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Microsatellite Markers Used for Genetic Monitoring of Exotic Common Carp Variants (Common Carp and Scale Carp) in the River Chenab, Punjab, Pakistan

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SP and LS design the study. FT and MFK wrote the manuscript text. FT, UB and MT analyzed the data. FT, MH and AS prepared figures. FT and MFK prepared the tables.

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Genetic structure, Exotic species, Scale carp, Common carp, Genetic diversity, Heterozygosity

ABSTRACT

Understanding the genetic composition and diversity of invasive species is crucial for the effective management and conservation of native wildlife. In this study, 300 samples of common carp (*Cyprinus carpio linnaeus*) and scale carp (*Cyprinus carpio communis*) from five natural populations in the Chenab River, Pakistan, were collected for genotyping and 10 microsatellite markers were used. The analysis revealed that both strains exhibited low to moderate levels of heterozygosity. The average FIS values for common carp ranged from 0.507 to 0.5914, while scale carp showed values ranging from 0.53 to 0.62. FST analysis indicated minimal genetic differentiation between the strains, with the greatest genetic differences observed between strains and the least within individual strain populations. According to AMOVA, 90.38% of the genetic variance in common carp was attributed to intra-population variability, while inter-population variability accounted for 9.62%. Conversely, in scale carp, intra-population variability contributed to 12.92% of the total genetic variance, with 87.08% attributed to inter-population diversity. Bayesian clustering analysis identified 10 distinct groups among the populations of both strains, with no genetic evidence of admixture found in the pure, original strains. The high observed heterozygosity compared to expected heterozygosity in common carp populations suggest the possibility of a bottleneck event. The directed relative migration network highlighted HT (common carp) as the core population, showing the highest genetic exchange with the other five peripheral populations. In contrast, except for the scale carp population in Head Khanki, no significant migration was observed in the other populations. A dendrogram constructed using the Unweighted Pair Group Method with Averages (UPGMA) distinguished common carp and scale carp as two separate groups. This study provides valuable insights that could enhance the management strategies for invasive species.

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INTRODUCTION

In freshwater conditions and aquaculture systems across Pakistan, the species *Cyprinus carpio*, sometimes referred to as the European or common carp, and its variation, *Cyprinus carpio communis*, are extensively distributed (Yaqoob, 2021). The native fish population has been steadily declining as a result of the introduction of foreign strains of common carp (Kang *et al*., 2023; Kovalenko *et al*., 2021). Originally found only in Central

Asia and a few European countries, the common carp is now found on every continent with the exception of Antarctica and Northern Asia. As of right now, it may be found spreading from Siberia to the Mediterranean in Southeast Asia, China, India, and Pakistan (Nakajima *et al*., 2019; Souza *et al*. 2022). In order to increase fish output in a variety of aquatic environments, including ponds, tanks, lakes, and reservoirs, common carp and scale carp were introduced to Pakistan using composite fish farming techniques. Although the recipient ecosystems may suffer from the introduction of non-native fish species, there may be significant economic benefits as well (Bilal and Khan, 2003). Additionally, carps developed themselves in Punjab, Pakistan's riverine system, bolstering a robust commercial fisheries operation.The organism's invasive characteristics such as its quick growth rate, strong reproductive potential, early sexual maturity, and tolerance to different food sources are primarily responsible for its success in a variety of environments (Khan *et al*., 2021; Hayat *et al*., 2020). On the other hand, the reduction of native fish species in these rivers may be caused by the introduction of carp into riverine system of Punjab, Pakistan (Imran *et al*., 2021a; Parveen *et al*. 2018). The introduction of a certain species has a clear correlation with the continued reduction of many fish species in the fauna (Barletta *et al*., 2010).

Because of the way carp eat in benthic habitats, sediments are disturbed, nutrients are released, and water turbidity is subsequently raised (Mutethya and Yongo, 2021). Some fish species capacity to forage is therefore restricted by elevated turbidity. Furthermore, carp may interfere with other fish species' ability to reproduce as well as compete for resources like food and habitat (Imran *et al*., 2021b). Because of factors like competition, hybridization, predation, and indirect effects; the introduction of non-native species frequently causes the number of native species in an environment to decrease (Chen *et al*., 2023; Erarto and Getahun, 2020). Because common carp strains seriously disturb aquatic populations and the environment, they are regarded as nuisance fish in many western nations (Sorensen, 2021; Yaqoob, 2021; Mutethya and Yongo, 2021). The most significant threat to the variety of plant and animal species in the ecosystems of shallow lakes and wetlands in the United States is generally acknowledged to be the common carp (Pearson *et al*., 2019). Fish species in Pakistan's natural reservoirs have declined substantially in recent years as a result of pollution, illicit fishing, natural catastrophes, habitat degradation, and the introduction of non-native species (Abro *et al*., 2020; Parveen *et al*., 2024).

Unintentional risks from exotic species on native fauna might result from fisheries management that ignores genetic diversity and population dynamics (Pyšek *et al*., 2020). There is a dearth of research on the genetic diversity patterns of exotic species, despite the vast study of invasion mechanisms. Initiating management initiatives to address their genetic diversity is crucial. An efficient way to evaluate the biodiversity of invasive species is through the use of microsatellite-based markers (Fazzi-Gomes *et al*., 2021; Pandolfi *et al*., 2021). The genetic variability of common carp has been extensively examined in the literature using microsatellite markers (Yick *et al*., 2021). Because of its impartiality in sample selection and the simplicity with which results derived from them may be cross-validated and compared across other populations, microsatellites are unique in the field of population research (Guo *et al*., 2022). Reducing and maintaining the quantity of scale carp and common carp below ecological thresholds might be important to enhance the quality of the water and boost the population of local fish species.

It has long been acknowledged that determining the fine-scale genetic structure is essential to developing policies for managing freshwater fisheries. We investigate the patterns of genetic diversity in populations of exotic common carp and scale carp in Punjab, Pakistan's River Chenab. Using microsatellite markers, our study investigates the organization and genetic diversity of the scale carp and common carp strains in the River Chenab and also analyzes the population structure of both represented species which play a crucial role in effectively controlling these strains within river ecosystems. Policies for the sustainable management of alien species' fisheries will benefit from this genetic data.

MATERIALS AND METHODS

Collecting samples and DNA extraction

Five sample locations (Head Muhammad Wala, Head Qadir abad, Head Khanki, Head Marala and Head Trimmu) along the River Chenab yielded a total of 150 specimens of all strains of carps (scale carp and common carp) (Fig. 1, Table I). Out of the five sample locations, thirty specimens of every carp strain were gathered, for a total of 150 specimens per strain. With a few minor adjustments, a typical phenol: chloroform technique was used to isolate genomic DNA (Ghatak *et al*., 2013). We used 1% agarose gel electrophoresis to evaluate the integrity of the DNA. Additionally, we used the Thermo Scientific, US NanoDrop 2000c spectrophotometer to measure the concentration and purity of the DNA. After being separated, the DNA was diluted to a final concentration of around 50 ng/µL and kept cold until the PCR amplification process.

