fishes

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Genetic Diversity and Genetic Structure among Four Selected Strains of Whiteleg Shrimp (*Litopenaeus vannamei*) Using SSR Markers

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Abstract: The whiteleg shrimp (*Litopenaeus vannamei*) is one of the most economically important aquaculture species in China. Genetic diversity is crucial for maintaining the gene pool of farmed shrimp. In this study, the effects of artificial selection on the genetic structure of four whiteleg shrimp strains were evaluated using microsatellite sequences. The results showed that the observed heterozygosity (H_o), expected heterozygosity (H_e), and the polymorphism information content (*PIC*) of the four selected strains ranged from 0.446 to 0.574, 0.450 to 0.566, and 0.435 to 0.509, respectively. All the selected strains maintained a moderate level of genetic diversity. Most inbreeding coefficients (F_{is}) in the four strains were positive but not significantly different from zero, indicating a relatively low degree of inbreeding within each strain. However, H_e and *PIC* in line 5 showed a decreasing trend from the 2017 to the 2019 generations, and F_{is} in line 10 showed a significant increasing trend across generations, indicating that measures must be taken to maintain the level of genetic diversity for lines 5 and 10. UPGMA cluster trees showed that the four breeding lines had apparent genetic differences, which could provide a genetic basis for studying crossbreeding between selective lines and the utilization of heterosis. This study will be useful for population genetic research and the breeding strategies of whiteleg shrimp.

Keywords: genetic diversity; artificial selection; microsatellite; whiteleg shrimp

Key Contribution: The four selected strains maintained a moderate level of genetic diversity and had apparent genetic differences by microsatellite analysis of whiteleg shrimp.

Citation: Zhang, Z.; Lu, C.; Lin, K.; You, W.; Yang, Z. Genetic Diversity and Genetic Structure among Four Selected Strains of Whiteleg Shrimp (*Litopenaeus vannamei*) Using SSR Markers. *Fishes* **2023**, *8*, 544. https://doi.org/10.3390/fishes8110544

Academic Editor: Manuel Manchado

Received: 21 September 2023
Revised: 27 October 2023
Accepted: 3 November 2023
Published: 6 November 2023

1. Introduction

Litopenaeus vannamei is native to the South Pacific and the coastal waters of America. Since its introduction to China in 1988, it has become a pillar of China's aquaculture industry after decades of development. With increasing industrial and social demands, the annual demand for seedlings in China exceeds 400 billion tails. However, in recent years, due to the invasion of various shrimp diseases and the degradation of germplasm caused by frequent inbreeding, problems have occurred in the culturing of *L. vannamei*, including miniaturization, slow growth rates, and poor disease resistance [1]. Therefore, it is urgent to characterize the genetic variation in the existing population at the molecular level and breed new strains suitable for farming.

Shrimp farming is an indispensable part of China's aquaculture industry. Almost all broods are produced in hatcheries using captive-cultured broodstock sources for artificial reproduction. In addition, current selection practices based on mass phenotypic selection are commonly applied, where rapidly growing individuals are particularly favored and then used as broodstock to produce the next generation of offspring. Thus, genetic diversity in hatchery stocks may be lost during such artificial propagation. The level of genetic diversity within a population closely relates to the animals' ability to adapt to new environments

and resist disease, and a reduction in genetic diversity can negatively impact production and hinder the development of the aquaculture industry [2].

Simple sequence repeats (SSRs) or microsatellite genetic markers are co-dominantly inherited in Mendelian patterns. They are widely used to estimate genetic distances, construct phylogenetic trees, and measure genetic diversity among species due to their diverse repeat motifs, high mutation frequency, rich polymorphism, codominant inheritance, and high versatility [3–5]. Although increasing attention has been paid to a new generation of molecular markers such as SNPs with the development of high-throughput sequencing, a previous study [6] suggested that SSRs provide more information concerning genetic diversity and perform better at estimating relative kinship than SNPs. SSRs have been used to monitor genetic variation in marine organisms such as Norway Lobster (*Nephrops norvegicus*) [7], Pacific oyster (*Crassostrea gigas*) [8], grooved carpet shell clam (*Ruditapes decussatus*) [9], Nile Tilapia (*Oreochromis niloticus*) [10], summer flounder (*Paralichthys dentatus*) [11], and pike-perch (*Sander lucioperca*) [12]. To date, hundreds of SSRs have been identified and utilized to examine the current status of germplasm resources of wild or hatchery stocks in shrimp species such as *Fenneropenaeus chinensis* [13], *F. indicus* [14], *Penaeus japonicus* [15], *P. monodon* [16], *P. notialis* [17], and *L. vannamei* [18]. Using eight polymorphic microsatellite markers, the genetic diversity and heterozygosity of four successive generations of *P. chinensis* were estimated and compared by Zhang et al. [19].

