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The Genetic Diversity of the Rice-crayfish Eco-farming *Procambarus clarkii* in Anhui Province, China

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Abstract

As a small economic aquatic animal for human consumption, the crayfish *Procambarus clarkii* breeding was vigorously promoted in Anhui Province. In the present study, genetic analysis was conducted on 12 cultured populations in Anhui Province by microsatellite. The results showed that all twelve populations were showed a high genetic diversity (H_e=0.483-0.660, PIC=0.422-0.588). Among which the genetic diversity of Quanjiao population is highest, while lowest in Changfeng population. AMOVA analysis showed that most of the genetic variation was found within *P. clarkii* population (91.08%), while only 8.92% was found among populations. Genetic differentiation and genetic distance analysis showed that the overall differentiation level was moderate (F_{st}=0.078). Phylogenetic tree showed that all groups in Taihu and Susong County have a close relationship, Huoqiu and Quanjiao populations formed a sister relationship, and genetic distance between Xuanzhou and other populations was furthest, which mainly related to the parents origin. Additionally, populations experience the bottleneck effect, and inbreeding existed within or among populations, especially in Chngfeng and Xuanzhou populations. The result indicated the germplasm resources of cultured crayfish in Anhui have a tendency of decline, the parents should be updated periodically, and genetic exchanges among the populations should be strengthened.

Introduction

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), was originally found in the southeastern United States but introduced to China in the early 20th century, and is nowadays the most cosmopolitan crayfish in the world (Gherardi, 2006; Li et al., 2012). In the 1950s, the pond culture of crayfish had been integrated with other farming operations, such as rice-crayfish co-culture, the mode which can produce both rice and crayfish in the limited space (Mcclain and Romaire, 2004; Si et al., 2017; Sun et al., 2021). As one of the most important freshwater aquaculture resources and well received by consumers in China, crayfish aquaculture has increased rapidly, and crayfish has become the highest-yield freshwater shrimp (Wang et al., 2021).

The artificial breeding of *P. clarkii* began in 1990s in Changfeng County of Anhui province. From 2005 to 2009, the cultivation area and scale gradually expanded, forming three main producing areas: Changfeng County, Quanjiao County and Huoqiu County. Due to its economic and ecological advantages, rice-crayfish eco-farming was greatly promoted. Since 2015, the culture of *P. clarkii* has shown an explosive growth in all cities of Anhui Province, especially in Anqing City. The total

output of Anhui Province is second in China only to Hubei Province in 2019, which become an important means of rural revitalization.

An important consideration when developing and managing captive populations, however, is the maintenance of genetic diversity to ensure that adequate variation exists to avoid the negative consequences of inbreeding (Miller et al., 2014). The decrease of genetic diversity can lead to the decrease of adaptability and survival of the population (Wang et al., 2017). Studies showed *P. clarkii* genetic diversity has been reduced and the germplasm has been narrowed in China, partly due to a long term of inbreeding, next to the founder effects that resulted from initially small number of individuals, and also for populations of subsequent generations exchange with other populations is minimal (Richards, 2000; Oficialdegui et al., 2019; Sun et al., 2021). Additionally, for captive populations, disorderly introduction, blindly releasing and escape to the natural water also probably occurred. Up to now, the genetic resource data of *P. clarkii* populations in China mainly focus on the wild population (Cao et al., 2010; Li et al., 2012), studies on the cultured populations are still scarce, Zhong et al. (2020) reported genetic diversity of *P. clarkii* populations in different areas of Guangxi Province, and there is no report on the genetic diversity of Anhui cultured populations, it is urgent to clarify the genetic background of the breeding population in Anhui. In the present study, the genetic diversity analysis was conducted on 12 populations of *P. clarkii* in Anhui Province by microsatellite, which aims to enhance the understanding of the genetic difference among various populations and provide basic data for resource protection, breeding and genetic modification, and also for rational and effective artificial breeding and management of the rice-crayfish symbiosis farming.

