair-wise F statistics (FST). Multiple regression analysis was performed to understand the relationship between Tf frequencies and environmental parameters.

Results

The distribution of Tf genotypes in common carp revealed substantial variation among individuals. With 45 individuals (Table 1), the DD genotype was the most frequent (Figure 1) followed by 34 FF and 24 GG. Less frequent genotypes included CC, CD, GD, and FD. Hardy-Weinberg equilibrium showed a marked difference from observed values with a high x2 value (251.57; P<0.001). This deviation indicated departure from random mating suggesting that selective forces, population structuring or environmental factors may have influenced Tf genotype distribution within the study population.

Genetic diversity indices derived from the Tf genotypes are summarized in Table 2. The observed heterozygosity (Ho = 0.2183) was notably lower that the expected heterozygosity (He = 0.6780) indicating a pronounced deficit of heterozygotes. The effective number of alleles (Ne = 3.195) and Shannon diversity index (I = 1.252) reflect substantial allelic variation despite this imbalance. The inbreeding coefficient (Fis = 0.6822) further supports the presence of nan-random mating or subpopulation differentiation. Collectively these findings point to a genetically diverse but structured population where certain Tf alleles may confer adaptive advantages under specific ecological or physiological conditions.

The distribution of Tf alleles across sampling site also showed noticeable heterogeneity in the population (Figure 2). The D allele was the most prevalent as was demonstrated by DD types while F and G alleles exhibited intermediate frequencies. The less frequent C allele appeared in small proportion as all sites. Such variation may reflect localized adaptation or environmental selection pressures acting upon Tf polymorphism. The allelic balance suggests a genetically diverse population with potential for resilience to ecological fluctuations such as a change in water temperature, pH and metal ion concentrations.

The deviation plot demonstrates the differences between observed and expected genotype counts for Tf loci (Figure 3). Several genotypes, notable DD and GG deviate markedly

from equilibrium expectation with an apparent excess of homozygotes and deficit of heterozygotes. This pattern implies that random mating is unlikely to be the sole driver of the populations genetic structure. Instead of environmental factors, such as temperature stress of iron availability, may be influencing allelic combinations possibly through differential survival or reproduction linked to Tf-mediated iron metabolism.

Table 1: Observed (O) and Expected (E) genotype counts, frequencies, and x2 distribution for transferrin (Tf) polymorphism in common carps under Hardy-Weinberg Equilibrium (HWE)

Genotype	Observed (O)	Expected (E)	O/N	E/N	χ^2 Contribution
CC	8	1.014	0.0563	0.0071	48.125
CD	8	10.225	0.0563	0.0719	0.484
CF	0	7.014	0	0.0494	7.014
CG	0	4.732	0	0.0333	4.732
DD	45	25.776	0.3169	0.1815	14.337
DF	0	35.363	0	0.2489	35.363
DG	0	23.859	0	0.1679	23.859
FF	34	12.129	0.2394	0.0854	39.441
FG	0	16.366	0	0.1152	16.366
GG	24	5.521	0.169	0.0388	61.848