Table I. Details of sampling sites.

Site code	City of sampling site	Sampling point	Geographic location	Date of capture	Sample size
Common carp					
HT	Jhang	Head Trimmu	31°8'41.23"N, 72°8'46.38"E	11/11/2021	30
HM	Sialkot	Head Marala	32°40'20.64"N, 74°27'51.8"E	21/12/2021	30
HK	Gujranwala	Head Khanki	32°24'09.65" N, 73°58'14.30" E	5/1/2022	30
HQ	Hafizabad	Head Qadirabad	32°19'60" N, 73°43'60" E	13/01/2022	30
HMW	Muzzafargarh	Head M. Wala	30° 18' 2N, 71° 20' 42E	3/2/2022	30
Scale carp					
HT	Jhang	Head Trimmu	31°8'41.23"N, 72°8'46.38"E	11/11/2021	30
HM	Sialkot	Head Marala	32°40'20.64"N, 74°27'51.8"E	21/12/2021	30
HK	Gujranwala	Head Khanki	32°24'09.65" N, 73°58'14.30" E	5/1/2022	30
HQ	Hafizabad	Head Qadirabad	32°19'60" N, 73°43'60" E	13/01/2022	30
HMW	Muzzafargarh	Head M. Wala	30° 18' 2N, 71° 20' 42E	3/2/2022	30

Table II. Characteristics of common carp specific microsatellite loci.

S. No.	Locus no.	Primer sequence 5`→3`	Fragment size	Annealing temperature	Allele no.
1	[A]MFW3	F:GATCAGAAGGTACAGAGAAG R:CCTTACAGAAAACCTGTTTGC	134-240	58	7
2	[A]MFW4	F:TCCAAGTCAGTTTAATCACCG R:GGGAAGCGTTGACAACAAGC	138-253	59	5
3	[A]MFW6	F:ACCTGATCAATCCCTGGCTC R:TTGGGACTTTTAAATCACGTTG	158-212	60	5
4	[A]MFW7	F:GATCTGCAAGCATATCTGTCG R:ATCTGAACCTGCAGCTCCTC	132-152	59	7
5	[A]MFW11	F:GCATTTGCCTTGATGGTTGTG R:TCGTCTGGTTTAGAGTGCTGC	110-196	60	5
6	[A]MFW13	F:ATGATGAGAACATTGTTTACAG R:TGAGAGAACAATGTGGATGAC	192-270	58	7
7	[A]MFW15	F:CTCCTGTTTTGTTTTGTGAAA R:GTTCACAAGGTCATTTCCAGC	159-283	59	5
8	[A]MFW17	F:CAGTGAGACGATTACCTTGG R:GTGAGCAGCCCACATTGAAC	254-312	60	6
9	[A]MFW26	F:CCCTGAGATAGAAACCACTG R:CACCATGCTTGGATGCAAAAG	151-221	60	7
10	[A]MFW31	F:CCTTCCTCTGGCCATTCTCAC R:TACATCGCAGAGAATTCGTAAG	283-305	60	3

A= Crooijmans *et al*. (1997); F, forward; R, reverse.

Genotyping of microsatellite loci

Using 10 species-specific primers and a gradient heat cycler (Cycler-25, CA.95070, USA), the target microsatellite loci were amplified. MFW3, MFW4, MFW6, MFW7, MFW11, MFW13, MFW15, MFW17, MFW26, and MFW31 are the primers that were identified from two common carp strains (Tóth *et al*., 2019). Additionally, these loci have been investigated for cross-species amplification in common carp and scale carp. The following is a summary of the specifics for all loci, including fragment sizes, primer-specific annealing and primer sequences demonstrated in the Table II.

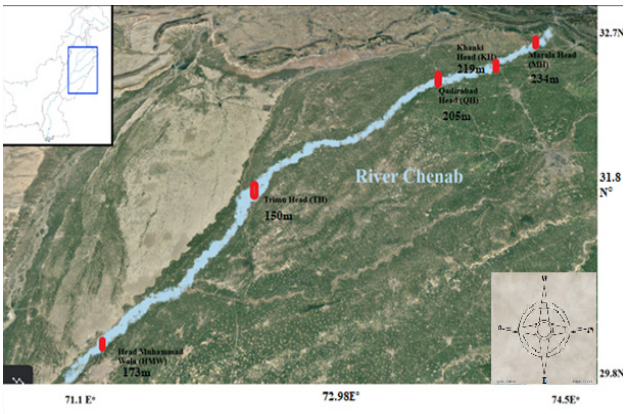

Fig. 1. Sampling locations of common carp and scale carp in Chenab River Punjab, Pakistan.

In the PCR cycle, double-stranded DNA is denaturized at 94°C after Taq polymerase activation takes place at 95°C during incubation. At 72°C, primers anneal, and eventually the cycle; DNA strand elongation carries out. 8% polyacrylamide gel electrophoresis (PAGE), which enables the detection of DNA bands of anticipated fragment sizes, was used for the analysis of amplicons. The information required for statistical analysis is provided by these bands. To act as a size marker for the amplicons, 3 µl of a sequence ladder was put into the center region of the gel. In addition, 5 µl of the PCR product from each sample was loaded in separate wells together with 1 µl of the loading dye, bromophenol blue. Following that, the PCR products underwent washing and were stained with silver. The gel was shaken continuously for 8–10 min with silver stain to visualize the PCR products. After that, gel findings were shown using a UV transilluminator (Sambrook *et al*., 1989).

Genetic diversity

The following summary statistics were used to quantify genetic variation: expected heterozygosity (He), observed heterozygosity (Ho), allelic richness (Ar), average number of alleles, and within-strain inbreeding coefficient (FIS). These statistics and the corresponding 95% confidence intervals for the statistical analysis were generated using the software programs GenAlEx 6.41, FSTAT v.2.9.3.2, and POPGENE 1.32.

Population structure

Using the FSTAT program version 5, the pairwise genetic differences between strains were calculated in accordance with the normal protocols outlined in the product instructions (García-Ramos and Kirkpatrick 1997). Nei's genetic distance approach was used to produce a genetic distance-based tree, which was then used to create the dendrogram (Nei, 1978) in the TFPGA software. The purpose of this investigation was to investigate the links between the two strains various populations. Using Arlequin 3.5.2.2, Analysis of Molecular Variance (AMOVA) was used to evaluate the genetic structural patterns in both carps strain populations, with a particular emphasis on allele frequencies in microsatellite data. To determine each pairwise comparison in order to achieve statistical significance, a thousand random permutations were carried out.