Studies on the genetic diversity of whiteleg shrimp for different geographic locations have also been reported. Li et al. [20] analyzed the genetic background of six populations of whiteleg shrimp in China, and the results showed that the genetic diversity of *L. vannamei* in different populations may be high, while the genetic relationship of *L. vannamei* in the same population might be quite close. Huang et al. [21] analyzed the genetic diversity of seedling samples collected from seven culture populations in three major shrimp production areas in Guangdong, and the results showed that the seven populations were clustered into three branches, indicating that the genetic characteristics vary in different cultured populations of *L. vannamei*. In the assessment of genetic diversity of whiteleg shrimp in Iran, researchers utilized four microsatellite loci to examine individuals from different farms and observed an overall average heterozygosity (Ho) between 0.450 and 0.479, which was lower than the expected value (0.789–0.794) [22].

In the present study, we used 12 microsatellite markers to analyze the genetic diversity within and between four selected strains (5, 8, 9, and 10) of whiteleg shrimp, including different generations of selected fast-growing strains. The objective of this study was to evaluate the impact of selection practices on population genetic diversity. The results of this study will help shrimp farmers to better understand the genetic structure of the selected strains, thereby improving management strategies for shrimp farming programs.

2. Materials and Methods

2.1. Sample Collection

Four lines, namely 5, 8, 9, and 10, were used in the present study. These lines have been continuously selected for two to four generations from 2016 to 2019. Four generations were analyzed for line 5 (strain 20160505, 20170505, 20180505 and 20190505), two generations for line 8 (strain 20180808 and 20190808), two generations for line 9 (strain 20180909 and 20190909), and three generations were analyzed for line 10 (strain 20171010, 20181010, 20191010). At least 300 pairs from each population were preserved for constructing the core germplasm resource bank.

2.2. DNA Extraction

Live adult shrimp were randomly sampled, and equal amounts of muscle tissue were sampled from each strain (N = 32) from 2016 to 2019. The samples were immediately fixed in 95% ethanol for subsequent experiments. Total genomic DNA for genotyping was extracted using the TIANamp Marine Animals DNA kit (Beijing, China) according to the manufacturer's guidelines. DNA quantification and qualification were performed using an

ND2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and DNA integrity was examined via 1% agarose gel electrophoresis.

2.3. Microsatellite Loci Amplification and Genotyping

Genetic diversity was examined using 12 pairs of highly polymorphic and well amplified microsatellite primers that have been developed in previous research [23] (Table 1). DNA amplification was performed in a 20 μL reaction mixture containing 2.0 μL of 10× Taq TM buffer (Takara, Dalian, China), 0.25 mM of each dNTP, 1 U of TaqTM polymerase (Takara), 20 pmol of each primer (the forward primer from each pair was 5′-end-labeled with 6-FAM, ROX, or HEX dye), and 1 μL of genomic DNA (approximately 50 ng/μL). Polymerase chain reactions were conducted using an ABI thermocycler (ABI, Veriti96, Waltham, MA, USA) under the following conditions: preheating at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, primer-specific annealing for 45 s (Table 1), and primer extension at 72 °C for 45 s followed by a final extension step at 72 °C for 5 min. PCR products were detected by 1% agarose gel electrophoresis, and then placed in the gel imaging system to observe whether the target fragment was amplified. The mixed PCR products were then sent to Shanghai Sangon Biotechnology Co., Ltd., (Shanghai, China) and the ABI 3500xl gene analyzer was used for capillary electrophoresis to detect fluorescence signals.

Table 1. Primer sequences, repeat motifs, and fluorescent labels used for whiteleg shrimp microsatellite loci.