Materials and Methods

Sample Collection

Focusing on Anhui Province, a total of 370 individuals of *Procambarus clarkii* were collected from 12 artificial cultured populations (Taihu-1, Taihu-2, Taihu-3, Taihu-4, Susong-1, Susong-2, Susong-3, Changfeng, Huoqiu, Chaohu, Xuanzhou and Quanjiao) in different regions of agricultural leading enterprises, breeding with large-scale production farms and foundation seed farms from April to June, 2020 (Figure 1). The muscles tissue samples were quickly taken and cleaned with 0.70% physiological saline, then stored at -20°C in 95% ethanol.

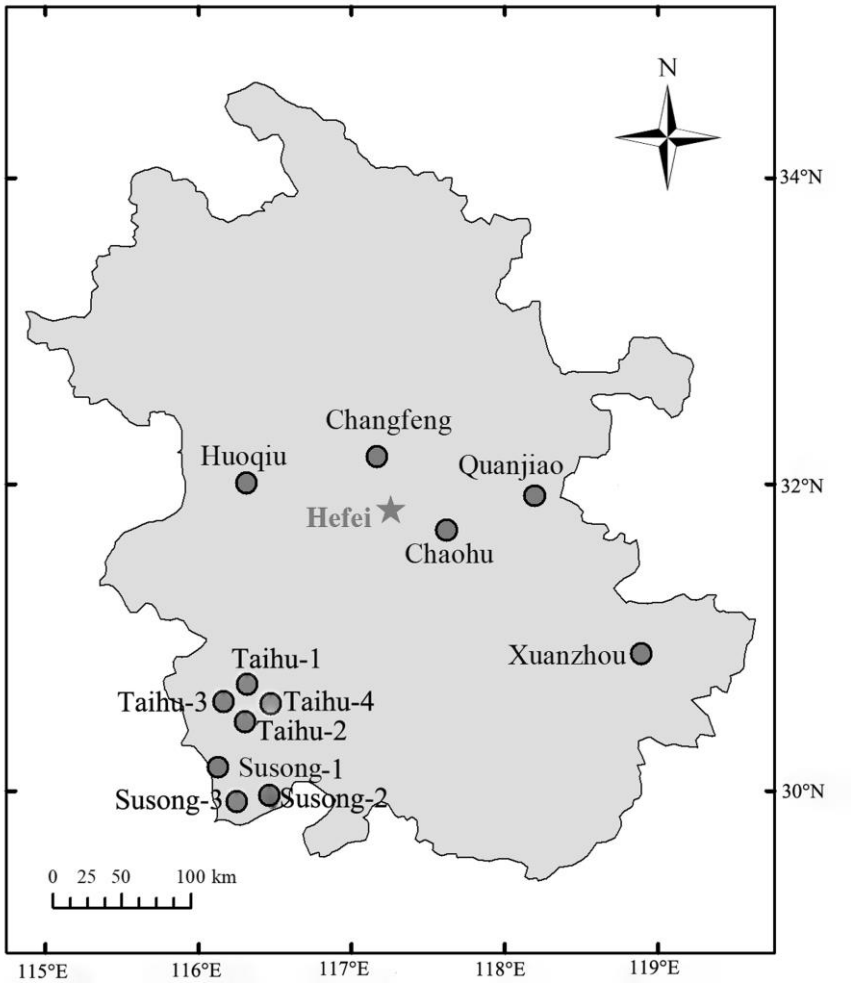

Figure 1. Sampling sites in Anhui Province

DNA Extraction and Genotyping

Genomic DNA was extracted using the DNeasy blood and tissue kit (Tiangen, Beijing, China) following manufacturer's instructions. A panel of nine microsatellite markers previously developed for *P. clarkii* were amplified by polymerase chain reaction (PCR) using the annealing temperatures 52-70 °C (Belfiore and May, 2000). The PCR reactions were conducted using a peltier thermal cycler using a 30 µl reaction mixture. Each reaction mixture contained 3 µl of 10× PCR buffer, and final concentrations of 2.5 µl (2.5 mmol/L) dNTPs, 1 µl (10 µmol/L) each of forward and reverse primer, 0.5 µl (5 U/µl) of Taq DNA polymerase (Transgen, Beijing, China), 1 µl (50 ng/µl) of template DNA that were added to 30 µl ddH2O. Temperature profiles for the PCR consisted of an initial denaturation at 94 °C for 5 min, then 32 cycles of 94 °C for 30 s, annealing at primer specific temperatures for 40 s at 72 °C for 50 s and a final extension at 72 °C for 10 min. Forward primers from each locus were labeled with different fluorescent dyes (Table 1).