The Bayesian approach presented in structure 2.3.4 was used to evaluate the genetic structures between strains. An admixture model with associated allele frequencies was used in the investigation. One million Markov Chain Monte Carlo (MCMC) repeats followed a burn-in phase of 100,000 iterations. For every K value between 1 and 10, which denotes the number of clusters, 10 separate runs were carried out in order to guarantee consistency and detect genetic clusters. One cluster was added to all strains for purpose to find the maximum number of clusters, which made it possible to identify possible substructures in the data. By utilizing Structure Harvester's Evanno approach to calculate ΔK, the ideal K genetic cluster was evaluated. The program CLUMPP 1.1.2 was then used to provide visual representations of the clustering findings and to evaluate greatest similarity coefficient for different values of K over several runs.

Bottleneck analysis

The two-phase model (TPM) was applied, containing 1000 replications and 90% single-step mutations (Yang *et al*., 2022) was used with the Bottleneck v1.2.02 program to determine if populations of common carp had undergone bottlenecks recently. To assess bottleneck effects in each population of common carp strains, the mode shift test was also performed.

Gene flow analysis

In order to investigate patterns of gene flow among the populations, relative migration rates were evaluated using the GST statistic technique (Nei, 1973). The divMigrate-online program (Sundqvist *et al*., 2016), available at https://popgen.shinyapps.io/divMigrate-online/, was used to perform this study. Additionally, 1000 bootstrap iterations were used to infer asymmetric relationships between population pairs.

RESULTS

Genetic diversity

Ten microsatellite loci unique to each species of both

represented species; common carp and scale carp were used to analyze five populations of each strain: MFW3, MFW4, MFW6, MFW7, MFW11, MFW13, MFW15, MFW17, MFW26, and MFW31. It was discovered that every locus under analysis was polymorphic and exhibited Mendelian inheritance patterns. There were on average between 3.8 and 6.0 alleles per locus. Each strain's five populations showed different quantities of alleles at each locus, indicating that each population's origins were different. The highest average allelic richness (Ar) value for common carp was found in the HT (Head Trimmu). The highest average Ar value for scale carp was discovered in both HT (Head Trimmu) and HK (Head Khanki). The strain with the lowest average Ar value was identified as HMW (Head Muhammad Wala) in both cases. Geographic differences among all the populations are indicated by the variances in average allelic richness (Ar) values. Low to moderate levels of heterozygosity were seen. The average observed heterozygosity (Ho) for common carp varied from 0.3167 to 0.443. In HQ, the lowest average value of Ho was discovered (Head Qadirabad). The highest average Ho value for common carp was discovered in HT (Head Trimmu). These Ho values show that HT had the highest average amount of genetic diversity among all common carp populations, whereas HQ (Head Qadirabad) had the lowest average level. The average observed values of Ho for scale carp varied from 0.3067 to 0.4067. HMW (Head Muhammad Wala) had the lowest genetic diversity among the five populations of scale carp, while HT had the highest genetic diversity. For both strains, the highest average value of Ho was discovered in HT. For every locus across all populations, the Ho in both strains was less than the corresponding predicted heterozygosity (He). For any population in either strain, there were no negative Ho/He values found. Both strains exhibit substantial levels of polymorphism at the MFW3 and MFW15 loci, suggesting that these loci have a major role in the total amount of genetic variation found. In contrast, both strains exhibit the least amount of variation at the chromosomal locus MFW31. Using FSTAT software, the study computed the very locus in every population as well as every sample within every population had its FIS population inbreeding coefficient determined. Findings show that various populations' average FIS levels vary. The average observed FIS values for common carp varied from 0.507 to 0.5914. Head Qadir Abad (HQ) had the highest average FIS value, while Head Trimmu (HT) had the lowest. The FIS averages for scale carp varied from 0.5310 to 0.6166. Supplementary Table S1 showed that HM (Head Marala) had the lowest average value of FIS and HMW (Head Muhammad Wala) had the highest average value.

Population genetic structure

The allelic data were analyzed using pairwise comparisons of each population to ascertain the genetic composition of the common carp and scale carp populations. Significant genetic difference between groups was shown by statistically significant results from several of these comparisons. Significant variation across all analyzed groups is indicated by the FST values. The 10 populations average FST values vary from 0.0471 to 0.173. In particular, the FST value of 0.173 showed complete isolation and no genetic material transfer between the two scale carp populations (HK and HMW). Conversely, a higher degree of genetic similarity between the common carp populations HT and HK indicated by the lowest FST value of 0.047 (Table III).

Table III. Pairwise measures of population differentiation (Garcia-Ramos *et al*.) below diagonal between populations of common carp and scale carp.

Populations	Populations				
	HT	**HM**	**HK**	**HQ**	**HMW**
Common carp					
HT	--				
HM	0.065	---			
HK	0.047*	0.074	---		
HQ	0.068	0.108	0.114	---	
HMW	0.100	0.155	0.139	0.089	---
Scale carp					
HT	---				
HM	0.077	---			
HK	0.128	0.111	---		
HQ	0.141	0.128	0.132	---	
HMW	0.142	0.140	0.173	0.116	---

HT, Head Trimmu; HM, Head Marala; HK, Head Khanki; HQ, Head Qadir Abad; HMW, Head Mohammad Wala

Genetic distance

The following measures were taken of the pairwise genetic identities and distances among all sample points as demonstrated in Table III. The population genetic identity values for the comparisons are as follows: HT against HK: 0.7739), HT vs HQ: 0.7235, HT vs HMW: 0.6551, HT vs HM: 0.7061). The genetic identity (similarity) between the HT population and each of the HM, HK, HQ, and HMW populations is shown by these values. For the aforementioned pairings, the genetic distances are: HT vs HK= 0.2563; HT vs HM= 0.3480; HT vs HQ= 0.3237; HT vs HMW= 0.4230, respectively. The genetic differences between each of the HM, HK, HQ, and HMW populations

and the HT population are represented by these values. Greater genetic divergence is indicated by higher genetic distance values, whereas lower genetic distance values implied closer genetic resemblance. The values of genetic distance and population genetic identity for the designated pairs of scale carp are as follows: (HT vs HM = 0.6970); (HT vs HK = 0.5294); (HT vs HQ= 0.4837); (HT vs HMW= 0.4971); (HT vs HM= 0.3610); (HT vs HK= 0.6359); (HT vs HQ= 0.7263); (HT vs HMW= 0.6990), within scale carp, these numbers represent the genetic distance (distance) and genetic similarity (identity) between the HT population and each of the HM, HK, HQ, and HMW populations as shown in the Table IV.

