fishes

MDPI

Article

Genetic Diversity and Population Structure of the Chinese Mitten Crab (*Eriocheir sinensis)* from Six Different Lakes Using Microsatellites

Shengyan Su [1,2], Jean Damascene Nsekanabo [1], Brian Pelekelo Munganga [1,3], Xinjin He [2], Jianlin Li [2], Fan Yu [2], Meiyao Wang [2] and Yongkai Tang [1,2,*]

[1] Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China; brijelimunga@gmail.com (B.P.M.)
[2] Key Laboratory of Integrated Rice-Fish Farming Ecology, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China
[3] Centre for Innovative Approach Zambia, Lusaka 00000, Zambia
* Correspondence: tangyk@ffrc.cn

Abstract: The Chinese mitten crab (*Eriocheir sinensis*) is among the most important species in China and other countries, and it contributes significantly to aquaculture and meeting protein demands for the fast-growing human population. To ensure their sustainable exploitation, management, and use in aquaculture, it is imperative to know their genetic diversity. Thus, we studied the genetic diversity of six populations of Chinese mitten crabs from six different lakes in the Yangtze River's drainage system. A total of 180 Chinese mittens crabs were collected from six lakes in China, with 30 being collected from each lake. Then, DNA was extracted using TaKaRa Dalian, genotyping was performed by the Gene Marker software for statistical analysis and the genetic parameters such as observed number of alleles (Na)were studied. A total of 87 alleles were observed in 180 individuals of six wild *Eriocheir sinensis* populations. The Fis results showed that six sites had negative values, and crab20 had the largest value. The results of Fit showed that the single locus had a negative value. All the Fst values among the populations were lower than 0.50, while an AMOVA analysis showed that 0.36% of the genetic variation came from among the six populations and 94.08% of the genetic variation was between individuals in a population. The six Chinese mitten crab populations showed higher genetic variability among individuals of the same population with very low genetic variability between the populations. Therefore, this illustrates that the crabs from the six lakes have similar genetic diversity and minor genetic differences among them.

Keywords: genetic diversity; microsatellite; linkage disequilibrium; heterozygosity; loci

Key Contribution: The six Chinese mitten crab populations showed higher genetic variability among individuals of the same population with very low genetic variability between the populations. The results could provide a theoretical basis for the rational exploitation, management, and protection of the natural Chinese mitten crab population, as well as valuable information for selective breeding programs.

check for updates

Citation: Su, S.; Nsekanabo, J.D.; Munganga, B.P.; He, X.; Li, J.; Yu, F.; Wang, M.; Tang, Y. Genetic Diversity and Population Structure of the Chinese Mitten Crab (*Eriocheir sinensis)* from Six Different Lakes Using Microsatellites. *Fishes* **2023**, *8*, 220. https://doi.org/10.3390/fishes8050220

Academic Editor: Joseph Quattro

Received: 29 September 2022
Revised: 9 April 2023
Accepted: 14 April 2023
Published: 23 April 2023

1. Introduction

The Chinese mitten crab is an important species in China, appreciated by consumers for its taste and nutritional value and its price by farmers and fishermen. In 2015, its contribution to the fisheries and aquaculture industry was approximately 820,000 tons, which accounted for more than 50 billion CNY in value [1,2]. This species will remain important towards meeting the protein needs of the ever-growing human population. Moreover, the wild population of the Chinese mitten crab serves as a broodstock resource base for aquaculture. The Chinese mitten crab (*Eriocheir sinensis*) is a catadromous species

that is widely distributed throughout rivers, lakes, and estuaries of the eastern region of China [3–5]. As a result of pollution, habitat loss, and overexploitation, there has been an unprecedented decline in the population of these crabs since the 1960s, making them prone to collapse if not well managed. Furthermore, just like other aquatic organisms, the Chinese mitten crab continually needs to cope with the effects of global climate change, which is causing increases in aquatic temperatures and has led to changes in dissolved oxygen availability, salinity, and circulation patterns in aquatic environments [6–8].

Over the past years, efforts have been put forward by the Chinese government through the Ministry of Fisheries to ensure the long-term sustainability of aquatic biodiversity in China, including stock management and the implementation of protected areas (MPAs) [9]. Within this context, the preservation of genetic resources plays a crucial role and is one of the factors that has always been taken into consideration to ensure sustainable utilization and optimized conservation actions of aquatic organisms [10,11]. One metric that may help us understand and predict whether or not species are most likely to adapt to future conditions is genetic diversity, as it influences species' capacity for resilience and their adaptative potential to environmental changes [12]. The patterns of population structure and genetic diversity inform us of the life histories, demography, ecology, and reproduction of a species [13,14]. Therefore, population genetic diversity and structure are particularly important for the protection and optimal use of genetic resources of *E. sinensis*.

So far, many scholars have studied the genetic structure and diversity of the Chinese mitten crab population in many water bodies in China. For example, Sui et al. [15] conducted a study on the genetic diversity and population structure of the Chinese mitten crab in its native range. In this study, it was found that neutral processes such as genetic drift and isolation have not resulted in significant genetic differentiation among the assessed geographic locations of this catadromous species. It was also found that a geographic population structure was absent. Another study conducted by Zhang et al. [16] assessed the genetic diversity and genetic structure of farmed and wild Chinese mitten crab (*E. sinensis*) populations from three major basins by mitochondrial *DNA COI* and *Cyt b* gene sequences. Since 2009, very few studies have assessed the genetic diversity and population structure of the Chinese mitten crab in its natural range in China. Moreover, it is very obvious that from that time up to the present, significant changes have occurred in these aquatic habitats. To ensure the effective and optimal utilization and conservation of the Chinese mitten crab, it is necessary to have recent genetic information about the population.

