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Genetic diversity of three consecutive selective breeding generations in *Pseudobagrus vachellii* (Actinopterygii: Siluriformes: Bagridae)

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Abstract

Pseudobagrus vachellii (Richardson, 1846) is a commercially important freshwater fish species in China. To understand the effects of artificial breeding on the genetic diversity of three consecutive *P. vachellii* breeding populations (F1, F2, and F3) since 2012, a genetic analysis was conducted using polymorphic microsatellite markers. The mean allele number, expected heterozygosity, observed heterozygosity, and the polymorphic information content from generation F1 to F3 decreased from 7.75 to 5.63, from 0.77 to 0.63, from 0.83 to 0.77, and from 0.72 to 0.58, respectively. Analysis of molecular variance showed greater genetic divergence within the three generations (93.67%) than that among the generations (6.33%), and the overall differentiation level was moderate. Additionally, the lowest genetic differentiation was between F2 and F3 (F_{st} = 0.0484), and the highest was between F1 and F3 (F_{st} = 0.12864). Inbreeding occurred in each generation and was the highest in generation F3. Structural analysis showed that the three *P. vachellii* generations were most likely divided into two different genetic clusters. Although genetic diversity declined slightly in the mass selection lines after three breeding generations, overall genetic diversity was maintained at a relatively high level. To minimize the loss of genetic diversity and inbreeding in the subsequent breeding process, a moderate number of parents can be used for each generation. Information regarding the genetic diversity and structure of the selective *P. vachellii* breeding generations obtained in this study will be useful for future broodstock management and selective breeding programs.

Keywords

genetic diversity, genetic structure, microsatellites, *Pseudobagrus vachellii*, selective breeding

Introduction

Pseudobagrus vachellii (Richardson, 1846) represents the family Bagridae and is an endemic freshwater fish species widely distributed in China. However, wild populations have rapidly declined owing to habitat destruction, water pollution, and overfishing. As an edible fish, it has the largest body size and is the fastest-growing group in the genus *Pseudobagrus*. Moreover, it is the male parent of the hybrid yellow catfish "Huangyou 1" (GS-02-001-2018). *Pseudobagrus vachellii* possesses high nutritional value and has a great taste, with low bone amounts in the muscle (Zheng et al. 2021). However, long farming periods can result in problems such as slow growth rate, differences in morphological traits, mottling of body color, short and thick body shapes (Fig. 1), and decreased stress resistance in the retained population. These problems greatly affect the market value of *P. vachellii*.

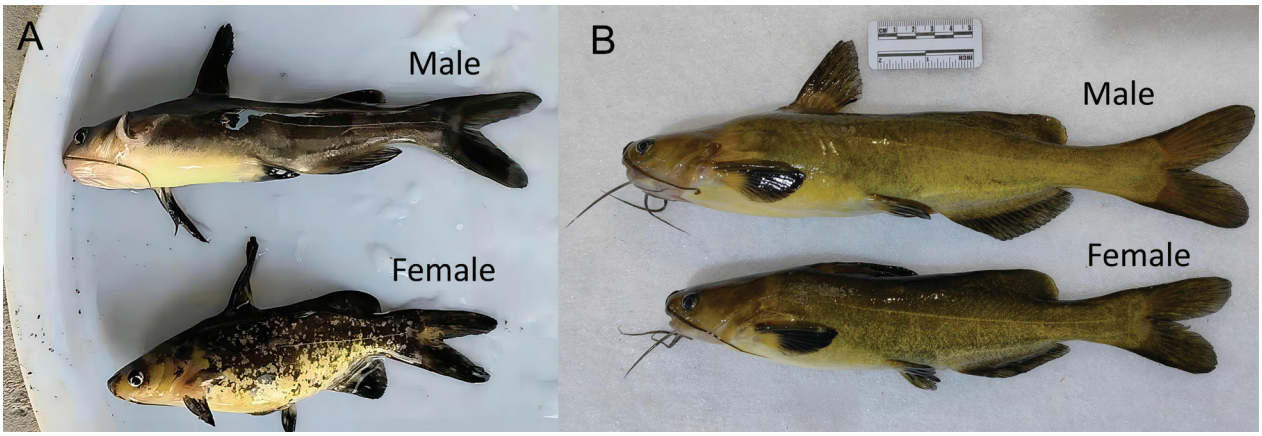

Figure 1. Comparison between *Pseudobagrus vachellii* populations. **(A)** Poor germplasm population and **(B)** mass-selected population by our project team.