Table IV. Genetic distance and identity between populations of common carp and scale carp.

Populations compared	Biased		Unbiased	
	Dist.	Ident.	Dist.	Ident.
Common carp				
HT vs. HM	0.3480	0.7061	0.3186	0.7272
HT vs. HK	0.2563	0.7739	0.2269	0.7970
HT vs. HQ	0.3237	0.7235	0.2960	0.7438
HT vs. HMW	0.4230	0.6551	0.3976	0.6719
HM vs. HK	0.3518	0.7034	0.3261	0.7217
HM vs. HQ	0.4985	0.6074	0.4747	0.6221
HM vs. HMW	0.7165	0.4884	0.6950	0.4991
HK vs. HQ	0.5303	0.5885	0.5064	0.6027
HK vs. HMW	0.5998	0.5489	0.5783	0.5609
HQ vs. HMW	0.3096	0.7337	0.2899	0.7483
Scale carp				
HT vs. HM	0.3610	0.6970	0.3356	0.7149
HT vs. HK	0.6359	0.5294	0.6120	0.5423
HT vs. HQ	0.7263	0.4837	0.7020	0.4956
HT vs. HMW	0.6990	0.4971	0.6757	0.5088
HM vs. HK	0.4802	0.6186	0.4579	0.6326
HM vs. HQ	0.5785	0.5607	0.5560	0.6735
HM vs. HMW	0.6347	0.5301	0.6131	0.5417
HK vs. HQ	0.5504	0.5767	0.5292	0.5891
HK vs. HMW	0.8116	0.4442	0.7913	0.4532
HQ vs. HMW	0.4554	0.6342	0.4349	0.6473

See Table III for abbreviation.

Hierarchal AMOVA analysis

Molecular variance analysis was performed with ARLEQUIN software (version 2.000) to assess overall population diversity. 90.38% of the variants in the species common carp were found inside populations, showing a high level of intra-population variety. On the other hand, 9.62% of the changes found were explained by inter-population variability. Within population diversity in common carp accounted for 12.92% of the observed differences, but inter-population diversity explained 87.08% of the variances, as reported in Table V.

Table V. Hierarchal AMOVA analysis of common carp and scale carp populations.

Strains	Source of variation	Sum of squares	Variance compo-nents	Percent-age of variation
Com-mon carp	Among populations	111.223	0.4007	9.62
	Within populations	1110.4	3.7641	90.38
	Total	1221.62	4.1647	
Scale carp	Among populations	146.453	0.5486	12.92
	Within populations	1090.6	3.697	87.08
	Total	1237.05	4.2456	

UPGMA dendrogram

A UPGMA dendrogram created with TFPGA software was used to evaluate the genetic links between each group, taking into account Nei's Genetic Distance (1972). This investigation revealed that both strains showed the segregation of three separate populations: HT, HM, and HK, in contrast to the other two populations, HQ and HMW. Two main clusters, each with two sub-clusters, are shown forming in the graphic. Individuals HT and HK of the species common carp are isolated from HM in the first cluster. On the other hand, HM and HT separated from HK in the first cluster in the subspecies scale carp, as depicted in Figure 2.

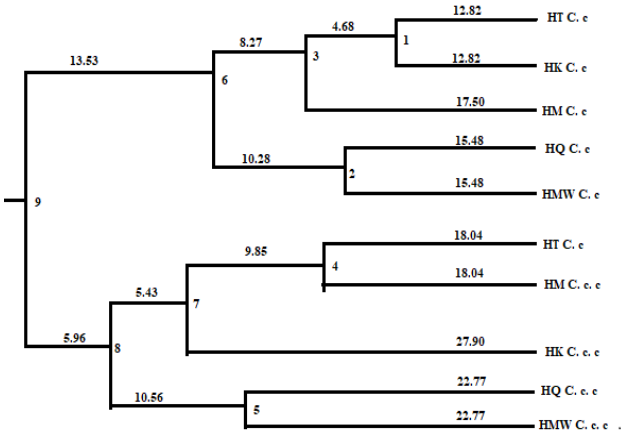

Fig. 2. UPGMA dendrogram showing the relationship between common carp and scale carp populations in Chenab basin. Tree generated on the basis of Nei's Genetic Distance (1978) in TFPGA software.

Linkage disequilibrium

Using chi-square software, we calculated linkage disequilibrium (LD) among all loci using Fisher's exact test. The loci (MFW3, MFW6); (MFW4, MFW7); (MFW3, MFW11); (MFW7, MFW11); (MFW3, MFW13); (MFW6, MFW15); (MFW13, MFW15); (MFW15, MFW17); and (MFW11, MFW26) showed significant non-random associations of alleles and genetic drift, as indicated by their non-significant results, according to the LD analysis.

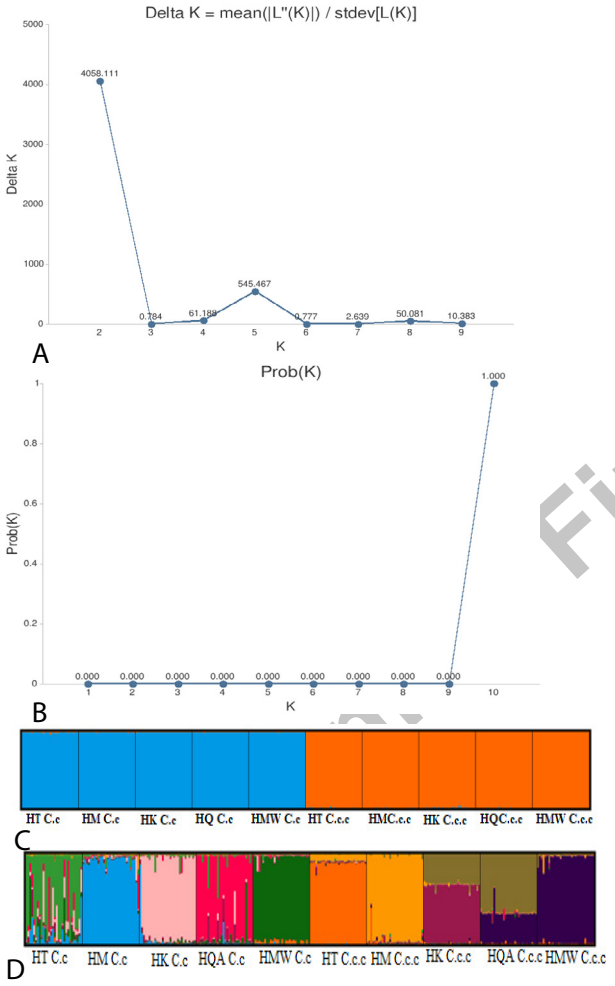

Fig. 3. A, B, C and D: The individuals were clustered using a Bayesian method and 10 microsatellites, with K values of 2 and 10. Each cluster was denoted by a distinct color. In this representation, it can be observed that each vertical column corresponds to each individual, while the division of the column into 10 distinct colors signifies the predicted coefficient of membership for each strain.