Generally, within each species, genetic resources can be partitioned not only among interbreeding individuals but also among populations. Population genetic diversity and population structure may emerge from several mechanisms which include reproductive isolation, geographic distance [17], biogeographic barriers (soft boundaries associated with hydrological processes or hard barriers such as land bridges [18]), or specific behavioral traits (e.g., dependability to natal spawning grounds [18]). Understanding the patterns of connectivity and the rate of genetic exchange among populations is therefore fundamental from a resource management perspective, as the effectiveness of MPA is intrinsically dependent not only on the species' ability to self-replenish but also on the net export of biomass (i.e., spillover) beyond their boundaries [19].

The main aim of the present study is to determine the genetic diversity and population structure of the Chinese mitten crab from six lakes that are part of the Yangtse drainage system using microsatellites. The results could provide a theoretical basis for the rational exploitation, management, and protection of natural Chinese mitten crabs, as well as valuable information for selective breeding programs.

2. Materials and Methods

2.1. Study Area and Sample Collection

A total of 180 Chinese mitten crabs were collected from six different lakes in Jiangsu province in China and brought to the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences. A total of 30 crabs of which 15 were females and 15 males

were collected from each lake. The lakes include Changdang Lake (CD) (32°59′–31°62′ N, 119°52′–118°60′ E), Gucheng Lake (GC) (31°14′–31°18′ N, 118°53′–118°57′ E), Gaoyou Lake (GY) (32°42′–33°41′ N, 119°06′–119°25′ E), Hong-tse Lake (HZ) (33°06′–33°40′ N, 118°10′–118°52′ E), Taihu Lake (TH) (30°55′40″–30° N, 119°52′32″–120° E), and Yangcheng Lake (YC) (30°55′40″–30° N, 119°52′32″–120° E); the locations of the lakes are shown in Figure 1. Upon arrival, the crabs were prepared, labeled, and stored in a −80 °C freezer.

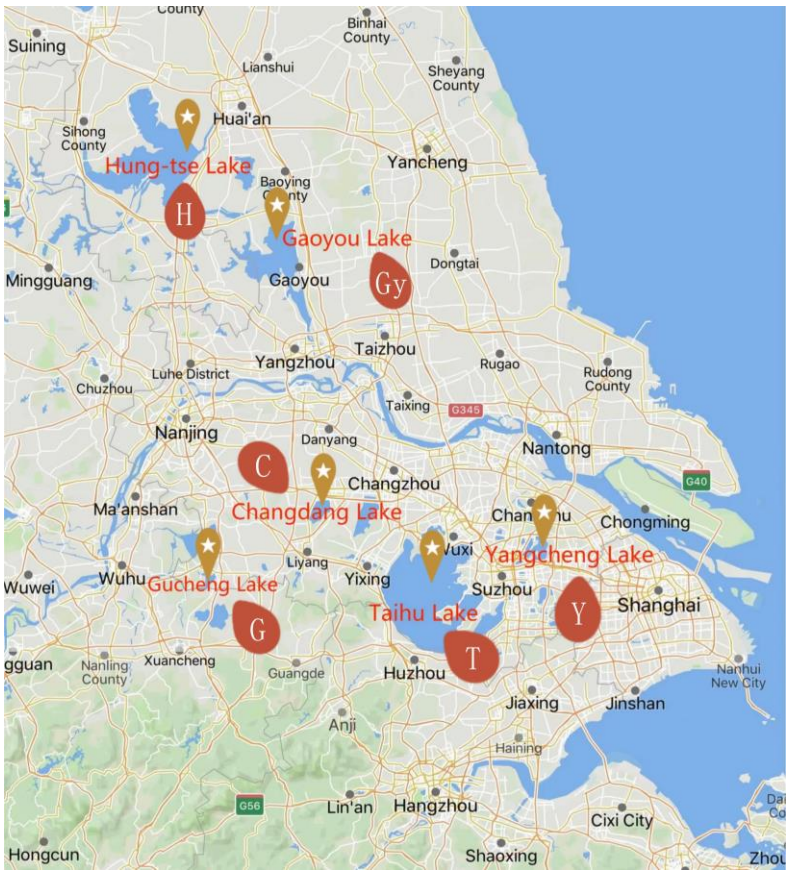

Figure 1. Map of the sampling sites.

2.2. DNA Extraction

DNA was extracted using an animal genomic DNA extraction kit (TaKaRa, Dalian, China). The DNA was tested for purity by agarose gel electrophoresis. The OD260 and OD280 concentrations were determined by a UV spectrophotometer. The qualified DNA dilution concentration was 50.00 ng/mL at −20 °C and stored for future use.

2.3. Microsatellite Primer Amplification

The 13 pairs of primers previously developed against microsatellites were used in the present study (Table S1). The FAM label at the 5′end of the forward primer was synthesized by Wuxi Tianlin Biological Co., Ltd. (Wuxi, China). The PCR reaction cycling was as follows: pre-denaturation at 94 °C for 5 min; pre-denaturation at 94 °C for 10 s and annealing for 10 s at 72 °C for 15 s in 30 cycles; and pre-denaturation at 72 °C for 10 min. The PCR products were detected by capillary electrophoresis using ABI3730XL sequencer (ABI, Foster City, CA, USA), and genotyping was performed by Gene Marker software for statistical analysis.

2.4. Genetic Diversity

The Popgene32 (v1.3.2) software [20] was used to calculate the allele number (Na), effective allele number (Ne), observed heterozygosity (Ho), Shannon Wiener index (I), and expected heterozygosity (He), to perform the likelihood ratio test, to calculate the

Hardy–Weinberg balance, genetic differentiation index, Fst (F-statistics, Fst), and Nei's standard genetic distance (Ds), and to perform neutral analysis detection. Genepop's contingency table method was used for a linkage disequilibrium analysis [21]. The Botstein method was used to calculate the polymorphic information content (PIC) of each locus. Finally, the Arlequin3.5 software [22] was used to complete the AMOVA analysis.