Locus	Forward Primer Fluorescent Label	Primer Sequence (5′→3′)	Ta/°C	Expected Size (bp)	GenBank Accession No.
TUMXLv10.200	FAM	F:GCAACAGACATAATGTAGGC R:AATGCTCGTGCCCTCATC	54	105	AF359958
TUMXLv7.97	HEX	F:TGTCGTTAGTGCAGCTCATTC R:GGGGAGGAATAAGAGGAAAGG	52	176	AF360057
TUMXLv7.74	ROX	F:CCTGCGCAATACTGGATATG R:CGAGGTGTAGTTGTGCTTTGG	54	214	AF360056
TUMXLv10.207	FAM	F:GATCACTAGCCATATTTCATCC R:ATCGCATAATGAGCAAACTGG	56	97	AF359963
Pvan1815	HEX	F:GATCATTCGCCCCTCTTTTT R:ATCTACGGTTCGAGAGCAGA	56	126–141	AY062925
TUMXLv7.121	ROX	F:GGCACACTGTTTAGTCCTCG R:CGAACAGAATGGCAGAGGAG	56	242	AF360043
TUMXLv10.311	FAM	F:CATCCACTTCTTCTCGTACCATC R:TCTCCATCCAGGTTCTGGG	58	105	AF359988
TUMXLv10.312	HEX	F:ATACGAAACACCCCATCCC R:GTGGTCTTACCTCGTGGCTC	58	179	AF359989
TUMXLv10.284	ROX	F:TCTTTAAAGGTCAGGTAAAGG R:CGGCCAGACTCCACAACTAC	58	205	AF359983
TUMXLv10.291	FAM	F:CCCTCAAACAGTCGCAGTG R:GTTGGGTGAGTCTTTAGGCG	58	140	AF359983
TUMXLv10.255	HEX	F:CTAAATAAATCACGGGTTGGG R:CCTTCTGGTTTACTGTTGAGGC	58	213	AF359977
TUMXLv10.364	ROX	F:TGAAAGCATTCTGGTAAGGC R:GAATAAAACAAGGGGTGAGGG	58	299	AF360000

2.4. Data Analysis

Allele size scoring was performed using GeneMapper ver. 4. Possible null alleles and genotyping errors were tested using MICRO-CHECKER ver. 2.2.3 (1000 randomizations) [24], and conformation to the Hardy–Weinberg equilibrium was tested in Arlequin [25] using the Markov chain method with 1,000,000 chain steps and an initial burn-in of 100,000 steps. Standard genetic diversity parameters, including the number of alleles (*Na*), the number of effective alleles (*Ne*), observed heterozygosity (H_o), expected heterozy-

gosity (H_e), and the fixation index (F), were determined for each sample at each locus using GenAlEx 6.41 [26]. Differences in diversity between populations were calculated through a Kruskal–Wallis test using SPSS 17.0. The inbreeding coefficient (mean F_{is}) was then calculated using FSTAT 2.9.3 [27]. Genetic distances among populations were estimated using Nei's genetic distance (GD). To examine genetic relationships among populations, a phylogenetic tree was constructed based on pairwise genetic distances for all samples using the UPGMA method as provided in the program MEGA5.2. The occurrence of recent bottlenecks was evaluated using a standardized differences test (assuming the infinite alleles model, 10,000 replicates at a nominal level of 5%) in BOTTLENECK v.1.2.02 [28].

3. Results

3.1. Genetic Diversity Analysis

No evidence of scoring errors due to stuttering or large allele dropout was identified by Micro-Checker, whereas there was evidence of null alleles at several loci for particular populations. All loci were polymorphic across strains, and the level of polymorphism varied among loci (Table 2).

Table 2. Summary statistics of variation detected in 12 microsatellite loci in four selected strains.