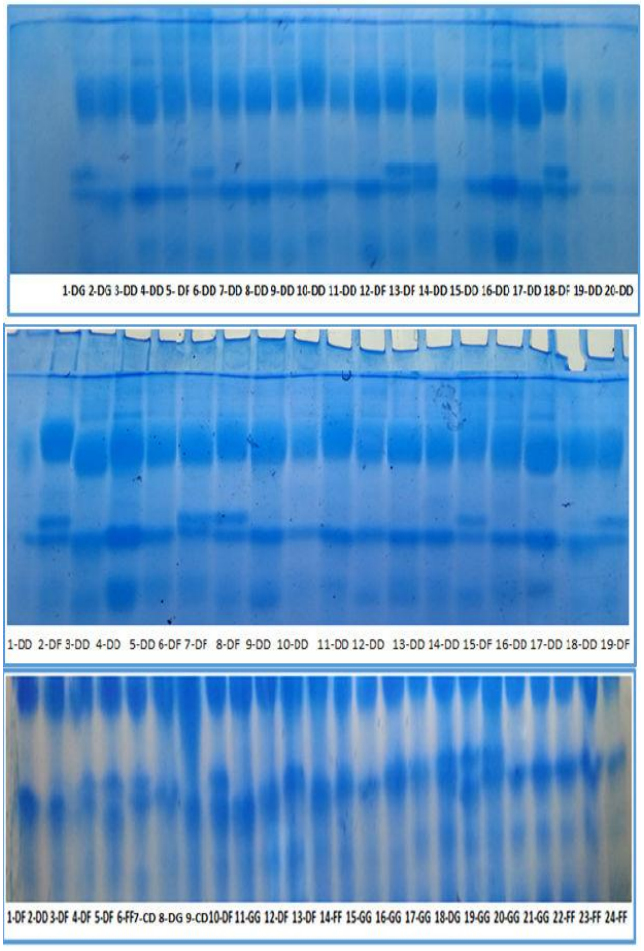

Figure 1: SDS PAGE Analysis of representative samples. Plasma samples from individual fish were electrophoresed and compared to detect allele type.

Table 2: Summary of genetic diversity at the transferrin (Tf) locus in Common carp

Index	Value
N (individuals)	142
Alleles (k)	4
Allele frequencies (C, D, F, G)	0.0845, 0.4261, 0.2923, 0.1972
Observed heterozygosity (Ho)	0.2183
Expected heterozygosity (He)	0.687
Effective number of alleles (Ne)	3.195
Shannon Index (I)	1.252
FIS	0.6822
χ^2 (HWE)	251.57 (df=6, p<0.001)

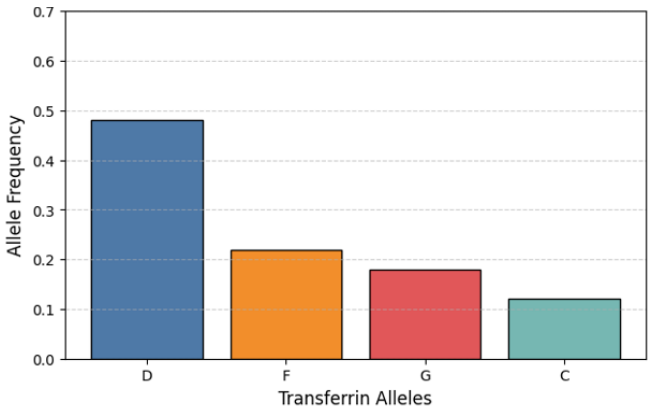

Figure 2: Allelic frequency distribution of transferrin genotypes in common carp. Relative frequencies of the alleles were calculated.

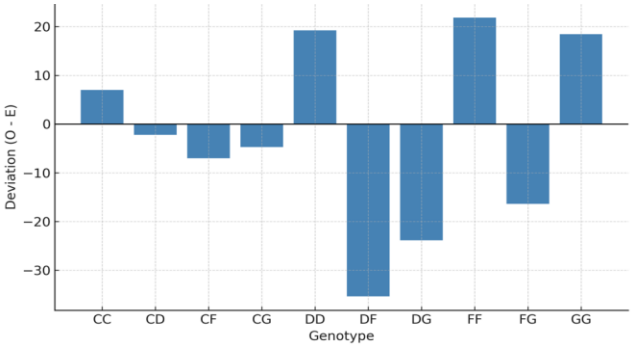

Figure 3: Deviation from Hardy-Weinberg Equilibrium in common carp Transferrin genotypes. Positive values show genotype found in excess, while negative values indicate underrepresented or absent genotypes.

Table 3: Physicochemical characteristics of the sampling sites

Site	Temperature (°C)	pH	Fe (mg/L)
Al-Basrah	29.5	8.5	0.2
Al-Diwaniya	28.5	7.6	0.35
Al-Hillah	27.5	7.8	0.35
Al-Kut	28	7.6	0.4

Water quality parameters varied moderately among the four sampling locations (Table 3). Temperature ranged from 27.5 ºC to 29.5 ºC reflecting the upstream-to-downstream thermal gradient typical of the Iraq riverine system. The pH values were slightly alkaline (7.6-8.5) across all sites consistent with carbonate-rich freshwater environments. Dissolved Iron concentration increased progressively across the downstream from 0.2 mg/L to 0.4 mg/L that may be attributed to agricultural run-off. This environmental gradient provided a meaningful context for assessing the influence of environmental and chemical factors on Tf allelic distribution within common carp population.

The inverse relationship between iron concentration in water and frequency of allele D across the sampling site (Figure 4) suggested a potential adaptive response of Tf alleles to iron bioavailability. This allelic adjustment can help minimize oxidative stress due to the presence of iron in water. While the positive correlation of allele D with temperature (Figure 5) demonstrated its thermal stability and hence fitness to bind with the iron. While water pH was associated positively with the prevalence of allele F (Figure 6), reflecting varied properties of different Tf alleles.