Structure-based analyses

The two strains are kept in a pure condition, and their different populations HM in common carp, HK in common carp, HMW in common carp, HT in scale carp, HM in scale carp, and HMW in scale carp showed genetic diversity in their development, according to the results of the Structure analysis (Fig. 3A-D). It is commonly known that heterozygous advantage is necessary for the maintenance of stable polymorphism. Four populations of common carp (HT in common carp; HQ in common carp; HK in scale carp; and HQ in scale carp) have shown genetic evidence of mixing. Although successful breeding programs can improve this state of affairs, they must be carefully considered because of the possibility of uncontrolled interbreeding (Fig. 3A-D).

Bottleneck analysis

The two-phase model (TPM) with a 90% single-step mutation rate and the mode shift test, which was established by Ye *et al.* (2022), were used in the study to evaluate possible recent bottlenecks in populations of common carp and scale carp strains. Over 1000 replications were also undertaken (Table VI). A possible bottleneck event may have occurred in some populations of common carp strains, as indicated by the notable difference between observed and anticipated heterozygosity. To be more precise, all 10 of the loci that were examined showed heterozygosity excess in the common carp populations, HQ common carp and HMW common carp, and no locus showed heterozygosity deficit. The two-phase mutation hypothesis explained these observations. By using the Wilcoxon test, it was found that in non-bottlenecked, equilibrium populations, the observed ratio of 9:1 substantially differed from the predicted ratio of 1:1. For the HQ common carp population,

Table VI. Heterozygosity excess under two-phase mutation model at ten microsatellite loci in each population of common carp strains.

Species	Populations	Sign test		Wilcoxon test
		Hex/Hd	P	P (One tail for Hex)
Common carp	HT	5/5	0.43	0.931
	HM	6/4	0.41	0.923
	HK	7/3	0.35	0.834
	HQ	9/1	0.34	0.055
	HMW	8/2	0.32	0.069
Scale carp	HT	5/5	0.42	0.542
	HM	6/4	0.28	0.451
	HK	7/3	0.26	0.067
	HQ	8/2	0.21	0.038
	HMW	9/1	0.16	0.037

P, probability; Hex, Heterozygosity excess; Hd, Heterozygosity deficiency. See, Table III for abbreviation.

Table VII. Directional relative migration network of the studied common carp strains populations constructed with divMigrate.

Strains	Pops	Common carp					Scale carp				
		HT	HM	HK	HQ	HMW	HT	HM	HK	HQ	HMW
Common carp	HT	0	0.368	0.32	0.243	0.117	0.074	0.062	0.105	0.042	0.04
	HM	0.799	0	0.347	0.181	0.113	0.07	0.055	0.059	0.039	0.031
	HK	1	0.552	0	0.193	0.12	0.061	0.051	0.102	0.054	0.045
	HQ	0.704	0.331	0.221	0	0.302	0.05	0.064	0.079	0.082	0.065
	HMW	0.863	0.248	0.336*	0.64	0	0.061	0.068	0.09	0.061	0.05
Scale carp	HT	0.091	0.058	0.057	0.044	0.037	0	0.426	0.139	0.134	0.13
	HM	0.117	0.09	0.049	0.078	0.054	0.392	0	0.235	0.177	0.14
	HK	0.258	0.097	0.129	0.104	0.067	0.212	0.214	0	0.226	0.178
	HQ	0.116	0.063	0.056	0.104	0.056	0.129	0.161	0.178	0	0.249
	HMW	0.081	0.064	0.057	0.076	0.069	0.18	0.178	0.101	0.328	0

See Table III for abbreviation.

the sign test produced a p-value of 0.34 and the one-tailed Wilcoxon test for heterozygosity excess (Hex) produced a p-value of 0.055. For the HMW scale carp population, the sign test produced a p-value of 0.16 and the one-tailed Wilcoxon test for heterozygosity excess (Hex) produced a p-value of 0.037. The HM common carp and HK scale carp populations records, on the other hand, did not exhibit a statistically significant increase of heterozygosity. The sign test produced p-values of 0.26 for HK common carp and 0.41 for HM scale carp. Moreover, HM common carp and HK scale carp yielded p-values of 0.923 and 0.067, respectively, from the one-tailed Wilcoxon test used to evaluate heterozygosity. These results imply that there could have been a bottleneck among the populations under consideration, albeit the data only sporadically support the importance of this conclusion. The Hex/Hd ratio of 5/5 indicates that there was no statistically significant excess of heterozygosity in the datasets derived from HT populations of strains.

Directional relative migration network

The directed relative migration network analysis among the populations of common carp strains tested indicated that the HT (common carp) population acted as the core population and showed considerable genetic exchange with six periphery populations (Supplementary Fig. S1). These peripheral populations are connected to the HQ, HMW, HM, and HK areas. There was little genetic exchange between the groups designated as HK common carp; HM common carp; HMW common carp; HMW scale carp; HQA scale carp; and HK scale carp and the core population. It is believed that the two strains of carps which are common carp and scale carp, came from different parts of the world. 10 populations in total (five for each strain) were examined in order to determine the directional relative migration network. Every population was recognized as a wild population. According to the research, there was the greatest relative migration value from HMW scale carp to HT common carp, and the lowest relative migration from HMW scale carp to HM common carp (0.08) (Table VII).

DISCUSSION

It is vitally important to comprehend the migration patterns, population structure and genetic diversity of invasive species in order to prevent their spread. The objective of this study was to examine the genetic variability and population composition of rare varieties of represented species common carp and scale carp in River Chenab of Punjab Pakistan. The recent study found that there were 3 to 8 alleles per locus. Five populations of all strains factually had varying numbers of alleles at all loci suggests that the populations came from distinct sources. The variances in Ar values across all groups show disparities in geography. The HT population of common carp had the highest average Ar value. The populations from HT and HK had the highest average Ar value for scale carp. The HMW population in both strains had the lowest average Ar value. This indicates that as there is a dearth of space and mating partners, HMW has relatively low genetic diversity for strains of common carp.