Strain	Locus	*N*	*Na*	*Ne*	H_o	H_e	F_{is}	*PIC*
20160505	TUMXLv10.207	32	3	1.408	0.281	0.294	0.029	0.256
	Pvan1815	32	10	4.154	0.656	0.771	0.136	0.725
	TUMXLv7.121	32	5	2.441	0.594	0.600	−0.006	0.548
	TUMXLv10.312	32	4	2.926	0.656	0.669	0.003	0.600
	TUMXLv10.284	32	5	2.723	0.500	0.643	0.210	0.581
	TUMXLv10.291	32	1	1.000	0.000	0.000	N/A	N/A
	TUMXLv10.255	32	2	1.992	0.938	0.506	−0.882	0.374
	TUMXLv10.364	32	3	1.547	0.188	0.359	0.470	0.309
	TUMXLv10.200	32	5	1.865	0.594	0.471	−0.280	0.423
	TUMXLv7.97	32	2	1.853	0.344	0.468	0.253	0.354
	TUMXLv7.74	32	2	1.438	0.313	0.310	−0.026	0.258
	Mean	32.000	3.818	2.123	0.460	0.463	−0.009	0.443
20170505	TUMXLv10.207	32	2	1.753	0.313	0.437	0.273	0.338
	Pvan1815	32	8	4.501	0.656	0.790	0.156	0.745
	TUMXLv7.121	32	5	2.656	0.563	0.633	0.098	0.584
	TUMXLv10.312	31	4	3.654	0.806	0.738	−0.110	0.676
	TUMXLv10.284	31	4	2.619	0.645	0.628	−0.044	0.566
	TUMXLv10.291	32	1	1.000	0.000	0.000	N/A	N/A
	TUMXLv10.255	32	2	2.000	1.000	0.508	−1.000	0.375
	TUMXLv10.364	32	3	1.979	0.344	0.502	0.305	0.444
	TUMXLv10.200	32	4	1.870	0.594	0.473	−0.276	0.425
	TUMXLv7.97	32	3	2.169	0.344	0.548	0.362	0.447
	TUMXLv7.74	32	2	1.600	0.250	0.381	0.333	0.305
	Mean	31.818	3.455	2.346	0.501	0.513	0.010	0.490
20180505	TUMXLv10.207	32	4	2.538	0.469	0.616	0.226	0.547
	Pvan1815	32	7	3.568	0.625	0.731	0.132	0.686
	TUMXLv7.121	32	4	3.442	0.625	0.721	0.119	0.659
	TUMXLv10.312	32	4	3.537	0.750	0.729	−0.046	0.666
	TUMXLv10.284	32	4	2.335	0.563	0.581	0.016	0.516
	TUMXLv10.291	32	5	1.380	0.094	0.280	0.660	0.259
	TUMXLv10.255	32	5	2.183	0.938	0.551	−0.730	0.441
	TUMXLv10.364	32	2	1.438	0.375	0.310	−0.231	0.258
	TUMXLv10.200	32	3	1.331	0.281	0.252	−0.132	0.230
	TUMXLv7.97	32	3	1.743	0.469	0.433	−0.100	0.348
	TUMXLv7.74	32	2	1.398	0.281	0.289	0.012	0.244
	Mean	32.000	3.909	2.263	0.497	0.499	−0.007	0.441

Table 2. *Cont.*

Strain	Locus	*N*	*Na*	*Ne*	H_o	H_e	F_{is}	*PIC*
20190505	TUMXLv10.207	32	3	1.809	0.500	0.454	−0.118	0.371
	Pvan1815	32	8	4.223	0.313	0.775	0.591	0.736
	TUMXLv7.121	32	3	1.208	0.125	0.175	0.275	0.162
	TUMXLv10.312	32	4	3.396	0.656	0.717	0.070	0.651
	TUMXLv10.284	32	6	2.994	0.938	0.677	−0.408	0.617
	TUMXLv10.291	32	4	1.136	0.063	0.122	0.478	0.118
	TUMXLv10.255	32	3	2.071	0.781	0.525	−0.511	0.434
	TUMXLv10.364	32	1	1.000	0.000	0.000	N/A	N/A
	TUMXLv10.200	32	4	1.581	0.438	0.374	−0.190	0.341
	TUMXLv7.97	32	5	2.622	0.594	0.628	0.040	0.586
	TUMXLv7.74	32	2	1.983	0.594	0.503	−0.198	0.373
	Mean	32.000	3.909	2.184	0.455	0.450	0.003	0.439
20180808	TUMXLv10.207	32	5	2.698	0.469	0.639	0.255	0.560
	Pvan1815	32	7	4.491	0.594	0.790	0.236	0.746
	TUMXLv7.121	32	3	1.629	0.406	0.392	−0.052	0.353
	TUMXLv10.312	32	3	2.848	0.813	0.659	−0.252	0.574
	TUMXLv10.284	32	6	4.452	0.781	0.788	−0.008	0.740
	TUMXLv10.291	32	2	1.032	0.031	0.031	−0.016	0.031
	TUMXLv10.255	32	3	2.186	0.906	0.551	−0.671	0.438
	TUMXLv10.364	32	3	1.627	0.219	0.391	0.432	0.352
	TUMXLv10.200	32	3	1.135	0.125	0.121	−0.053	0.115
	TUMXLv7.97	32	6	1.853	0.313	0.468	0.321	0.436
	TUMXLv7.74	32	2	1.932	0.500	0.490	−0.036	0.366
	Mean	32.000	3.909	2.353	0.469	0.484	0.014	0.428
20190808	TUMXLv10.207	32	6	2.424	0.406	0.597	0.308	0.556
	Pvan1815	32	14	7.557	0.469	0.881	0.460	0.856
	TUMXLv7.121	32	8	2.525	0.563	0.614	0.069	0.572
	TUMXLv10.312	32	4	2.786	0.719	0.651	−0.121	0.572
	TUMXLv10.284	32	7	3.690	0.813	0.741	−0.115	0.685
	TUMXLv10.291	32	1	1.000	0.000	0.000	N/A	N/A
	TUMXLv10.255	32	4	2.395	0.906	0.592	−0.556	0.495
	TUMXLv10.364	32	3	1.415	0.281	0.298	0.042	0.265
	TUMXLv10.200	32	3	1.099	0.094	0.092	−0.038	0.088
	TUMXLv7.97	32	6	2.498	0.438	0.609	0.270	0.554
	TUMXLv7.74	32	2	1.822	0.563	0.458	−0.247	0.349
	Mean	32.000	5.273	2.656	0.477	0.503	0.007	0.499
20180909	TUMXLv10.207	32.000	4.000	2.727	0.406	0.643	0.359	0.561
	Pvan1815	32	7	4.719	0.563	0.801	0.286	0.758
	TUMXLv7.121	32	4	1.801	0.406	0.452	0.087	0.404
	TUMXLv10.312	32	3	2.817	0.625	0.655	0.031	0.570
	TUMXLv10.284	32	6	4.911	0.625	0.809	0.215	0.765
	TUMXLv10.291	32	2	1.064	0.000	0.062	1.000	0.058
	TUMXLv10.255	32	3	2.176	0.875	0.549	−0.619	0.437
	TUMXLv10.364	32	3	1.331	0.094	0.252	0.623	0.230
	TUMXLv10.200	32	4	1.595	0.438	0.379	−0.173	0.353
	TUMXLv7.97	32	6	1.858	0.406	0.469	0.121	0.439
	TUMXLv7.74	32	2	1.853	0.469	0.468	−0.018	0.354
	Mean	32.000	4.000	2.441	0.446	0.504	0.174	0.448
20190909	TUMXLv10.207	32	4	2.407	0.594	0.594	−0.016	0.519
	Pvan1815	32	11	6.380	0.344	0.857	0.592	0.825
	TUMXLv7.121	32	6	2.158	0.469	0.545	0.126	0.508
	TUMXLv10.312	32	5	3.098	0.656	0.688	0.031	0.618
	TUMXLv10.284	32	6	3.969	0.844	0.760	−0.128	0.706