2.5. Population Structure and Genetic Differentiation

In this study, Arlequin v3.5 [22] was used for the analysis of the pairwise Fst for each pair of populations. An exact test with 10,000 permutations was used to determine statistical significance. The genetic differentiation was estimated using AMOVA after analysis using Arlequin v3.5 [22]. Tests for genetic differentiation among the crab populations were carried out at three hierarchical levels of variation: among individuals, within populations (FIS), and within individuals (FIT). With the help of these findings, we evaluated population structure and mobility by using gene flow and then the BayesAss v3 [23] software. Additionally, the theory of the Bayesian method allocated samples into clusters. Different values of the length of the burn-in period (10,000–100,000) and MCMC repetitions (10,000–100,000) were used in every run and the most likely value for K was calculated using the ΔK method [24].

3. Results

3.1. Microsatellite

The information about the detected microsatellite markers is summarized in Table 1. The 13 microsatellite loci tested were found to be highly polymorphic across the six populations. A total of 87 alleles were detected in 180 individuals of the six wild *Eriocheir Sinensis* populations. No null alleles were detected across the populations. The observed allele number ($\boldsymbol{A_O}$) for the 13 detected microsatellite markers ranged from 1 for *crab2* to 19 for *Esin42* and *HLJEsa42*, with an average of 6.69 alleles per locus (Table 1). The effective number of alleles ($\boldsymbol{A_E}$) ranged from 1.02 for *Crab2* to 11.96 for *Esin42*. The average observed heterozygosity ($\boldsymbol{H_O}$) was 0.56, with a range of 0.01 for *Crab2* to 0.79 for *Crab1* (Table 1). The average expected heterozygosity was 0.62, with a range of 0.01 for *Crab2* and 0.92 for *Esin42* ($\boldsymbol{H_E}$).

3.2. Genetic Diversity

The analysis of genetic diversity indicators such as the observed number of alleles ($\boldsymbol{A_O}$), the number of effective alleles ($\boldsymbol{A_E}$), Hardy Weinberg's balanced likelihood ratio test outcome (*HWE*), observed heterozygosity (H_O), and expected heterozygosity (H_E) are presented in Table 1, while the levels and patterns of genetic diversity for the six crab populations are summarized in Table 1. The observed heterozygosity and expected heterozygosity were similar among the six crab populations. Crabs from CD had the highest observed heterozygosity (0.59) while those from GC had the lowest (0.53). The expected heterozygosity was highest (0.64) in crabs from GC while those from CD had the lowest (0.61).

A total of 87 alleles were observed in 180 individuals of the six wild *Eriocheir sinensis* populations with an overall mean of 6.69 alleles per locus ranging from 1 to 15. The number of effective alleles ranged from 1 to 11.95 with the most being at *Esin 42*. The numbers of observed and expected loci in six populations were obtained so that correlations with possible variations could be observed in the populations in the six lakes.

Table 1. Genetic diversity indicators.

	Crab1	***Crab20***	***Crab2***	***Crab11***	***Crab18***	***Crab15***	***Crab6***	***Crab17***	***Crab8***	***Crab12***	***Esin42***	***HLJEsa42***	***Crab21***
A_O													
GC	2.00	5.00	1.00	6.00	7.00	8.00	5.00	6.00	9.00	10.00	14.00	10.00	5.00
HZ	2.00	6.00	2.00	6.00	6.00	7.00	3.00	5.00	8.00	9.00	17.00	9.00	6.00
YC	5.00	5.00	2.00	7.00	8.00	7.00	4.00	5.00	11.00	9.00	15.00	11.00	8.00
CD	2.00	5.00	1.00	8.00	7.00	7.00	4.00	5.00	8.00	6.00	13.00	15.00	6.00
GY	3.00	5.00	1.00	6.00	7.00	7.00	4.00	5.00	10.00	9.00	13.00	12.00	4.00
TH	2.00	7.00	1.00	5.00	7.00	5.00	3.00	4.00	10.00	7.00	15.00	14.00	6.00
All	5.00	9.00	2.00	10.00	13.00	8.00	5.00	6.00	15.00	13.00	19.00	19.00	8.00
A_E													
GC	1.98	3.25	1.00	2.03	3.86	4.05	1.70	3.32	3.75	6.14	11.11	5.59	2.85
HZ	1.86	2.89	1.06	2.98	3.44	2.31	1.18	2.86	4.09	5.80	10.84	6.33	3.57
YC	2.13	2.65	1.03	2.40	4.35	2.44	1.45	2.47	4.87	6.16	9.62	8.03	3.46
CD	1.98	2.14	1.00	2.51	2.62	3.07	1.46	2.45	3.94	4.54	9.72	8.45	3.23
GY	1.82	2.25	1.00	2.57	3.07	2.72	1.18	2.61	4.74	5.73	9.83	8.78	2.82
TH	1.89	3.61	1.00	3.19	3.60	1.92	1.22	2.31	4.23	5.57	10.05	9.00	3.44
All	1.96	2.88	1.01	2.63	3.71	2.75	1.36	2.73	4.38	6.14	11.95	8.47	3.25
H_O													
GC	0.90	0.46	0.00	0.53	0.40	0.33	0.33	0.60	0.76	0.76	0.83	0.40	0.56
HZ	0.73	0.43	0.06	0.83	0.40	0.36	0.16	0.53	0.70	0.76	0.83	0.53	0.80
YC	0.86	0.33	0.03	0.63	0.40	0.46	0.36	0.46	0.80	0.83	0.83	0.53	0.86
CD	0.9	0.36	0.00	0.63	0.60	0.46	0.36	0.40	0.70	0.80	0.90	0.90	0.70
GY	0.63	0.40	0.00	0.56	0.63	0.60	0.16	0.23	0.73	0.93	0.76	0.93	0.53
TH	0.70	0.33	0.00	0.70	0.66	0.46	0.20	0.43	0.80	0.90	0.66	0.93	0.80
All	0.78	0.38	0.01	0.65	0.51	0.45	0.26	0.44	0.75	0.83	0.80	0.70	0.71
H_E													
GC	0.50	0.70	0.00	0.51	0.75	0.76	0.42	0.71	0.74	0.85	0.92	0.83	0.66
HZ	0.47	0.66	0.06	0.67	0.72	0.57	0.15	0.66	0.76	0.84	0.92	0.85	0.73
YC	0.54	0.63	0.03	0.59	0.78	0.60	0.31	0.60	0.80	0.85	0.91	0.89	0.72
CD	0.50	0.54	0.00	0.61	0.62	0.68	0.32	0.60	0.75	0.79	0.91	0.89	0.70
GY	0.45	0.56	0.00	0.62	0.68	0.64	0.15	0.62	0.80	0.83	0.91	0.90	0.65
TH	0.48	0.73	0.00	0.69	0.73	0.48	0.18	0.57	0.77	0.83	0.91	0.90	0.72
All	0.49	0.65	0.01	0.62	0.73	0.63	0.26	0.63	0.77	0.83	0.91	0.88	0.69
HWE													
GC	0.00	0.04	-	0.92	0.00	0.00	0.68	0.19	0.90	0.58	0.77	0.00	0.09
HZ	0.00	0.05	0.85	0.16	0.00	0.05	0.94	0.44	0.63	0.43	0.99	0.01	0.65
YC	0.02	0.00	1.00	0.99	0.00	0.15	0.89	0.38	0.97	0.48	0.99	0.06	0.22
CD	0.00	0.17	-	0.91	0.87	0.11	0.89	0.45	0.63	0.44	0.82	0.99	0.40
GY	0.03	0.06	-	0.39	0.42	0.39	0.99	0.00	0.91	0.86	0.45	0.74	0.00
TH	0.00	0.00	-	0.90	0.88	0.67	0.90	0.50	0.96	0.54	0.95	0.99	0.49
All	0.00	0.00	0.89	0.99	0.00	0.00	0.32	0.00	0.20	0.54	0.20	0.03	0.01