Genetic diversity

The heterozygosity levels, which varied from low to moderate, were evaluated. We found that (Tóth *et*

al., 2020), the observed heterozygosity of common carp strains was comparatively low. For common carp, the estimated values of heterozygosity varied from 0.3167 to 0.443, while for scale carp, they ranged from 0.3067 to 0.4067. On the other hand, (Napora-Rutkowski *et al*., 2017) the estimated value of heterozygosity, which varied from 0.418 to 0.781, was reported with somewhat higher values. In comparison to predicted heterozygosity (He), the study observed heterozygosity (Ho) results were somewhat lower. The genetic diversity of the populations under investigation was lowest in the HQ and HMW strains of common carp and scale carp, and highest in the HT strains. Every one of the 10 microsatellite loci showed polymorphism across the groups; MFW 3 and MFW 15 had significant genetic variation, whereas MFW 31 showed the least amount of polymorphism in both strains. The study used FSTAT software to determine the FIS population inbreeding coefficient for every locus in each population. The obtained values were recorded, exposing variations in average FIS values throughout populations (Napora-Rutkowski *et al*., 2017; Tóth *et al*., 2020) according to the theory behind the inbreeding coefficient, a large number of fish strains have an excess of heterozygotes, which predicts a decreased risk of inbreeding depression. Low to moderate heterozygosity levels in the study; might be the result of restricted allele variety and a potentially small sample size; prior research indicates that around 25–30 individuals per community are required for reliable estimations of genetic diversity derived from microsatellite data (Hale *et al*., 2012). Previous studies has demonstrated that there are very slight advantages to expanding the sample size in these types of studies with regard to projected heterozygosity and allele frequency (Sánchez-Montes *et al*., 2017). Ultimately, the primary objective is to precisely estimate the frequency and diversity of alleles; identifying every allele is not as important as identifying relevant information since rare alleles could not have a substantial impact on the assessment of population genetic diversity or structure. The average FIS values for common carp varied from 0.507 to 0.5914, with the greatest FIS values seen in the HQ, HMW, HK, HM, and HT populations, in that order. The average FIS values in scale carp were found to vary from 0.5310 to 0.6166, with the HMW population displaying the greatest FIS, followed by the HQ, HK, HM, and HT populations, following this order. The populations with the highest FIS scores indicated unfavorable environmental circumstances, which included things like low levels of nutrients and minerals, poor soil texture, and deteriorated water quality. These high FIS values also suggested fewer brooders and less-than-ideal management techniques.

Population structure

Each population pairwise, the study investigated genetic difference within populations. Significant variation across all populations is indicated by the FST values. Our results are in line with previous studies that found little genetic difference across several common carp strains in the Czech Republic (Tóth *et al*., 2020; mean Fst = 0.183) and France (Napora-Rutkowski *et al*., 2017; mean Fst = 0.250). Numerous reasons, like small number of strains were examined, the existence of null alleles, or genotyping mistakes, might be the cause of the low FST values. It is essential to acknowledge that these variables may impact the uniqueness of the strains that were sampled (Napora-Rutkowski *et al*., 2017). The five populations' average FST values range from 0.0471 to 0.173. Significant isolation and restricted gene flow between populations HK and HMW are indicated by a high FST score of 0.173. Conversely, populations HT and HK have a lower FST value of 0.047, which indicates a tighter genetic link between these two groups. In light of these observations (Tóth *et al*., 2020) Based on allelic frequency data, we calculated genetic distance (D) across all populations using POPGENE software. In common carp, populations HT and HMW had the greatest genetic difference (D = 0.4230), whereas populations HT and HK had the shortest genetic distance (D = 0.1919). The populations of HT and HQA in scale carp showed the greatest genetic difference (D = 0.7263), whereas the populations of HT and HM showed the least genetic distance (D= 0.3610) (Tóth *et al*., 2019), Arlequin (version 2.000) software was used to conduct an analysis of molecular variance (AMOVA) and undertake other analysis. Higher levels of variation within populations and smaller levels of variation across populations were seen in both strains. These outcomes agree with the conclusions of earlier research (Napora-Rutkowski *et al*., 2017), who noticed a comparable pattern of variation between populations in Polish-bred strains of common carp. Most loci revealed deviations from linkage disequilibrium (LD), although several loci yielded noteworthy findings (Xu *et al*., 2019) looked at seven fish populations with sufficient sample sizes to investigate linkage disequilibrium (LD). As the distance between populations grew, the LD patterns changed. The study investigated genetic differentiation among seven fish populations, observing varying patterns of linkage disequilibrium influenced by geographical distances between populations. According to a previous study (Ye *et al*., 2022), breeding introductions significantly influenced the genetic structure of carp populations co-cultivated in southern Zhejiang Province paddies (Zhu *et al*., 2022) discovered that the predicted genetic clusters had high probability, with either two or four clusters detected, based on delta K values. Every person showed a combination of genetic clusters in different ratios.

Genetic bottleneck and contemporary gene flow

Genetic bottlenecks decrease genetic variety and impede population survival and adaptability. They are frequently associated with habitat fragmentation. Unexpectedly high levels of heterozygosity in the data point to recent bottlenecks. Common carp populations that are declining exhibit high heterozygosity despite differences in the results of genetic testing; this is probably because to the factors including habitat degradation and overexploitation. This emphasizes how serious bottlenecks may be (Thai *et al*., 2007) using microsatellite DNA markers, it investigated bottleneck effects in endangered common carp populations. Their genetic information research improves the accuracy and efficiency of managing invasive species by helping to pinpoint high-risk locations and create focused management plans. Furthermore, knowledge of genetic linkage across groups might facilitate direct efforts to prevent future spread and use resources more wisely.

Conservation implications

The outcomes of this study have important implications for the conservation and management of invasive represented species in the River Chenab. Based on the genetic diversity and important role in gene flow, it is crucial to prioritize HT population. It is important to develop this approach to retain the genetic diversity and connectedness in the river systems by ensuring the health of carp populations in the region. Recognizing health of the HT population is crucial because it is not only beneficial for their own population's health but also for other populations that depend on the gene flow in the River Chenab (Sanda *et al*., 2024).

Genetic bottlenecks inbreeding, and isolation in the HQ and HMW populations, it is important to observe effective conservation measures. Such measures might involve restoring habitats to improve between populations, establishing genetic links to enhance the flow of genes, and starting programs to increase population densities, thereby reducing the effects of inbreeding and genetic drift (Paganelli *et al*., 2024). Furthermore, managing the impacts of the changes in environment like preservation of habitats could support in alleviating the genetic challenges that these populations are experiencing. Such measures would ensure the sustainability and ability of the HQ and HMW populations to survive and thrive in the future.