Artificial selective breeding is an effective way to improve performance traits, breed excellent aquatic varieties, and increase productivity; however, it also decreases genetic diversity in breeding populations (Gjedrem et al. 2012; Chen et al. 2017; Phuc et al. 2021; Swain et al. 2022). As a raw material, for artificial selection in captive populations, maintaining the genetic diversity of the breeding population is a prerequisite for the continuous utilization of aquatic germplasm resources, and genetic assessments should be conducted periodically (Diyie et al. 2021). For many fish species, usually in the late stages of artificial selection breeding, the number of breeding individuals gradually decreases, which leads to increased inbreeding, decreased population genetic diversity, and loss of genetic variability in artificially bred seed populations. This eventually leads to the loss of product development and utilization (Ortega-Villaizan et al. 2011; Liu et al. 2018).

Because of the long-term artificial breeding of *Pseudobagrus vachellii*, nonstandard conservation, and germplasm degradation have occurred in nursery farms. The mass selection program of *P. vachellii* derived from the wild Huai River and Yangtze River populations for growth traits and morphological characteristics has been conducted since 2012 for three generations. Compared with that of the unselected *P. vachellii*, the growth rate increased by 15 percentage points, which showed obvious advantages (Duan et al. 2023). However, the changes in the genetic variation and diversity among these three breeding generations are unknown.

The microsatellite marker technique is a sensitive, simple, and efficient method for studying the genetic diversity of aquatic animals (Fu et al. 2017). Some microsatellite markers have been successfully developed for the genetic analysis of *P. vachellii* (see Wang et al. 2021; Zheng et al. 2020). Eight polymorphic microsatellite loci were used to monitor changes in genetic diversity and structure during the selection process in this study. This useful information can be used to evaluate whether the levels of variation are appropriate for a long-term selective breeding program. Moreover, it provides guidelines for avoiding genetic variation loss and inbreeding for future *P. vachellii* selective breeding programs and promotes the sustainable and healthy development of the *P. vachellii* industry.

Materials and methods

Mass selection process and pond culture management. In our selective breeding program, three generations of *Pseudobagrus vachellii* were produced between 2012 and 2021 at the Fishery Research Institute of the Anhui Academy of Agricultural Sciences in Hefei, Anhui, China (Fig. 2). From the founder group comprising more than 800 *P. vachellii* individuals, 80 individuals with large sizes and similar morphological traits were screened as breeding parents. The female-to-male ratio was approximately 1:1, and they were artificially bred in May 2014. Approximately 300 000 F1-generation hatchlings, which were cultivated using natural bait in the ponds (rotifers, branchiostomatids, copepods, etc.) for one week, were fed compound feed powder (with a protein content of