Table 2. *Cont.*

Strain	Locus	*N*	*Na*	*Ne*	H_o	H_e	F_{is}	*PIC*
20190909	TUMXLv10.291	32	2	1.032	0.031	0.031	−0.016	0.031
	TUMXLv10.255	32	4	2.844	0.906	0.659	−0.398	0.581
	TUMXLv10.364	32	2	1.205	0.188	0.173	−0.103	0.156
	TUMXLv10.200	32	4	1.339	0.281	0.257	−0.110	0.242
	TUMXLv7.97	32	6	2.876	0.375	0.663	0.425	0.601
	TUMXLv7.74	32	3	2.050	0.500	0.520	0.024	0.397
	Mean	32.000	4.818	2.669	0.472	0.522	0.039	0.471
20171010	TUMXLv10.207	32	4	1.830	0.313	0.461	0.311	0.422
	Pvan1815	32	12	7.447	0.563	0.879	0.350	0.851
	TUMXLv7.121	32	6	2.606	0.594	0.626	0.036	0.563
	TUMXLv10.312	32	5	3.215	0.750	0.700	−0.089	0.633
	TUMXLv10.284	32	6	4.008	0.719	0.762	0.042	0.710
	TUMXLv10.291	32	3	1.919	0.188	0.487	0.609	0.401
	TUMXLv10.255	32	3	2.817	0.719	0.655	−0.114	0.570
	TUMXLv10.364	32	3	1.210	0.188	0.177	−0.079	0.166
	TUMXLv10.200	31	5	1.453	0.290	0.317	0.068	0.295
	TUMXLv7.97	32	8	2.619	0.406	0.628	0.343	0.597
	TUMXLv7.74	32	3	2.022	0.344	0.513	0.320	0.393
	Mean	31.909	5.273	2.831	0.461	0.564	0.163	0.509
20181010	TUMXLv10.207	32	3	2.293	0.438	0.573	0.224	0.485
	Pvan1815	32	10	4.655	0.531	0.798	0.323	0.756
	TUMXLv7.121	32	9	1.960	0.531	0.498	−0.085	0.474
	TUMXLv10.312	32	4	2.738	0.750	0.645	−0.182	0.571
	TUMXLv10.284	32	7	4.267	0.813	0.778	−0.061	0.728
	TUMXLv10.291	32	2	1.280	0.000	0.222	1.000	0.195
	TUMXLv10.255	32	2	1.998	0.969	0.507	−0.939	0.375
	TUMXLv10.364	32	3	1.697	0.156	0.417	0.620	0.357
	TUMXLv10.200	32	4	1.339	0.219	0.257	0.135	0.241
	TUMXLv7.97	32	7	1.845	0.344	0.465	0.249	0.431
	TUMXLv7.74	32	3	2.163	0.563	0.546	−0.046	0.435
	Mean	32.000	4.909	2.385	0.483	0.519	0.113	0.459
20191010	TUMXLv10.207	32	3	2.107	0.313	0.534	0.405	0.416
	Pvan1815	32	8	2.926	0.375	0.669	0.430	0.616
	TUMXLv7.121	32	4	1.958	0.656	0.497	−0.341	0.435
	TUMXLv10.312	32	4	3.465	0.906	0.723	−0.274	0.660
	TUMXLv10.284	32	5	3.543	0.875	0.729	−0.219	0.668
	TUMXLv10.291	32	2	1.438	0.000	0.310	1.000	0.258
	TUMXLv10.255	32	2	1.969	0.875	0.500	−0.778	0.371
	TUMXLv10.364	32	3	2.169	0.500	0.548	0.072	0.446
	TUMXLv10.200	32	4	1.514	0.406	0.345	−0.197	0.307
	TUMXLv7.97	32	4	2.258	0.781	0.566	−0.402	0.470
	TUMXLv7.74	32	3	1.820	0.625	0.458	−0.387	0.363
	Mean	32.000	3.818	2.288	0.574	0.534	−0.063	0.455