The PCR products were separated and sized on an ABI 3730xl automated sequencer with ROX 500 size standard, and the resulting genotype traces scored in GeneMapper 3.7 (all Applied Biosystems). The presence of null alleles, large allele dropouts, scoring of stutter peaks and typographic errors were assessed using a Micro-checker (Van Oosterhout et al., 2004).

Data Analysis

The microsatellite data were analyzed using web-based Genepop software (http://genepop.curtin.edu.au/), with Markov chain parameters of 10,000 dememorisation, 500 batches and 5000 iterations per batch to determine whether each locus deviated from the Hardy-Weinberg equilibrium and to test the linkage equilibria. The number of alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), shannon's information index (I), gene flow (N_m) and Nei's genetic distance (D_a) values were calculated using Popgene 1.32, respectively (Nei, 1972; Yeh & Boyle, 1997).

The genetic differentiation coefficient (F_{st}) and genetic variation were analyzed by AMOVA (analysis of molecular variance) using Arlequin 3.5 software (Excoffier & Lischer, 2010). UPGMA phylogenetic tree based on Nei's genetic distance was constructed by using MEGA 7.0 software (Kumar et al., 2016). The polymorphism information content (PIC) of each locus and each population was calculated using Cervus 3.0 (Kalinowski, 2007).

The genotypes that were determined utilizing COANCESTRY (V1.0.1.1; Wang, 2011) were used to measure relatedness estimates (R) between the populations and within population genotypes using previously described procedures of Wang (2002) and the paternal inbreeding coefficient (F) using previously described procedures of Ritland (1996).

The bottleneck effect was analyzed based on three mutation models, infinite allele model, two phase mutation model and step-wise mutation model using Bottlenecks 1.2. Moreover, mutation-drift equilibrium of the population was analyzed by Sign test and Wilcoxon test to estimate whether the population had heterozygosity excess or deficiency (Luikart & Cornuet 1998; Piry et al., 1999).

Result

Summary Statistics

No evidence of allelic stutter or large allele dropout was found in the dataset, and no null alleles were

Table 1. Summary details of microsatellite loci used in this study.

Loci	Primer sequences (5'→3')	Annealing temperature	Repeat motif
PclG02	F: CTCCCCATGCACTCTGGCTCTGT—FAM R: TGGCGAATTTTGCCTGTTTCTGTC	66 °C	$(GATA)_3GAGAA(GATA)_5$
PclG03	F: CTCTCCACCAGTCATTTCTT—FAM R: AAGCTTACAATAAATATAGATAGAC	52 °C	$(TCTA)_{20}$
PclG04	F: TATATCAGTCAATCTGTCCAG—FAM R: TCAGTAAGTAGATTGATAGAAGG	54 °C	$(TCTA)_3…(TCTA)_2…(TCTA)_{29}…(TCTA)_2$
PclG07	F: CCTCCCACCAGGGTTATCTATTCA—HEX R: GTGGGTGTGGCGCTCTTGTT	63 °C	$(TCTA)_8$
PclG09	F: TATGCACCTTTACCTGAAT—HEX R: TGTTGGTGTGGTCATCA	60 °C	$(TCTA)_{14}$
PclG15	F: GGCGTGACGCCAACGTGTCTT—HEX R: GGCTGGCCACTTTGTTAGCCTGAG	70 °C	$(TATC)_2TGTC(TATC)_{17}TATT(TATC)_3$
PclG17	F: GTCGGGAACCTATTTACAGTGTAT—FAM R: AAGAGCGAAGAAAGAGATAAAGAT	57 °C	$(TCTA)_{14}$
PclG29	F: GAAAGTCATGGGTGTAGGTGTAAC—HEX R: TTTTTGGGCTATGTGACGAG	65 °C	$(TATC)_9$
PclG33	F: TTCGAGGCGTTGCTGATTGTAAGT—FAM R: CAAGGAAGCGTATAGCCGGAGTCT	68 °C	$(GT)_{21}$

detected at any of the nine loci. Almost of all nine loci were found to be highly polymorphic (*PIC*>0.5). Summary details of the nine microsatellite loci were given in Table 2.