When Tf allelic frequencies were investigated in relation to their spatial distribution, it was observed that the D allele was predominant at all locations with the highest frequency in a comparatively warmer and low-iron habitat (Table 4). In comparatively cold- and iron-rich sites of Al-Hillah and Al-Kut, F and G alleles were common. Observed heterozygosity (Ho = 0.2 – 0.23) remained lower than expected (He = 0.66-0.69) yielding consistently positive inbreeding populations, possibly due to limited gene flow or localized selective pressures linked to physicochemical conditions. Moreover, the consistent heterozygote deficit (high F_{is} values) across all sites indicates that population sub-structuring or localized pressures are may be linked to physicochemical variability and play a more significant role than random mating alone in maintaining the genetic heterogeneity.

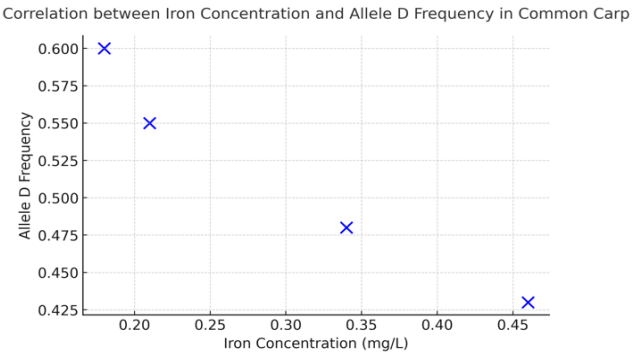

Figure 4: Correlation between iron concentration and allele D frequency in common carp.

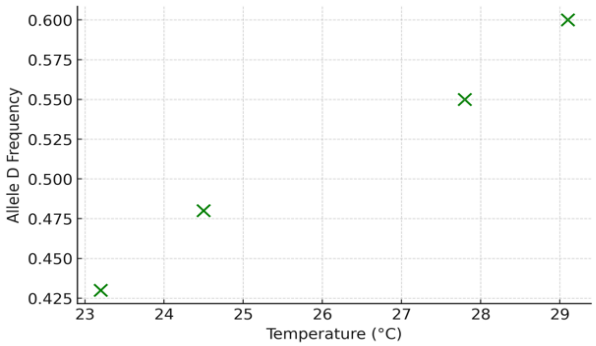

Figure 5: Correlation between temperature and allele D frequency in common carp.

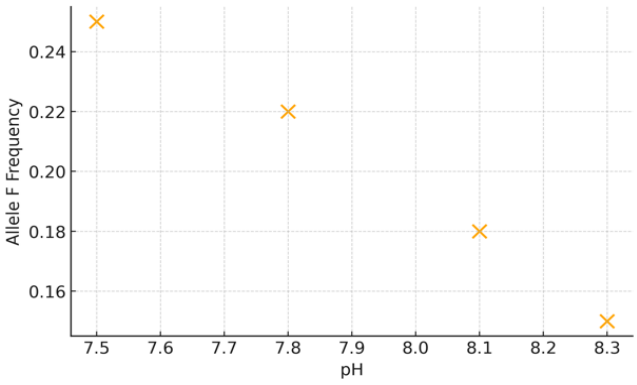

Figure 6: Correlation between water pH and allele F frequency in common carp.

The data was assessed by considering analysis of molecular variance, fixation index and correlation analysis (Table 5). It indicates moderate population differentiation with 7.4% of genetic variance occurring among the sampling site and several significant pairwise FST values (Table 5). Strong positive correlation between iron concentration and allele D was observed highlighting impact of iron availability as a dominant environmental predictor of allele distribution. While temperature has a weak negative influence while pH has not conclusive impact.