Na: number of alleles; *Ne*: number of effective alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; *PIC*: polymorphism information content.

The results showed that in the five generations of line 5, the *Na*, *Ne*, H_e, and *PIC* values ranged from 3.455 to 3.909, 2.123 to 2.346, 0.450 to 0.513, and 0.435 to 0.490, respectively. In the three generations of line 8, the averages for *Na*, *Ne*, H_e, and *PIC* ranged from 3.909 to 5.273, 2.353 to 2.656, 0.484 to 0.523, and 0.428 to 0.509, respectively. In the three generations of line 9, the averages of *Na*, *Ne*, H_e, and *PIC* ranged from 4.000 to 4.818, 2.441 to 2.669, 0.504 to 0.535, and 0.448 to 0.471, respectively. In the four generations of line 10, the averages of *Na*, *Ne*, H_e, and *PIC* ranged from 3.818 to 5.273, 2.288 to 2.831, 0.519 to 0.566, and 0.455 to 0.515, respectively. These results indicated a mid-level genetic diversity and a rich polymorphism information content of these core populations.

3.2. Genetic Relationships among Populations

The UPGMA cluster trees of the four selected populations based on genetic distance are shown in Figure 1. The results showed that the genetic distances among breeding generations of line 5 and other lines were the largest, clustering into a single group at present. By contrast, the other three breeding lines showed significant genetic differences, providing a basis for the study of cross-breeding between lines and the utilization of heterosis. At present, no genetic markers have been found to distinguish the four lines, but the number of alleles in each line at pvan1815 was different, and the numbers of alleles in lines 8 and 9 were significantly higher than those in line 10 groups.

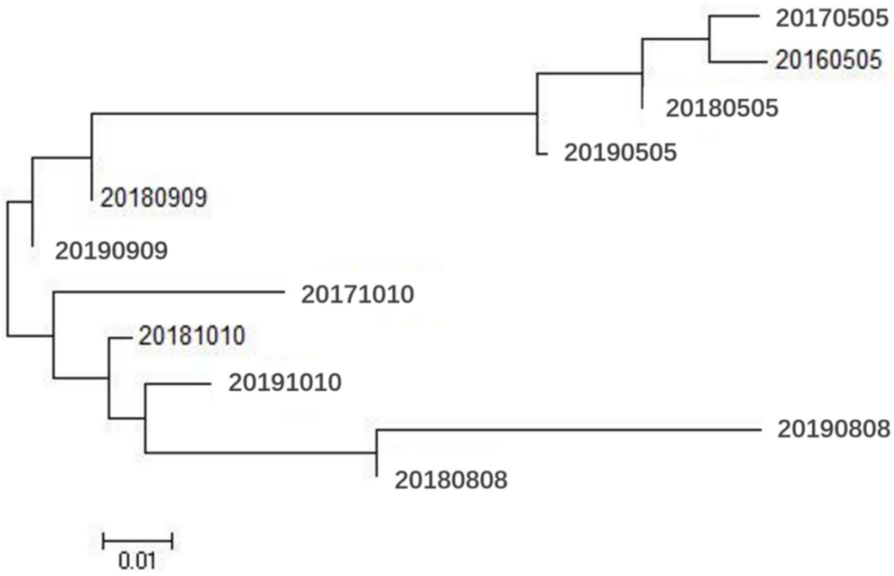

Figure 1. UPGMA tree for the four selected strains of the whiteleg shrimp based on Nei's genetic distance.