Of the 9 loci assessed for Hardy-Weinberg equilibrium (HWE) were observed using the probability test (P<0.05), five loci in Taihu-4, Huoqiu and Quanjiao, four loci in Changfeng and Chaohu, three in Xuanzhou, Taihu-2, Taihu-3, Susong-1 and Susong-2, two in Susong-3 and one locus in Taihu-1 showed significant departures from HWE. When heterozygote excess was assessed using HWE procedures, no disequilibrium was detected in all populations, additionally, no linkage disequilibrium was detected between any loci (P>0.05). The comparison of genetic information of different *P. clarkii* breeding populations in Anhui Province was showed in Table 3. All twelve populations were showed a high genetic diversity (H_e=0.483～0.660, PIC=0.422～0.588). Among which the genetic diversity of Quanjiao population is highest, while lowest in Changfeng population.

Genetic Variation and Differentiation

The result of AMOVA showed that the variation among populations is only 8.92%, while variation within populations is 91.08%. Wright (1965) proposed that the genetic differentiation coefficient F_{st} < 0.05 was low differentiation, 0.05 < F_{st} < 0.15 was moderate differentiation, F_{st} > 0.15 was high differentiation. The whole F_{st} value is 0.078, which means a moderate differentiated degree (0.05<F_{st}<0.15) (Table 4).

The F_{st} value of the 12 populations is from 0.0017 to 0.2513. The lowest genetic differentiation of which was between the populations of Taihu-2 and Susong-2 (F_{st}=0.002), with a smallest genetic distance (D_a=0.030), while the highest genetic differentiation of which is between Changfeng and Xuanzhou populations (F_{st}=0.251), with a biggest genetic distance (D_a=0.481) (Table 5).

UPGMA phylogenetic tree based on Nei's genetic distance (Nei 1972) indicated the 12 populations have a common root, which can divide the populations into two

Table 2. Genetic information of *Procambarus clarkii* breeding populations in Anhui Province based on microsatellite markers.

Locus	*Ho*	*He*	*PIC*	*Na*	*Ne*	*I*	*Nm*
PclG02	0.5568	0.5712	0.477	6	2.3277	0.9373	2.0035
PclG03	0.6189	0.7615	0.725	14	4.1744	1.6531	2.151
PclG04	0.4784	0.6704	0.611	9	3.0255	1.2946	1.8418
PclG07	0.6000	0.6292	0.566	8	2.6905	1.2357	3.3567
PclG09	0.3297	0.7294	0.684	11	3.6814	1.5382	3.409
PclG15	0.5189	0.5623	0.511	8	2.2806	1.1106	2.9832
PclG17	0.6081	0.659	0.593	9	2.925	1.2353	1.8401
PclG29	0.4703	0.7547	0.713	6	4.0605	1.4855	2.6032
PclG33	0.7703	0.6262	0.553	14	2.6691	1.2177	6.0427
Mean±SD	0.550±0.123	0.663±0.074	0.604±0.083	9.444±3.005	3.093±0.713	1.301±0.223	2.915±1.249

Table 3. Comparison of genetic information of *Procambarus clarkii* breeding populations in Anhui Province.

Sampling site	n	Na	*Ho*	*He*	*PIC*	I
Changfeng	30	3.556±0.882	0.437±0.208	0.483±0.193	0.422±0.158	0.841±0.318
Xuanzhou	30	4.333±1.581	0.507±0.179	0.570±0.129	0.498±0.122	1.018±0.298
Chaohu	30	4.667±1.225	0.507±0.237	0.627±0.116	0.560±0.104	1.145±0.236
Huoqiu	30	5.556±2.297	0.603±0.198	0.641±0.118	0.576±0.118	1.215±0.321
Quanjiao	40	5.222±1.394	0.550±0.160	0.660±0.056	0.588±0.068	1.208±0.186
Taihu-1	30	4.889±1.453	0.607±0.185	0.655±0.069	0.582±0.074	1.196±0.187
Taihu-2	30	5.222±1.787	0.622±0.210	0.628±0.114	0.556±0.119	1.156±0.306
Taihu-3	30	4.667±1.000	0.496±0.155	0.634±0.117	0.564±0.115	1.160±0.278
Taihu-4	30	4.778±1.481	0.582±0.185	0.619±0.108	0.541±0.117	1.118±0.296
Susong-1	30	5.556±0.727	0.563±0.164	0.616±0.109	0.548±0.100	1.157±0.218
Susong-2	30	5.000±1.118	0.541±0.208	0.618±0.135	0.549±0.125	1.139±0.288
Susong-3	30	5.000±1.323	0.585±0.149	0.600±0.097	0.535±0.100	1.119±0.247

Table 4. Analysis of molecular variance (AMOVA) results for *Procambarus clarkii* in Anhui Province using 9 microsatellite loci.