Genetic diversity seems to be less in River Chenab populations than the Jhelum River. This implies that the Chenab populations might be more sensitive to the environmental changes as well to anthropogenic pressures. There is a need for effective conservation of these populations in order to address genetic and ecological challenges that these populations encounter to ensure viable and sustainable future of these species. Therefore, understanding the environment in its broader sense by considering the conservation measures that the invasive and native species have the possibility of crossbreeding, which may be important in dealing with issues of genetic diversity. The insights of these factors is vital to define strategies and management techniques that would protect genetic integrity and overall viability of both invasive and native species in the Chenab River system (Gozlan *et al*., 2024).

Importance of study and future research directions

Inbreeding of represented species populations may have potential negative genetic impacts on local strains. Future research is crucial to evaluate changes in the genetic diversity and population structure of these carp populations over time to compare the effectiveness of conservation approaches and to determine other potential genetic shifts that may take place. This is specifically important to understand changes that occur in genetic diversity, inbreeding and gene flow over time in order to evaluate the prospects of long-term conservations of such populations and make informed decisions on their management. In addition, understanding the environmental and ecological factors that may be influential of the observed genetic patterns including the fragmentation of habitats, the quality of water, and intensity of fishing which can provide more focused and efficient mitigation approaches. More populations are needed from different river systems in order to have further improvement on the genetic analysis. This will enable us to place the conclusions derived from the present study on the Chenab River in a broader context, and may even reveal other trends of genetic diversity and population structure of the invasive carp in the region. Further research is much needed to examine the impact of climate change on the genetic diversity and population dynamics of the represented species. Climate change may thus affect the distribution range of these species, their breeding time, and availability of the proper habitat. They could have a significant effect on their genetic diversity and population structure. Developing adaptive management strategies is essential to the continued sustainability of these populations and mechanism of sustainable management flexibly adopting to the dynamic nature of the production environments.

In conclusion, this study provides an understanding of genetic variations and population dynamics of common carp and scale carp in the Chenab River with important consequences for their conservation. These, in turn, underscore the need to conserve technologically distinct though however small, such as HT, and specifically target other at-risk populations. Therefore, research and conservation should sustain for the protection of these populations in future and for less impact of invasive

species on the Chenab River ecosystem.

CONCLUSION

Concerning the spread and regulating the effects of invasion species requires a thorough understanding of the represented species' genetic diversity, population structure, and movement patterns. In the present study aimed to understand the population structure and genetic diversity of invasive strains of common carp and scale carp in the river Chenab in Punjab, Pakistan. Outcomes of the study showed that these strains demonstrate from low to moderate level of heterozygosity. The average FIS values varied in a range between 0.51 to 0.59 for common carp and from 0.53 to 0.62 for scale carp. FST analysis showed moderate genetic differentiation between the strains. In common carp, 9.62% of the genetic variation was attributed to inter-population differences, while 90.38% was due to intra-population variability. For scale carp, AMOVA results showed that 87.08% of the genetic variation was due to inter-population differences, with 12.92% attributed to intra-population diversity. Bayesian clustering analysis identified 10 distinct categories among the populations of both strains, with no genetic evidence of admixture found in the pure original strains. The high observed heterozygosity compared to expected heterozygosity in common carp populations suggest the presence of a bottleneck event. The directed relative migration network highlighted HT common carp as the core population with the greatest genetic exchange, while no significant migratory events were observed in other scale carp populations, except for the HK population. The findings of this genetic study contribute to identifying high-risk areas and guiding targeted management strategies, thereby improving the effectiveness and precision of controlling invasive species. Additionally, the available genetic connectivity data between populations can inform resource allocation and mitigation strategies for future dispersal. Understanding the origin populations of non-native species is critical for comprehending their introduction routes and developing effective countermeasures. This knowledge facilitates the creation of programs aimed at regulating further investigations and managing invasive species effectively.

DECLARATIONS

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Ethical statement

All experimental study was approved and reviewed by the Department of Animal Welfare and Ethical Committee of Department of Zoology, Government Sadiq College Women University Bahawalpur 63100, Pakistan.

Data availability statement

The data supporting this study's findings are included in the manuscript or the supplementary information.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20241005051145

Generative AI and AI-assisted technology statement

During the preparation of this article, the author did not utilize generative AI and AI-assisted technology.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Microsatellite Markers Used for Genetic Monitoring of Exotic Common Carp Variants (Common Carp and Scale Carp) in the River Chenab, Punjab, Pakistan

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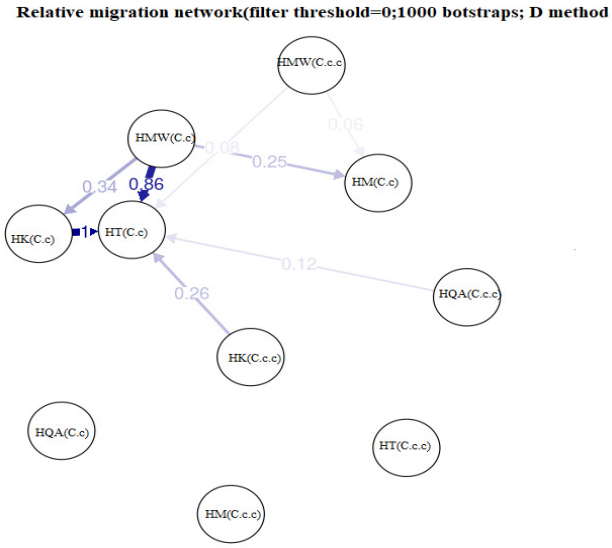

Supplementary Fig. 1. The directional relative migratory network of the populations of the common carp strains under study was created using the divMigrate method. The numerical values depicted on the arrows correspond to the relative migration coefficients that have been obtained from D statistics. The intensity of gene flows is positively correlated with the degree of line shading and thickness.

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Supplementary Table SI. Genetic diversity parameters of five populations at ten microsatellite loci of *C. carpio* and *C. c. communis* in Chenab basin of Punjab, Pakistan.