4. Discussion

The assessment of genetic diversity is essential for the conservation and utilization of germplasm resources. In selective breeding, genes related to target traits are often retained, while adverse alleles tend to be eliminated, resulting in decreased genetic diversity. Furthermore, unbalanced parental contributions and small effective population sizes are associated with selective breeding programs for shrimp promote inbreeding, leading to a rapid decline in genetic diversity and inbreeding depression [29]. Therefore, monitoring the genetic diversity and understanding changes in the genetic structure in generations of different hatchery stocks is important to improve management strategies for shrimp breeding programs.

Differences in H_o, H_e, and *PIC* values reflect differences in the included accessions, as well as the polymorphism of the included SSR markers. In this study, H_o, H_e, and *PIC* values for the four selected strains ranged from 0.446 to 0.574, 0.450 to 0.566, and 0.435 to 0.509, respectively. Compared with previous reports on the genetic diversity of the whiteleg shrimp *L. vannamei*, similar levels of these same parameters were reported by Huang et al. [21] for the first filial generation of seven introduced populations in Guangdong, China (H_o: 0.370–0.505; H_e: 0.477–0.670; *PIC*: 0.414–0.623). The levels of genetic diversity seemed slightly higher than those of four selected stocks (H_o: 0.3833–0.4444; H_e: 0.4214–05289; *PIC*: 0.3813–0.4478) in Zhanjiang, China [30], as well as nine breeding populations (H_o: 0.2225–0.3662; H_e: 0.3317–0.4539) in Shanghai, China [31]. However, the parameter values in this study were lower than those reported by Lima et al. [32], Zhang et al. [33], and Ren et al. [18], with the range of variation in H_e being 0.632–0.709, 0.600–0.802, and 0.58–0.78, respectively. This analysis suggests that each of the selected strains maintained a moderate level of genetic diversity. In addition, most of the strains in this study had $H_e > H_o$,

indicating that a certain degree of inbreeding already exists within the population, which reminds us that the richness of the base population can be appropriately increased during artificial selection to avoid the loss of genetic diversity.

H_e and *PIC* are optimal parameters for measuring the genetic diversity of a population at multiple loci. In this study, H_e and *PIC* in the 2017–2019 generations of line 5 showed a decreasing trend from year to year, with a total decrease rate of about 0.6 over the three years. In lines 8, 9, and 10, the values of H_e and *PIC* in consecutive generations fluctuated, and H_e was greater than 0.5 in all lines except line 8 (0.484) in 2018, indicating a relatively stable level of genetic diversity. Loss of genetic diversity during selective breeding has been previously observed in *P. monodon* [34], *P. chinensis* [19], and *Macrobrachium rosenbergii* [35]. Sbordoni et al. [29] reported an evident reduction in genetic variation over time in hatchery stocks of *P. japonicus* using allozyme markers, with H_o decreasing from 0.102 in F_1 to 0.039 in F_6. The result was attributed to a bottleneck effect resulting from an effective breeding population as low as four. Smaller breeding population sizes and higher selection intensity may increase genetic gains but can also magnify sibship among broodstock, resulting in inbreeding and a loss of genetic diversity. This, in turn, can reduce resistance to environmental fluctuations and the potential for future generations to respond to selection pressures.