Source of variation	D.F.	Sum of squares	Variance components	Percentage
Among populations	11	193.184	0.23539	7.84
Within populations	728	2010.433	2.76638	92.16
Total	739	2203.617	3.00177	100

Notes： *Fst*=0.078.

Table 5. Genetic differentiation F_{st} values and Nei's genetic distance among populations of *Procambarus clarkii* in Anhui Province.

	Changfeng	Xuanzhou	Taihu-3	Taihu-1	Taihu-4	Taihu-2	Susong-3	Chaohu	Susong-2	Huoqiu	Susong-1	Quanjiao
Changfeng	****	**0.251**	0.160	0.179	0.195	0.159	0.221	0.078	0.198	0.128	0.197	0.078
Xuanzhou	0.481	****	0.104	0.132	0.134	0.138	0.144	0.134	0.138	0.093	0.106	0.102
Taihu-3	0.282	0.214	****	0.011	0.038	0.019	0.060	0.059	0.022	0.045	0.019	0.042
Taihu-1	0.341	0.295	0.050	****	0.022	0.010	0.036	0.075	0.009	0.070	0.009	0.058
Taihu-4	0.360	0.279	0.095	0.068	****	0.029	0.063	0.091	0.025	0.082	0.022	0.065
Taihu-2	0.277	0.296	0.063	0.047	0.079	****	0.055	0.050	**0.002**	0.070	0.023	0.047
Susong-3	0.420	0.292	0.133	0.090	0.137	0.122	****	0.134	0.025	0.112	0.030	0.093
Chaohu	0.120	0.284	0.141	0.184	0.207	0.119	0.308	****	0.084	0.041	0.094	0.029
Susong-2	0.369	0.287	0.067	0.044	0.070	0.030	0.066	0.191	****	0.089	0.015	0.066
Huoqiu	0.214	0.193	0.116	0.180	0.192	0.169	0.256	0.104	0.209	****	0.065	0.026
Susong-1	0.364	0.212	0.060	0.043	0.063	0.067	0.074	0.212	0.051	0.153	****	0.064
Quanjiao	0.120	0.218	0.109	0.153	0.156	0.119	0.213	0.081	0.158	0.077	0.153	****

Note: F_{st} (above diagonal) and Nei's genetic distance (below diagonal).
The bolded values showed highest and lowest F_{st} values.

clades. The analysis revealed a strong clustering of populations in Taihu and Susong County including Taihu-1, Susong-1, Taihu-2, Susong-2, Taihu-3, Taihu-4 and Susong-3, then clustered with the populations of Changfeng, Chaohu, Huoqiu and Quanjiao. Of which, Huoqiu and Quanjiao formed a sister relationship, they were the most related, then clustered with Chaohu and Changfeng. The tree also showed that the genetic distance between Xuanzhou and other groups was furthest (Figure 2).

Partner Relatedness and Inbreeding Coefficient Analysis

Taihu County and Susong County belong to Anqing City, located in the southwest of Anhui Province. The populations in the two counties revealed a strong clustering according to the above analysis, here, populations from the two counties were combined into Anqing population to calculate the partner relatedness (*R*) and inbreeding coefficient (*F*) analysis. The *R* within each population showed that *R* value is positive in Changfeng and Xuanzhou, and the value is biggest in Changfeng. However, *R* value is negative in Chaohu, Huoqiu, Quanjiao and Anqing populations. The result also showed the *F* value in each of the six populations is positive, with a biggest value in Xuanzhou and smallest value in Anqing (Table 6). The *R* value between populations is negative except that in Changfeng and Chaohu is positive. The *F* value in Anqing and other five populations are negative, and also negative in Xuanzhou and Chaohu, Xuanzhou and Quanjiao populations (Table 7).