Table 4: Transferrin allelic frequencies and diversity indices of common carp across sampling sites

Site	Allele C	Allele D	Allele F	Allele G	H_o	H_e	F_{is}
Al-Basrah	0.06	0.45	0.33	0.16	0.23	0.68	0.66
Al-Diwaniya	0.07	0.43	0.31	0.19	0.22	0.69	0.68
Al-Hillah	0.09	0.41	0.29	0.21	0.21	0.67	0.69
Al-Kut	0.1	0.38	0.27	0.25	0.2	0.66	0.7

Table 5: Combined statistical summary presenting Analysis of molecular variance (AMOVA), Fixation index (FST), Correlation and Regression analyses for transferrin (Tf) alleles in common carp

Analysis component	Statistic/Parameter	Value
AMOVA	Variation among populations	7.4%
	p-value	0.021*
Pairwise FST	Ranges	0.021-0.081
	Significant population pairs	4 to 6
Pearson Correlations	Allele D Vs Fe	r = 0.78 (*p*=0.001*)
	Allele F vs Fe	r = 0.52 (*p*=0.018*)
	Allele D vs Temperature	r = 0.42 (*p*=0.041*)
	Allele G vs pH	r = 0.44 (*p*=0.052)
Regression Model	Fe Concentration	0.711 (*p*=0.0002)
	Temperature	-0.281 (*p*=0.042*)
	pH	-0.142 (*p*=0.324)
	Model R^2	0.63

Discussion

Tf is a vital iron binding glycoprotein in vertebrates that plays an important role in iron homeostasis and innate immunity (31). In fish species, its genetic variants have been documented earlier. In common carp multiple co-dominant alleles have been reported to segregate at a single Tf locus (29). The electrophoretic profile of Tf alleles as revealed in this study corroborates earlier findings, where 6-8 alleles were reported in Europe and other regions of Asia (23). Our findings about the prevalence of D allele and outnumbering of heterozygotes by homozygotes were consistent with other studies where population specific dominant of particular Tf alleles have been reported (32-34), yet the magnitude of homozygosity was higher in our study. That can be linked with ecological niche and different physicochemical parameters. This justification was strengthened by the calculated association between allele frequencies and environmental factors that also suggested influence of local ecological factors over the distribution and maintenance of Tf alleles in carp populations.

The dominance of D allele may provide ecological advantage to the fish species as Tf alleles differ in their iron binding affinity, stability and expression level. In one study, DD genotype harboring carp individuals were found to be resistant to infection by *Trypanoplasma borreli*; it also reported improved survival probability of the fish indicating about the role of this genotype in fitness of the species (22). Likewise, in other species including Salmonids and croakers, Tf alleles have been reported to be evolved under positive selection with amino acid substitutions in exposed binding site (29,34). However, further investigations involving a wide range of parasites and pathogens are required to ascertain the positive role of D allele in carp population from Iraqi waters.

Our data significantly deviated from Hardy-Weinberg equilibrium indicated that the common carp population is not panmictic and the Tf locus is acted upon by selection, population structure or inbreeding (35). The marked deficit of heterozygotes may be attributed to Wahlund effect which shows that a single population may represent a mixture of partially isolated subpopulations with distinct allele frequencies (36,37). Considering that this effect has been reported in other aquatic species where environmental heterogeneity or restricted dispersal creates microgeographic structuring, this can be a plausible justification in our study too. However, genetic analysis of large datasets involving multiple genes is required to affirm this hypothesis. The

presence of non-random mating or inbreeding can be another possible reason for less heterozygous nature of our population. Moreover, carps are known for site fidelity during spawning (38). Limited gene flow between spawning grounds could lead to assortative mating and an increased frequency of homozygotes (39). Also, there may be undetected null alleles or scoring errors in gels that can give false results of homozygote counts. Yet confirmation after repeated experiments render this option less likely. Nonetheless, higher fitness exhibited by DD Tf allele under given environmental and pathogenic conditions rendered a high homozygote frequency that contributed to Hardy-Weinberg disequilibrium.

The impact of environmental conditions on allele distribution was obvious as D allele was more common in warmer water but its frequency decreased with increasing iron concentration. The negative association of this allele with iron may reflect functional trade-off inherent in iron binding proteins as in high-iron environment, excessive iron binding can lead to oxidative stress through Fenton reaction (40). On the other hand, in low-iron environment, alleles that efficiently scavenge iron from scarce resources provide additional benefits. It is also worth mentioning that temperature influences both, the metabolic rate and solubility of iron. Hence, temperature can alter the physiological demands placed on Tf mediated iron transport. Protein adapted to higher temperatures exhibit more stability and have improved kinetic properties (41). This interpretation in the context of aquatic animals and Tf has been strengthened by earlier studies where thermal adaptation at protein loci was documented in fish population inhabiting variable thermal environment (42).

While F allele has a mild positive association with pH, changes in the form of iron under the influence of pH can have an impact over Tf's iron binding capacity. Iron is converted to insoluble hydroxides at higher pH and becomes unavailable to the living systems. In this case, a Tf variant that can maintain strong binding under reduced iron solubility could provide selective advantage. A previous study also indicated about the environment specific associations between Tf alleles and water chemistry in some teleost (43). Likewise, a modest but consistent association of F allele with slightly alkaline pH highlights stability and binding efficiency of this allele under such conditions.