The inbreeding coefficients (F_{is}) for the four strains were mostly positive (except for groups 20180505 and 20191010), but they were not significantly different from zero, suggesting relatively low degrees of inbreeding in each group. These results for F_{is} also indicate the maintenance of a moderate level of genetic diversity. More significant F_{is} values have been reported by Valles-Jimenez et al. [36] in wild populations of *L. vannamei* from Mexico to Panama (F_{is} = 0.533) and by Atencia-Galindo et al. [17] in natural populations of *P. notialis* in Colombia (F_{is}: 0.597–0.720). Tong et al. [37,38] have reported germplasm degradation in successive generations as a result of the gradual loss of genetic diversity and relatively high levels of inbreeding in *L. vannamei*, as evidenced by size inequality and slow growth. In this study, we noted that F_{is} in each generation of line 10 was significantly higher than in the other three lines, indicating that inbreeding and a loss of genetic diversity may occur more easily in this strain. Therefore, to maintain the level of genetic diversity, especially for lines 5 and 10, it will be necessary to preserve a sufficient number of effective broodstock using a balanced sex ratio, a large broodstock size, and low selection pressure in the selective breeding program [39]. The breeding can also be improved by introducing an outgroup with high genetic diversity for hybridization.

Broadcast spawning in commercial hatcheries and unequal parental contributions increase the risk of unintentional inbreeding in aquatic animals, which can adversely affect production [2,40]. It has been reported that genetic diversity is lost when the effective number of parents is reduced to <100 [24,41]. Furthermore, artificial and natural selection in the culture environment may have altered the overall allelic composition of the farmed populations [42,43]. The present results indicate that the selected strains have lost considerable genetic variation during domestication. A 22–44% loss of alleles has been reported for hatchery-cultured silver-lipped pearl oysters, *Pinctada maxima* [2]. Substantial losses in genetic diversity in long-term mass selection or hatchery lines have also been observed in the Eastern oyster [44,45] the Pacific oyster [46], and cultured abalones [47–49]. Several factors, such as null alleles, nonrandom mating, admixture of independent populations, or artificial and natural selection during seed production and cultivation, could lead to heterozygote deficiency. The Wahlund effect refers to the reduction in observed heterozygosity caused by (cryptic) population substructure [50]. In the presence of the Wahlund effect, the power to detect heterozygote deficits depends on the effective population size, migration rate, the number of demes being pooled, and the sample size per deme [51].

The idea that highly fecund marine animals might have smaller-than-expected effective population sizes due to high variance in individual reproductive success has been described as sweepstake reproductive success (SRS) [52]. Reproductive success in marine organisms may at times resemble a sweepstake lottery, in which there are a few big winners and

many losers. SRS, which is conceptually distinct from a bottleneck, produces a dynamic, mutation-drift equilibrium driven by the environmental vagaries of reproductive success and maintains much less genetic diversity than expected based on a large census size. Recent studies have found evidence for reduced variation within cohorts and enhanced variation among cohorts compared to variation among adult populations [52,53]. This pattern would increase the impact of inbreeding depression at the farm level during aquaculture. The results suggested a slight but not significant decline in heterozygosity and a reduction in differentiation and variation in the genetic structure over time under increased selection.

Several strategies exist concerning how to maintain the genetic diversity of the nucleus population. Cross-breeding with wild animals may be an effective method [54]. However, there are also distinct disadvantages. Wild animals are genetically unimproved, and thus their addition to the breeding nucleus could degrade the genetic selection responses already achieved, and the introduction may introduce pathogens [55]. Another method was suggested by Knibb [56]. In his plan, a line is divided into various sublines, and although there is a loss of variation in each subline, the loss is for different alleles or haplotypes. Overall, across the different sublines, variations approaching that in the ancestral population can be retained. In addition, the decrease in genetic variation may be delayed by lower selection intensity. A previous study on a species of clam (*Meretrix petechialis*) showed that observed heterozygosity was reduced by 11.48% after one generation of selection using a selection intensity of the top 15%, and this was alleviated under lower selection intensity (top 30%) over four generations [57,58].

5. Conclusions

In conclusion, the present study has revealed that artificial selection negatively influences genetic variation and differentiation in cultured populations and the four selected lines had apparent genetic differences. These findings indicate the importance of balancing the relationship between enhancing economic production traits and maintaining sufficient genetic diversity in a selective breeding program for aquatic animals. Cross-breeding and utilization of heterosis might be an efficient method for the shrimp breeding program in China.

Author Contributions: Conceptualization, W.Y. and Z.Y.; methodology, C.L.; investigation, Z.Z.; data curation, K.L.; supervision, W.Y.; project administration, Z.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants from the seed industry innovation and industrialization project in Fujian Province (2017FJSCZY02).

Institutional Review Board Statement: The sample collection and experimental protocols were approved by the Animal Care and Use Committee of the Fisheries Research Institute of Fujian (Animal Ethics No. 2021-2). All animal handling and methods were performed according to the relevant guidelines.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors acknowledge help from Wenchao Yu with the data collection and manuscript preparation.

Conflicts of Interest: The authors declare no conflict of interest.

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