Mutation-drift Equilibrium Analysis

According to allele frequency of microsatellite loci and based on three different hypotheses models of IAM, TPM and SMM, mutation-drift equilibrium of *P. clarkii* populations in Anhui Province were tested, and the results are shown in Table 8. When using Sign test, Xuanzhou and Taihu-4 populations showed significant deviation from mutation-drift equilibrium (P<0.05), Quanjiao and Taihu-1 populations showed extremely significant deviation from mutation-drift equilibrium under IAM model (P<0.01). Only Susong-1 population was showed significant deviation from mutation-drift equilibrium under TPM model (P<0.05). Only Susong-3 population was showed significant deviation from mutation-drift equilibrium under SMM model (P<0.05).

However, when using two tails for heterozygosity excess and deficiency of Wilcoxon test, Xuanzhou, Taihu-2 and Taihu-4 populations were showed significant deviation from mutation-drift equilibrium (P<0.05), while Chaohu, Quanjiao, Taihu-1 and Taihu-3 showed extremely significant deviation from mutation-drift equilibrium under IAM model (P<0.01). None of any population was deviation from mutation-drift equilibrium under TPM model. However, Susong-1 (P<0.01) and Susong-3 (P<0.05) populations were deviation from mutation-drift equilibrium under SMM model.

Discussion

Genetic Diversity

Genetic diversity means genetic variation and adaptation of species' ability to the environment in the long-term evolutionary process (Liu et al., 2020). In general, an invasive alien species often can avoid inbreeding defects and adapt well to a new environment, thereby resulting in high genetic diversity (Zhong et al., 2020). Since multiple paternity was detected in *Procambarus clarkii*, which is also a compensation mechanism to maintain genetic diversity (Yue et al., 2010; Wang et al., 2017). Studies showed the genetic diversity of 25 wild *P. clarkii* populations in China was still high (Liu et al., 2020). With the promotion of eco-farming *P. clarkii* and rice in China, the production of the cultured crayfish has been greatly increased, while genetic evaluation of the cultured crayfish is less understood.

The genetic diversity of *P. clarkii* cultured populations in different areas of Guangxi Province showed H_e=0.5692-0.6544, PIC=0.4899-0.5843 (Zhong

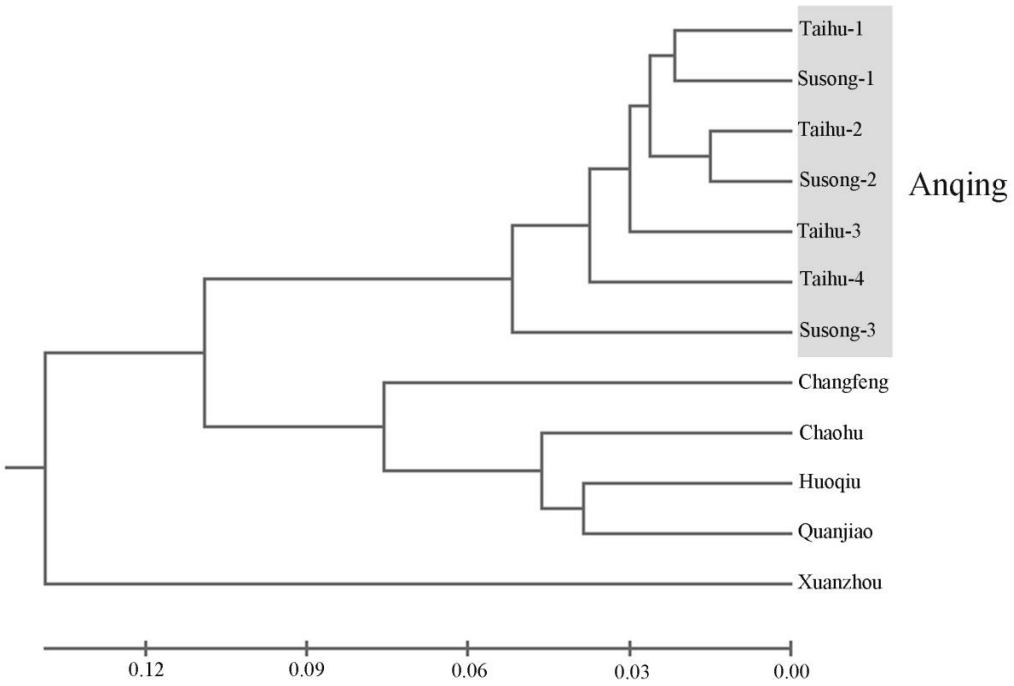

Figure 2. Nei's UPGMA tree of 12 *Procambarus clarkii* populations based on microsatellites. Note: Bar is the genetic distance.