3.3. Hardy–Weinberg Equilibrium and Mutation–Drift Equilibrium Significant Test Using IAM, TPM, and SMM Model

The Hardy–Weinberg equilibrium test and linkage disequilibrium (LD) test are widely used in analyzing population evolution and structure, and locating important functional genes [25]. In the detection of the Hardy–Weinberg linkage disequilibrium in the six crab populations, a total of 78 gene locus pairs were detected, of which 10 were detected in the linkage disequilibrium state, including *crab11/crab18*, *crab20/crab15*, *crab15/crab6*, *crab20/crab12*, *crab20/Esin42*, *crab8/Esin42*, *crab12/Esin42*, *crab12/HLJEsa42*, *Esin42/HLJEsa42*, *crab12/crab21* (Table S2).

Through the mutation–drift balance test of the three models, it was found that the crabs in Yangcheng Lake (YC) had the highest number of gene loci with the expected number of loci having an excess of heterozygosity, followed by crabs from Hongze Lake. For the observed number of loci with an excess of heterozygosity, crabs in Yangcheng Lake (YC) were also showed the least. Only under the infinite alleles model (IAM) model, the crabs in Yangcheng Lake (YC) were in a drifting equilibrium state. Regardless of the model, the crabs of GC, GY, and TH confirmed the drift equilibrium state (Table 2).

Table 2. Mutation–drift equilibrium significant test using IAM, transcript per million (TPM), and single-step mutation (SMM) model.

	IAM	TPM	SMM
Expected number of loci with excess of heterozygosity	42.67	42.86	43.11
Observed number of loci with excess of heterozygosity	55.00	40.00	21.00
Observed number of loci with deficient heterozygosity	19.00	34.00	53.00
All loci fit mutation–drift equilibrium	1.30	2.09	0.37

Genetic variability and identity have many contributions to the domestication process, and the more variations there are in populations, the greater an individual is likely to adapt and pass traits on to the next generation. From the Shannon index (Table 3), the genetic diversity is in the order of Yangcheng Lake hairy crabs, Gucheng Lake hairy crabs, Hongze Lake hairy crabs, Taihu hairy crabs, Changdang Lake hairy crabs, and Gaoyou Lake hairy crabs. From the perspective of polymorphic information content, in crabs from six different lakes, 10 sites were at a high polymorphism level, 2 were at a moderate polymorphism level, and 1 was at a low polymorphism level (Table 4).

Table 3. Shannon's information index of six crab populations.

	Pop	Crab1	Crab2	Crab6	Crab8	Crab11	Crab12	Crab15	Crab17	Crab18	Crab21	Esin42	HLJEsa42
Shannon's Information index	GC	0.68	0.00	0.85	1.62	1.09	1.99	1.66	1.36	1.58	1.26	2.51	1.94
	HZ	0.65	0.14	0.34	1.62	1.32	1.88	1.25	1.20	1.45	1.44	2.56	2.00
	YC	0.89	0.08	0.61	1.88	1.20	1.95	1.25	1.05	1.64	1.51	2.42	2.21
	CD	0.68	0.00	0.65	1.61	1.33	1.63	1.44	1.07	1.33	1.33	2.39	2.38
	GY	0.73	0.00	0.37	1.81	1.26	1.88	1.34	1.12	1.42	1.16	2.41	2.29
	TH	0.66	0.00	0.37	1.74	1.36	1.81	0.96	0.98	1.52	1.38	2.47	2.39
	All	0.74	0.04	0.61	1.82	1.33	1.97	1.43	1.19	1.63	1.39	2.62	2.40

Table 4. PIC index of six crab populations.

	Pop	Crab1	Crab2	Crab6	Crab8	Crab11	Crab12	Crab15	Crab17	Crab18	Crab21	Esin42	HLJEsa42
PIC	GC	0.37	0.00	0.31	0.74	0.60	0.77	0.67	0.58	0.62	0.69	0.90	0.88
	HZ	0.37	0.00	0.41	0.73	0.51	0.83	0.75	0.69	0.73	0.64	0.90	0.81
	YC	0.45	0.00	0.14	0.79	0.61	0.82	0.63	0.62	0.67	0.64	0.90	0.88
	CD	0.35	0.05	0.14	0.75	0.66	0.82	0.57	0.65	0.70	0.71	0.90	0.84
	GY	0.36	0.00	0.17	0.76	0.68	0.81	0.48	0.56	0.72	0.70	0.90	0.89
	TH	0.52	0.03	0.30	0.79	0.58	0.83	0.59	0.59	0.77	0.71	0.89	0.87

The Fis results showed that six sites had negative values while *crab20* had the largest value (Table 5). The results of Fit revealed that the single locus had a negative value, which was also the largest at crab20 locus. All the Fst values among the populations were lower than 0.50. The gene flow value between populations was between 6.77 and 35.27 (Table 5).