Species	Population	Parameters	MFW 3	MWF 4	MWF 6	MWF 7	MWF 11	MWF 13	MWF 15	MWF 17	MWF 26	MWF 31	Average
C. carpio	HT	N_a	8	4	6	4	5	6	7	6	6	4	6
		A_r	8.000	4.000	6.000	4.000	5.000	6.000	7.000	6.000	6.000	4.000	5.600
		H_o	0.533	0.367	0.433	0.333	0.400	0.467	0.600	0.467	0.467	0.367	0.443
		H_e	0.866	0.747	0.834	0.768	0.798	0.834	0.854	0.838	0.844	0.714	0.810
		F_{IS}	0.461	0.551	0.521	0.609	0.542	0.481	0.409	0.483	0.522	0.490	0.507
		Ne	6.222	3.665	5.391	3.948	4.426	5.408	5.629	5.497	5.600	3.352	4.914
	HM	N_a	6	5	6	3	4	6	6	5	6	4	5
		A_r	6.000	5.000	6.000	3.000	4.000	6.000	6.000	5.000	6.000	4.000	5.100
		H_o	0.533	0.367	0.433	0.267	0.400	0.433	0.467	0.467	0.400	0.333	0.4100
		H_e	0.843	0.749	0.786	0.644	0.755	0.786	0.808	0.760	0.836	0.693	0.7659
		F_{IS}	0.484	0.557	0.533	0.590	0.553	0.453	0.465	0.390	0.599	0.523	0.5147
		Ne	5.116	3.602	4.000	2.723	3.621	4.412	4.634	3.956	5.315	3.141	4.0520
	HK	N_a	7	4	5	4	4	5	5	4	6	4	4.8000
		A_r	7.000	4.000	5.000	4.000	4.000	5.000	5.000	4.000	6.000	4.000	4.800
		H_o	0.433	0.267	0.400	0.367	0.433	0.400	0.467	0.400	0.400	0.267	0.3833
		H_e	0.851	0.773	0.751	0.760	0.699	0.807	0.784	0.687	0.824	0.699	0.7634
		F_{IS}	0.530	0.739	0.509	0.561	0.531	0.508	0.409	0.422	0.593	0.622	0.5424
		Ne	6.007	3.881	3.713	3.840	3.031	4.839	4.358	3.082	4.391	3.197	4.0880
	HQ	N_a	5	3	5	4	4	5	5	4	6	4	4.5000
		A_r	5.000	3.000	5.000	4.000	4.000	5.000	5.000	4.000	6.000	4.000	4.5000
		H_o	0.400	0.267	0.300	0.267	0.200	0.367	0.433	0.433	0.233	0.267	0.3167
		H_e	0.794	0.646	0.801	0.649	0.658	0.786	0.747	0.727	0.818	0.742	0.7368
		F_{IS}	0.541	0.591	0.629	0.593	0.748	0.538	0.424	0.449	0.757	0.644	0.5914
		Ne	4.313	2.740	4.700	2.761	2.691	4.412	3.774	3.357	4.947	3.696	3.7390
	HMW	N_a	4	4	4	3	4	4	4	3	5	3	3.8000
		A_r	4.000	4.000	4.000	3.000	4.000	4.000	4.000	3.000	5.000	3.000	3.8000
		H_o	0.400	0.333	0.300	0.267	0.167	0.333	0.400	0.333	0.400	0.267	0.3200
		H_e	0.742	0.668	0.699	0.638	0.644	0.698	0.752	0.590	0.786	0.667	0.6884
		F_{IS}	0.549	0.600	0.614	0.678	0.744	0.609	0.472	0.439	0.572	0.604	0.5881
		Ne	3.329	2.609	3.150	2.497	2.723	3.004	3.838	2.381	4.116	2.908	3.0554
C. c. carpio	HT	N_a	6	4	5	4	4	6	7	4	5	3	4.8000
		A_r	6.000	4.000	5.000	4.000	4.000	6.000	7.000	4.000	5.000	3.000	4.8000
		H_o	0.500	0.300	0.400	0.267	0.367	0.433	0.500	0.433	0.400	0.400	0.4000
		H_e	0.824	0.749	0.774	0.775	0.738	0.840	0.854	0.711	0.785	0.708	0.7759
		F_{IS}	0.471	0.644	0.526	0.739	0.544	0.523	0.493	0.467	0.534	0.562	0.5503
		Ne	4.915	3.633	4.024	3.960	3.586	5.644	5.702	3.207	4.216	2.976	4.1862
	HM	N_a	6	4	5	3	3	5	7	5	4	4	4.6000
		A_r	6.000	4.000	5.000	3.000	3.000	5.000	7.000	5.000	4.000	4.000	4.6000

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Species	Population	Parameters	MFW 3	MWF 4	MWF 6	MWF 7	MWF 11	MWF 13	MWF 15	MWF 17	MWF 26	MWF 31	Average
		H_o	0.467	0.300	0.367	0.300	0.333	0.400	0.500	0.400	0.400	0.400	0.4067
		H_e	0.816	0.721	0.784	0.637	0.684	0.793	0.852	0.787	0.734	0.716	0.7525
		F_{IS}	0.254	0.626	0.613	0.579	0.557	0.616	0.417	0.572	0.502	0.574	0.5310
		Ne	4.810	3.378	4.116	2.568	2.972	4.107	6.164	4.148	3.426	2.946	3.8633
	HK	N_a	8	4	5	3	3	5	6	6	5	3	4.8000
		A_r	8.000	4.000	5.000	3.000	3.000	5.000	6.000	6.000	5.000	3.000	4.8000
		H_o	0.467	0.267	0.367	0.300	0.267	0.400	0.500	0.433	0.367	0.300	0.3667
		H_e	0.811	0.735	0.791	0.673	0.434	0.801	0.826	0.777	0.710	0.693	0.7250
		F_{IS}	0.462	0.641	0.581	0.648	0.389	0.580	0.434	0.482	0.531	0.656	0.5404
		Ne	4.890	3.607	4.280	2.718	1.744	4.429	5.191	4.174	3.144	2.953	3.7130
	HQ	N_a	4	4	4	3	7	5	6	5	6	3	4.7000
		A_r	4.000	4.000	4.000	3.000	7.000	5.000	6.000	5.000	6.000	3.000	4.7000
		H_o	0.333	0.267	0.367	0.333	0.267	0.367	0.433	0.400	0.333	0.200	0.3300
		H_e	0.715	0.764	0.765	0.502	0.728	0.796	0.743	0.779	0.836	0.675	0.7303
		F_{IS}	0.538	0.696	0.563	0.379	0.716	0.582	0.421	0.572	0.643	0.752	0.5862
		Ne	3.365	3.867	3.921	1.931	3.439	4.450	3.711	3.843	5.391	2.885	3.6802
	HMW	N_a	5	3	4	3	3	5	5	5	5	3	4.1000
		A_r	5.000	3.000	4.000	3.000	3.000	5.000	5.000	5.000	5.000	3.000	4.1000
		H_o	0.300	0.267	0.300	0.233	0.267	0.367	0.433	0.367	0.267	0.267	0.3067
		H_e	0.765	0.626	0.615	0.671	0.666	0.796	0.811	0.759	0.728	0.697	0.7133
		F_{IS}	0.650	0.665	0.516	0.701	0.648	0.584	0.506	0.560	0.680	0.656	0.6166
		Ne	3.849	2.497	2.528	2.827	2.780	4.369	4.806	3.797	3.378	2.820	3.375

NA, mean number of alleles per strain; Ar, mean values of allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding coefficient.