Table 6. The relationship (*R*) and inbreeding coefficient (*F*) within each of the six *Procambarus clarkii* populations in Anhui Province.

	Changfeng	Xuanzhou	Chaohu	Huoqiu	Quanjiao	Anqing
R	0.29975	0.14865	-0.01723	-0.00258	-0.05079	-0.00392
F	0.14764	0.18692	0.09405	0.08605	0.04278	0.0142

Table 7. The relationship (*R*) and inbreeding coefficient (*F*) between the six *Procambarus clarkii* populations in Anhui Province.

Populations	Changfeng	Xuanzhou	Chaohu	Huoqiu	Quanjiao	Anqing
Changfeng	****	-0.20372	0.01984	-0.05283	-0.00924	-0.15993
Xuanzhou	0.04372	****	-0.15316	-0.08773	-0.13569	-0.1445
Chaohu	0.04578	-0.02187	****	-0.07932	-0.07894	-0.14568
Huoqiu	0.00058	0.00643	0.00361	****	-0.07513	-0.13527
Quanjiao	0.03815	-0.00778	0.00677	0.00748	****	-0.13543
Anqing	-0.03127	-0.02254	-0.02419	-0.02297	-0.02213	****

Note: The inbreeding coefficient (*F*) value is below diagonal and the relationship (*R*) value is above diagonal.

Table 8 Mutation-drift equilibrium analysis of *Procambarus clarkii* populations in Anhui Province.

	SIGN TEST						WILCOXON TEST		
Population	IAM		TPM		SMM		IAM	TPM	SMM
	H_E/H_D	*P*	H_E/H_D	*P*	H_E/H_D	*P*	*P*	*P*	*P*
Changfeng	6/3	0.33758	5/4	0.58229	5/4	0.58069	0.35938	0.57031	0.73438
Xuanzhou	8/1	0.03754*	7/2	0.20160	4/5	0.27209	0.01367*	0.30078	0.35938
Chaohu	8/1	0.05029	7/2	0.20610	3/6	0.10564	0.00586**	0.12891	0.42578
Huoqiu	7/2	0.19690	6/3	0.44552	3/6	0.10996	0.12891	0.65234	0.49609
Quanjiao	9/0	0.00615**	7/2	0.20963	4/5	0.27686	0.00195**	0.12891	0.42578
Taihu-1	9/0	0.00662**	6/3	0.46841	4/5	0.27730	0.00195**	0.16406	0.73438
Taihu-2	6/3	0.42531	5/4	0.54626	3/6	0.10268	0.02734*	0.82031	0.16406
Taihu-3	8/1	0.05154	6/3	0.46564	5/4	0.53188	0.00977**	0.25000	0.91016
Taihu-4	8/1	0.04457*	5/4	0.55201	4/5	0.27332	0.01953*	0.65234	0.35938
Susong-1	6/3	0.46649	2/7	0.02733*	1/8	0.00375	0.25000	0.25000	0.00977**
Susong-2	7/2	0.18918	6/3	0.46619	3/6	0.09878	0.16406	0.49609	0.30078
Susong-3	6/3	0.43315	4/5	0.29621	2/7	0.02803*	0.12891	0.73438	0.01953*

Note: H_E/H_D means the ratio of loci number with heterozygosity excess to heterozygosity deficiency; * means significant deviation from mutation-drift equilibrium (P<0.05); ** means extremely significant deviation from mutation-drift equilibrium (P<0.01).

et al., 2020). Our present study also showed a similar result, H_e=0.483-0.660, PIC=0.422-0.588. However, this result is lower compared with the previous study of wild population. Nine wild populations from the Yangtze, Huaihe, and Xin'anjiang River basins in Anhui Province showed a high genetic diversity (H_e =0.78) (Cao et al., 2010). Li et al. (2012) reported 35 wild populations from China showed a relatively high diversity (N_a=6.4-11.8, H_e=0.7002-0.8214), which is lower than that in Louisiana (USA) and Saitama (Japan) population. Some recent studies indicated the genetic diversity of the crayfish in China was decreased, with a medium genetic diversity among 14 populations by using fifty polymorphic SSR markers (H_e=0.39, PIC=0.29) (Oficialdegui et al., 2019; Sun et al., 2021).