Table 5. Summary of F-Statistics and gene flow for six crab populations.

	Crab1	Crab20	Crab2	Crab11	Crab18	Crab15	Crab6	Crab17	Crab8	Crab12	Esin42	HLJEsa42	Crab21
Fis	−0.62	0.38	−0.02	−0.06	0.26	0.27	−0.03	0.28	0.01	−0.01	0.10	0.18	−0.03
Fit	−0.60	0.40	−0.01	−0.04	0.29	0.29	0.00	0.29	0.02	0.01	0.12	0.200	−0.02
Fst	0.01	0.03	0.01	0.01	0.03	0.03	0.03	0.02	0.01	0.02	0.02	0.01	0.01
Nm *	20.78	7.04	12.50	13.64	7.08	7.84	6.77	11.04	24.85	13.06	15.33	13.42	35.27

* Nm = gene flow estimated from Fst = 0.25 (1 − Fst)/Fst.

The neutral test analysis results of 1000 repeated simulations of 13 microsatellites showed that all 12 sites were within the 95% confidence interval and belonged to neutral sites. Only the *Esin42* site was not within the 95% confidence interval (Table 6).

Table 6. Ewens–Watterson test for neutrality in six crab populations.

Locus	Obs. F	Min F	Max F	Mean	SE	L95	U95
crab1	0.50	0.20	0.97	0.57	0.03	0.29	0.93
crab20	0.34	0.11	0.95	0.38	0.02	0.18	0.72
crab2	0.98	0.50	0.99	0.84	0.02	0.50	0.99
crab11	0.37	0.10	0.95	0.34	0.017	0.17	0.69
crab18	0.26	0.07	0.93	0.27	0.01	0.14	0.53
crab15	0.36	0.12	0.96	0.41	0.02	0.21	0.78
crab6	0.73	0.20	0.97	0.56	0.03	0.28	0.92
crab17	0.36	0.16	0.97	0.50	0.03	0.25	0.88
crab8	0.22	0.06	0.92	0.24	0.00	0.12	0.51
crab12	0.16	0.07	0.93	0.27	0.01	0.14	0.56
Esin42	0.08	0.05	0.90	0.19	0.00	0.10	0.38
HLJEsa42	0.11	0.05	0.90	0.18	0.00	0.10	0.38
crab21	0.30	0.12	0.96	0.40	0.02	0.20	0.78

3.4. Population Structure of Six Crab Populations

The pairwise Fst values across all six populations are given in Table 5. The pairwise Fst values ranged from −0.00 (CD vs. GY) to 0.01 (GC vs. TH). All the pairwise Fst values were not statistically significant ($p > 0.05$). The genetic differentiation between the six populations was very low. Although not statistically significant, the genetic differentiation between GC and TH (0.002) was the highest and the lowest was between CD and TH (−0.00). To further understand the genetic differentiation among the six crab populations, we performed a genetic identity and distance analysis (Table 7). All the populations were highly similar, with similarities ranging from 94.54% for GY vs. GC to as high as 97.57% for GY vs. CD. Furthermore, STRUCTURE v 2.3.4 [26] was used to study the population structure and genetic relations of the six different populations. The model-based clustering analysis suggested k values ranging from two to six to possibly identify population structure; however, no evidence of population genetic structure could be observed, and the genetic structure of crabs in six different water bodies was found to be not significantly different (Figure 2). Furthermore, an analysis of molecular variance (AMOVA) enabled us to understand the nature and level of genetic variance and differentiation within and among the crab populations. The results showed very little (0.36%) variation among the crab populations but a high variation (94.08%) between individuals in the populations (Table 8). This shows that the populations were not reasonably genetically differentiated based on the lake of origin, and that genetic variation was among individuals within each population. This is in agreement with pairwise Fst, genetic identity analysis, distance analysis, and model-based clustering analysis results showed that no reasonable genetic differentiation and structure could be observed among the six populations.

Table 7. Genetic identity and genetic distance of six crab populations.

	GC	HZ	YC	CD	GY	TH
GC		0.95	0.95	0.95	0.94	0.94
HZ	0.04		0.97	0.96	0.97	0.96
YC	0.04	0.02		0.95	0.96	0.95
CD	0.05	0.04	0.04		0.97	0.95
GY	0.05	0.02	0.03	0.02		0.97
TH	0.05	0.03	0.04	0.04	0.02	