The integrated effect of environmental factors on Tf polymorphism and its relationship with adaptive advantages has been hinted out earlier (43). The consistent findings across aquatic species strengthen our findings on common carp population from this region. However, there are certain limitations associated with this study. First, it is a single temporal snapshot ignoring multi-year and seasonal variations. Secondly reliance on SDS-PAGE to determine allelic forms may have brought a bias and the silent alleles remained undetected. A comprehensive genetic analyss is required to ascertain these findings. Moreover, the data given in this study should be considered cautiously as the correlation between allele frequencies and environmental parameters may not necessarily reflects causation and hence functional validation is essentially required. Also, the biochemical assays for iron-binding kinetics, structural stability across temperatures and pH ranges and expression studies should be conducted in future studies to ascertain the conclusions of this study. In addition, investigating other genes impacting Tf expression and variation is mandated to get a holistic view of the Tf role and functions in common carp present in varying environments.

Conclusion

The present study contributes to the understanding of natural variation of Tf alleles in common carp highlighting the utility of classical protein markers in ecological and evolutionary genetics. The dominance of D allele with a significant deviation from Hardy-Weinberg equilibrium and its association of iron concentration, water temperature and pH collectively suggest that Tf locus is influenced by natural selection rather than neutral processes alone. Our results reinforce earlier findings that Tf in common carp is functionally relevant and linked to immunity and environmental adaptation. Future studies integrating molecular characterization, experimental validation and multivariate environmental modelling will further explore the mechanistic basis of Tf adaptive diversity.

Conflict of interest

The authors do not declare any conflict of interest.

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تردد الأليلات والأنماط الجينية للترانسفيرين في الكارب الشائع في جنوب العراق

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الخلاصة

يُعدّ الكارب الشائع (*Cyprinus carpio*) واسع الانتشار في آسيا وأوربا، ويشتهر بقدرته على تحمّل الضغوط الفسيولوجية والبيئية. دُرست الأليلات المشفرة للترانسفيرين (Tf) في الكارب الشائع لدورها في تحديد النمط المُتحمّل ودراسة أنماط التكاثر. مع ذلك، فإن البيانات المتعلقة بالأليلات المختلفة للترانسفيرين في الكارب الشائع في العراق قليلة. لذا، هدفت هذه الدراسة إلى تحديد ترددات أليلات الترانسفيرين المختلفة في ١٤٢ عينة من الكارب الشائع جُمعت من ٣٢ موقعًا مختلفًا. تم الكشف عن أشكال الأليلات باستخدام تقنية الفصل الكهربائي الهلامي SDS-PAGE، ورُبطت وفرتها بالمعايير الفيزيائية والكيميائية للمياه المأخوذة. أشارت نتائجنا إلى وجود درجة من تعدد الاشكال الجيني للترانسفيرين في الكارب الشائع، حيث تم الكشف عن وجود الأليلات C وD وF وG. حيث وُجد أن الأليل DD هو الأكثر شيوعًا، يليه FF، بينما وُجدت أليلات الأخرى بترددات منخفضة. أظهر توازن هاردي-واينبرغ اختلافًا ملحوظًا عن القيم الملحوظة، مع قيمة χ^2 عالية (٢٥١,٥٧؛ $P < 0.001$)، مما يشير إلى أن قوى الانتقاء، أو بنية التجمعات السكانية، أو العوامل البيئية قد أثرت على توزيع النمط الجيني للترانسفيرين (Tf) داخل مجتمع الدراسة. يعكس انخفاض التغاير الزيجوتي الملحوظ مقارنةً بالتغاير الزيجوتي المتوقع ومؤشر شانون للتنوع تباينًا أليليًا كبيرًا. كما ارتبطت سيادة الأليل بدرجة حرارة الماء وتركيز الحديد. لذا، قد تكون بنية تجمعات الكارب الشائع مرتبطة بالتغيرات الفيزيائية والكيميائية أكثر من ارتباطها بالتزاوج العشوائي. يتطلب دور الترانسفيرين (Tf) في استقلاب الحديد والجهاز المناعي مزيدًا من الدراسات لدعم الاستزراع المائي والحفاظ على إمداد مستدام من البروتين الحيواني.

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