In the present study, the genetic diversity of Changfeng population is lowest, while highest in Quanjiao population. As Changfeng County is first to set up the rice-crayfish eco-farming base in Anhui Province, and become an important base of introduction, like aquaculture enterprises from Chaohu and Huoqiu introduced multiple times. While a self-propagating and self-sustaining model in Changfeng might lead to the decrease of genetic diversity. Quanjiao foundation seed farm is the only one *P. clarkii* foundation seed farm in Anhui Province, which has a mature standard management mode and maintain a high genetic diversity those years.

Genetic Structure, Variation and Inbreeding

Human mediated dispersal had played an important role in the population expansion and genetic differentiation (Yue et al., 2010). In the present study, parental origin is the primary factor of genetic structure. In recent years, the rice-crayfish eco-farming was vigorously promoted and has become an important source of economy in Taihu and Susong County of Anqing City, situated in southwestern part of Anhui Province. We found populations in Susong and Taihu Counties clustered together, this mainly due to *P. clarkii* in the two counties was introduction from Hubei Province. The whole F_{st} showed a moderate differentiated degree, which is similar to the study in Guangxi Province (Zhong et al., 2020).

In addition, the population inbreeding coefficient in Anqing city is small compare with other five populations. Genetic distance between Xuanzhou and other populations was furthest, the parental origin of Xuanzhou population is from the local Nanyi Lake, a tributary of the Yangtze River. The partner relatedness of the *P. clarkii* is closest in Changfeng population, and that is also closer in Xuanzhou population. Additionally, there is a risk of inbreeding in each inner-farm especially Xuanzhou population. The long-time sealing culture mode should be change in Changfeng and Xuanzhou populations according to this result.

Commonly, introduced populations carry only a portion of the source population's genetic variability, and genetic variability can be further reduced by stochastic events such as genetic bottlenecks and/or founder effect in new habitats (Almerao et al., 2018). The populations of *P. clarkii* in Anhui Province except Changfeng, Huoqiu and Susong-2 populations probably suffering bottleneck effect under IAM, TPM and SMM by Sign test and Wilcoxon test. However, studies have shown that many microsatellite data are more in line with TPM model, which has been recommended to test the population bottleneck effect (Cornuet et al., 1996). In the present study, mutation-drift had little impact on the populations if only use TPM model.

Conclusion

In conclusion, the present study is investigated the genetic diversity and structure of artificial cultured populations located in different areas of Anhui. The results showed the genetic diversity of the cultured *P. clarkii* is lower than wild populations in China, and also lower than that in USA and Japan. Particularly, there is a need to improve the germplasm resources of *P. clarkii* in Changfeng and Xuanzhou. Genetic variation is mainly within populations, and genetic differentiation is moderate. Meanwhile, inbreeding existed within or among populations. The result suggested that the cultured *P. clarkii* populations in Anhui Province should update the parents and strengthen genetic exchanges among the populations, for example, introduce good varieties from other provinces or abroad to decrease the inbreeding coefficient and maintain the genetic diversity of the populations.

Ethical Statement

The guidelines established by the Administration of Affairs Concerning Animal Experimentation state that approval from the Science and Technology Bureau of China and the Department of Wildlife Administration is not necessary when the animals in question are neither rare nor near extinction (first- or second-class state protection level). Therefore, approval was not required for the artificial cultured *Procambarus clarkia* conducted in this study.

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Author Contribution

All authors are responsible for the general design of the manuscript. WH analyzed the data and wrote the manuscript. JH contributed to data analysis and revised the manuscript. SG, LJ and PT collected the samples and revised the manuscript. HY, ZH and YM revised the manuscript. DG supervised the whole project. All authors contributed on specific aspects.

Conflict of Interest

The authors declare no conflict of interest.

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