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

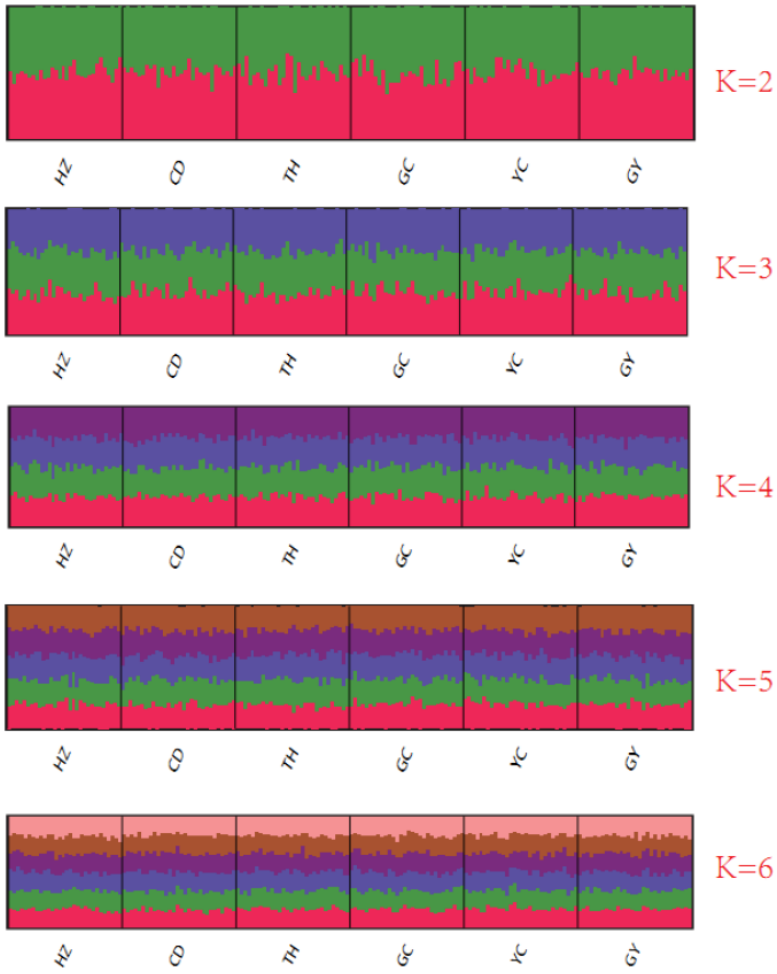

Figure 2. Model-based clustering analysis showing relationship among Chinese mitten crab populations from six lakes. Vertical bars represent individuals in the population, and the color proportion for each bar represents the likelihood of assignment of each individual to the different groups (k) inferred. At the bottom is the name of the lake from which the crabs were collected. All the k values did not show clear structure grouping or a structure based on geographic or genetic background.

Table 8. AMOVA analysis of six crab populations.

Source of Variation	d.f	Sum of Squares	Variance Components	Percentage of Variation
Among populations	5	22.91	0.013	0.36
Among individuals Within populations	174	662.62	0.20	5.56
Within individuals	180	613.00	3.41	94.08
Total	359	1298.53	3.62	

4. Discussion

Genetic diversity, species diversity, and ecosystem diversity are constituents of biodiversity, which is the basis of the evolution of life and species diversity [27,28]. Exploring genetic diversity enables us to know the origin of species diversity, variation, and evolution [29–31]. Several studies have been conducted on the genetic diversity of the Chinese mitten crab mainly for different populations in China [16,32,33]. The present study was aimed at assessing the genetic diversity of the Chinese mitten crab populations by microsatellite makers. The results obtained showed that the average Fst value at eight mi-

crosatellite loci, which revealed low genetic differentiation among the six crab populations and high variation within individuals in the populations and a low genetic flow [34].

4.1. Chinese Mittens Crab Diversity

By considering different genetic diversity parameters, a pairwise study of loci, the Shannon index, and the linkage disequilibrium, we found that 10 pairs of loci confirmed the linkage disequilibrium, which means that the populations deviated from the Hardy–Weinberg principle. Since the pairs of the loci are in linkage disequilibrium, there is a likelihood of genetic drift in the Chinese mitten crab population which can affect gene flow. The Fst values showed a moderate genetic difference among the different loci. The low genetic variation observed can be attributed to the geographic connectivity of the six crab populations, based on the genetic distance in the Chinese mitten crab population [34].

4.2. Mutation–Drift Equilibrium Significant Test Using IAM, TPM, and SMM Model

Three evolutionary models were used for the analysis of the mutation–drift equilibrium. In addition, the IAM model based on multi-step mutations is more suitable than the single-step mutation (SMM) model of genes [35]. However, TPM has both single-step and multi-step mutations, so researchers believe that microsatellites are more suitable for bottleneck analysis using TPM [36]. Previous studies have shown that environmental temperature has a great influence on animals [37–39]. However, the crabs in the six lakes are found in the Yangtze River drainage systems, and thus there are very little variations in the temperatures between them (Figure 1). Since temperature differences are not a large influence, there is little difference in the size of adult crabs. At the same time, the results show low genetic differentiation between the six crab populations and high variation within individuals in the populations. Nonetheless, judging from the experimental data collected, Yangcheng Lake crabs have richer biodiversity and better reproductive potential and growth performance.

4.3. Genetic Distance of Chinese Mitten Crabs Population

Previous studies showed that there was weak genetic differentiation among populations of crabs for several reasons [15,40,41], including their low migratory capacity, which hinders gene flow. In addition, the market demand for crabs can have significant impacts on genetic variability among Chinese mittens crabs [40], as market demand increases the translocation of crabs from one province to another. From our results, a higher genetic distance was found in HZ and YC, which means gene flow in their population is lower compared to that of other Chinese mittens crabs in other lakes. However, crab populations from YC and HC showed no significant genetic distance because the geographical distance between them is small; thus, they had more to mix genetically. The reason is presumably due to the absence of physical barriers to migration between the two lakes.

5. Conclusions

In conclusion, the study has shown that the six Chinese mittens crab populations have high genetic variability among individuals of a population and low genetic diversity between the six populations. In addition, no population structure was observed. Genetic diversity studies are necessary to assess the genetic diversity of Chinese mitten crabs after being subjected to mass selection and environmental change. The of the present study are important towards sustainable exploitation of the Chinese mitten crab. They also provides information for the selection process of germplasm resource for breeding Chinese mitten crabs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8050220/s1. Table S1: 13 Pairs of microsatellite primers used to detect the genetic diversity of Chinese mittens crabs; Table S2: Genotypic linkage disequilibrium for each locus pair across all populations.

Author Contributions: S.S., B.P.M. and Y.T. conceived the study and contributed to the designing of the experiments. J.D.N., B.P.M., X.H., J.L., F.Y. and M.W. performed crab data collection. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Central Public-interest Scientific Institution Basal Research Fund, Freshwater Fisheries Research Center, CAFS (2020TD36); the Key Project for Jiangsu Agricultural New Variety Innovation (PZCZ201749); Jiangsu Revitalization of Seed Industry (JBGS(2021) 031).

Institutional Review Board Statement: This study does not involve any species at risk of extinction. The permission to collect crab samples from the lakes was obtained from the Bureau of Fisheries, Ministry of Agriculture; however, no written authorization was given because the species is not endangered. All experiments were conducted in accordance with the guidelines for the care and use of animals in experiments (Ministry of Science and Technology of China, 398th file in 2006 (the code for the application or the authorization related to animal management protocols of the Committee of Ethics and Animal Care)).

Data Availability Statement: All data generated or analyzed during this study are included in this article.

Acknowledgments: The authors gratefully acknowledge Jean Damascene, Xinjin He, and the staff in the fish breeding and genetics lab for all their assistance during the time we were conducting the experiments for this study.

Conflicts of Interest: The authors declare no competing financial interest.

References

1. Fishery Bureau. *China Fishery Statistical Yearbook 2016*; China Agriculture Press: Beijing, China, 2016.
2. Dong, J.; Li, X.; Zhang, R.; Zhao, Y.; Wu, G.; Liu, J.; Zhu, X.; Li, L. Comparative analysis of the intestinal bacterial community and expression of gut immunity genes in the Chinese Mitten Crab (*Eriocheir sinensis*). *AMB Express* **2018**, *8*, 192. [CrossRef] [PubMed]
3. Reverter, M.; Tapissier-Bontemps, N.; Sasal, P.; Saulnier, D. Use of Medicinal Plants in Aquaculture. *Diagn. Control. Dis. Fish Shellfish.* **2017**, *9*, 223–261.
4. Sui, L.; Wille, M.; Cheng, Y.; Wu, X.; Sorgeloos, P. Larviculture techniques of Chinese mitten crab *Eriocheir sinensis*. *Aquaculture* **2011**, *315*, 16–19. [CrossRef]
5. Zhang, T.; Li, Z.; Cui, Y. Survival, growth, sex ratio, and maturity of the Chinese mitten crab (*Eriocheir sinensis*) reared in a Chinese pond. *J. Freshw. Ecol.* **2001**, *16*, 633–640. [CrossRef]
6. Crozier, L.G.; Hutchings, J. Plastic and evolutionary responses to climate change in fish. *Evol. Appl.* **2014**, *7*, 68–87. [CrossRef]
7. Levitus, S.; Antonov, J.I.; Boyer, T.P.; Baranova, O.K.; Garcia, H.E.; Locarnini, R.A.; Mishonov, A.V.; Reagan, J.R.; Seidov, D.; Yarosh, E.S.; et al. World ocean heat content and thermosteric sea level change (0–2000 m), 1955–2010. *Geophys. Res. Lett.* **2012**, *39*, 1–5. [CrossRef]
8. O'Reilly, C.M.; Sharma, S.; Gray, D.K.; Hampton, S.E.; Read, J.S.; Rowley, R.J.; Schneider, P.; Lenters, J.D.; McIntyre, P.B.; Kraemer, B.M.; et al. Rapid and highly variable warming of lake surface waters around the globe. *Geophys. Res. Lett.* **2015**, *42*, 10773–10781. [CrossRef]
9. Miao, H.; An, C. Challenges for Protected Areas Management in China. *Sustainability* **2020**, *12*, 5879.
10. Zaya, D.N.; Molano-Flores, B.; Feist, M.A.; Koontz, J.A.; Coons, J. Assessing genetic diversity for the USA endemic carnivorous plant Pinguicula ionantha R.K. Godfrey (*Lentibulariaceae*). *Conserv. Genet.* **2016**, *18*, 171–180. [CrossRef]
11. Frankham, R. Genetics and extinction. *Biol. Conserv.* **2005**, *126*, 131–140. [CrossRef]
12. Sean, H.; Bruford, M.W.; Chris, F.W.; Peter, G.; Patrick, G.M.; Grueber, C.E.; Myriam, H.; Hunter, M.E.; Christina, H.; Kalamujic, S.B. Global Commitments to Conserving and Monitoring Genetic Diversity Are Now Necessary and Feasible. *Bioscience* **2021**, *71*, 964–976.
13. Monika, S.; Gabor, S.; Katarzyna, W.O.; Jakub, S.; Dusan, G. Genetic Diversity and Population Structure of the Rare and Endangered Plant Species *Pulsatilla patens* (L.) Mill in East Central Europe. *PLoS ONE* **2016**, *11*, e0151730.
14. Páez-Triana, L.; Muoz, M.; Herrera, G.; Moreno-Pérez, D.A.; Rmírez, J.D. Genetic diversity and population structure of *Rhipicephalus sanguineus* sensu lato across different regions of Colombia. *Parasites Vectors* **2021**, *14*, 424. [CrossRef] [PubMed]
15. Sui, L.; Zhang, F.; Wang, X.; Bossier, P.; Sorgeloos, P.; Hänfling, B. Genetic diversity and population structure of the Chinese mitten crab *Eriocheir sinensis* in its native range. *Mar. Biol.* **2009**, *156*, 1573–1583. [CrossRef]
16. Zhang, C.; Li, Q.; Wu, X.; Liu, Q.; Cheng, Y. Genetic diversity and genetic structure of farmed and wild Chinese mitten crab (*Eriocheir sinensis*) populations from three major basins by mitochondrial DNA COI and Cyt b gene sequences. *Mitochondrial DNA A DNA. Mapp. Seq. Anal.* **2017**, *29*, 1081–1089.
17. Cunningham, G.M.; Roman, M.G.; Flores, L.C.; Hubbard, G.B.; Salmon, A.B.; Zhang, Y.; Gelfond, J.; Ikeno, Y. The paradoxical role of thioredoxin on oxidative stress and aging. *Arch. Biochem. Biophys.* **2015**, *576*, 32–38. [CrossRef]

18. Cowman, P.F.; Bellwood, D.R. The historical biogeography of coral reef fishes: Global patterns of origination and dispersal. *J. Biogeogr.* **2013**, *40*, 209–224. [CrossRef]
19. Harrison, H.B.; Williamson, D.H.; Evans, R.D.; Almany, G.R.; Thorrold, S.R.; Russ, G.R.; Feldheim, K.A.; van Herwerden, L.; Planes, S.; Srinivasan, M.; et al. Larval Export from Marine Reserves and the Recruitment Benefit for Fish and Fisheries. *Curr. Biol.* **2012**, *22*, 1023–1028. [CrossRef]
20. Yeh, F.C.; Yang, R.-C.; Boyle, T.; Ye, Z.; Xiyan, J. *POPGENE 32, Microsoft Windows-Based Freeware for Population Genetic Analysis*; University of Alberta: Edmonton, AB, Canada, 2000.
21. Rousset, F. Equilibrium Values of Measures of Population Subdivision for Stepwise Mutation Processes. *Genetics* **1996**, *142*, 1357–1362. [CrossRef]
22. Excoffier, L.; Smouse, P.E.; Quattro, J.M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **1992**, *131*, 479–491. [CrossRef]
23. Wilson, G.A.; Rannala, B. Bayesian Inference of Recent Migration Rates Using Multilocus Genotypes. *Genetics* **2003**, *163*, 1177–1191. [CrossRef] [PubMed]
24. Evanno, G.S.; Regnaut, S.J.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
25. Austin, Z.; Sutton, J. Qualitative research: Getting started. *Can. J. Hosp. Pharm.* **2014**, *67*, 436. [CrossRef] [PubMed]
26. Pritchard, J.K.; Stephens, M.J.; Donnelly, P.J. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef]
27. Simental-Rodríguez, S.L.; Quiñones-Pérez, C.Z.; Moya, D.; Hernández-Tecles, E.; López-Sánchez, C.A.; Wehenkel, C. The Relationship between Species Diversity and Genetic Structure in the Rare Picea chihuahuana Tree Species Community, Mexico. *PLoS ONE* **2014**, *9*, e111623. [CrossRef]
28. Stange, M.; Barrett, R.D.H.; Hendry, A.P. The importance of genomic variation for biodiversity, ecosystems and people. *Nat. Rev. Genet.* **2020**, *22*, 89–105. [CrossRef]
29. Amos, W.; Harwood, J. Factors affecting levels of genetic diversity in natural populations. *Philos. Trans. R. Soc. B Biol. Sci.* **1998**, *353*, 177–186. [CrossRef]
30. Coates, D.J.; Byrne, M.; Moritz, C. Genetic Diversity and Conservation Units: Dealing with the Species-Population Continuum in the Age of Genomics. *Front. Ecol. Evol.* **2018**, *6*, 165. [CrossRef]
31. Gaggiotti, O.E.; Chao, A.; Peres-Neto, P.; Chiu, C.H.; Edwards, C.; Fortin, M.J.; Jost, L.; Richards, C.M.; Selkoe, K.A. Diversity from genes to ecosystems: A unifying framework to study variation across biological metrics and scales. *Evol. Appl.* **2018**, *11*, 1176–1193. [CrossRef]
32. Wang, C.; Chenhong, L.I.; Sifa, L.I. Mitochondrial DNA-inferred population structure and demographic history of the mitten crab (*Eriocheir sensu stricto*) found along the coast of mainland China. *Mol. Ecol.* **2008**, *17*, 3515–3527. [CrossRef]
33. Jun, W.; Lei, H.; Qixuan, C.; Guoqing, L.; Chenghui, W. Complete mitochondrial genomes of three mitten crabs, Eriocheir sinensis, *E. hepuensis*, and *E. japonica*. *Mitochondrial DNA Part A* **2016**, *27*, 1175–1176.
34. Wang, H.; Feng, G.; Zhang, Y. Studies on genetic diversity of Chinese mitten crab Eriocheir sinensis of Yangtze River system based on mitochondrial DNA control region. *J. Phys. Conf. Ser.* **2020**, *1549*, 032010. [CrossRef]
35. Estoup, A.L.; Garnery, L.; Solignac, M.; Cornuet, J.M. Microsatellite variation in honey bee (*Apis mellifera* L.) populations: Hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* **1995**, *140*, 679–695. [CrossRef] [PubMed]
36. Cornuet, J.M.; Luikart, G. Description and Power Analysis of Two Tests for Detecting Recent Population Bottlenecks from Allele Frequency Data. *Genetics* **1996**, *144*, 2001–2014. [CrossRef]
37. Wu, Z.; Yu, D.; Wang, Z.; Li, X.; Xu, X. Great influence of geographic isolation on the genetic differentiation of *Myriophyllum spicatum* under a steep environmental gradient. *Sci. Rep.* **2015**, *5*, 15618. [CrossRef]
38. Prentice, M.B.; Bowman, J.; Khidas, K.; Koen, E.L.; Row, J.R.; Murray, D.L.; Wilson, P.J. Selection and drift influence genetic differentiation of insular Canada lynx (*Lynx canadensis*) on Newfoundland and Cape Breton Island. *Ecol. Evol.* **2017**, *7*, 3281–3294. [CrossRef]
39. Wu, Z.; Xu, X.; Zhang, J.; Wiegleb, G.; Hou, H. Influence of environmental factors on the genetic variation of the aquatic macrophyte *Ranunculus subrigidus* on the Qinghai-Tibetan Plateau. *BMC Evol. Biol.* **2019**, *19*, 237. [CrossRef]
40. Cheng, Y.; Wu, X.; Yang, X.; Hines, A.H. Current Trends in Hatchery Techniques and Stock Enhancement for Chinese Mitten Crab. *Eriocheir. Jpn. Sin. Rev. Fish. Sci.* **2008**, *16*, 377–384. [CrossRef]
41. Huang, W.; Zhao, X.; Zhao, X.; Li, Y.; Lian, J. Effects of environmental factors on genetic diversity of Caragana microphylla in Horqin Sandy Land, northeast China. *Ecol. Evol.* **2016**, *6*, 8256–8266. [CrossRef]