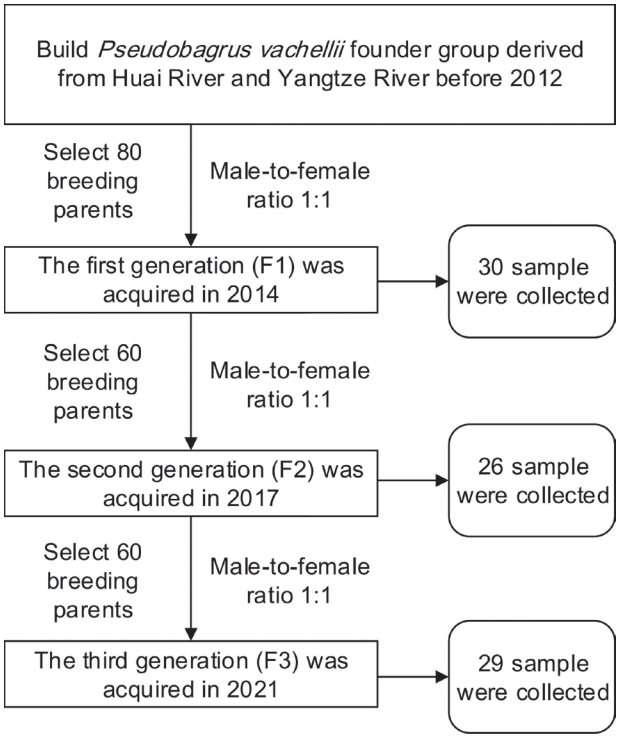

Figure 2. Mass selection of *Pseudobagrus vachellii* and sample collection.

45%) for cultivation and then changed to compound feed granules (with a protein content of 40%–45%). When the fish were half a month old, they were fed compound feed pellets (protein content of 40%–45%), and selection was carried out at 1 month, 12 months, and 32 months of age, respectively, using growth rate and morphological traits as the main selection indexes. Healthy and disease-free individuals with fast growth rates and similar morphological traits were selected and retained for cultivation. In June 2017, 60 breeding parents with a good degree of sexual maturity and morphology were selected from the F1 generation at a male-to-female ratio of approximately 1:1. They were then artificially inseminated to obtain approximately 220 000 F2 generation first hatchery fry. The fry were bred in the same way as the F1 generation and were selected at one, 12, and 32 months of age, using growth rate and morphological traits as the selection indices. In June 2021, 60 breeding parents with a good degree of sexual maturity and morphology were selected from the F2 generation at a female-to-male ratio of approximately 1:1. Artificial insemination was then performed to obtain approximately 220 000 fry in the F3 generation. The fry cultivation method was the same as that of the F1 generation, and selection was carried out at 1 month and 12 months of age, respectively, using growth rate, morphological traits, and hypoxia tolerance as selection indices. The number of individuals in the selection group was then approximately 7500. The female breeding parents of the F1, F2, and F3 generation all weighed over 150 g and the males weighed not less than 250 g. During breeding, oxytocin was injected artificially, and after the effect time was reached, insemination was performed by squeezing the eggs artificially, and the fertilized eggs were incubated on a mesh. Each female fish produced 6000~9000 eggs.

Fish materials and sample collection. The care and use of experimental animals in this study complied with the guidelines and policies approved by the Experimental Animal Welfare and Ethical Committee of the Anhui Academy of Agricultural Sciences. Twenty-six to thirty individuals from each generation were sampled randomly, and 85 unrelated individuals were randomly selected from each generation (Fig. 2). The fish body length was 31.77 ± 9.99 cm and the body weight was 434.24 ± 291.48 g. Muscle tissue samples were quickly removed, cleaned with 0.70% physiological saline, and stored at 4°C in 95% ethanol for subsequent experiments.

DNA extraction and genotyping. Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Tiangen, Beijing, China) following the manufacturer's instructions. A panel of eight microsatellite markers previously developed for *Pseudobagrus vachellii* were amplified using polymerase chain reaction (PCR) at annealing temperatures (Zheng et al. 2020; Wang et al. 2021) (Table 1). 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^{-1}) deoxynucleotide triphosphates, 1 μL (10 μmol · L^{-1}) of each forward and reverse primer, 0.3 μL (5 U · μL^{-1}) of Taq DNA polymerase (Transgen, Beijing, China), and 1–2 μL (50 ng · μL^{-1}) of template DNA that was added to 30 μL double-distilled H_2O. Temperature profiles for the PCR consisted of an initial denaturation at 94°C for 5 min, 31–34 cycles of 94°C for 30 s, annealing at primer-specific temperatures (53–60°C) for 40 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min (Wang et al. 2022). The PCR products were separated and sized on an ABI 3730xl automated sequencer with a ROX 500 size standard, and the resulting genotype traces were 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).

Table 1. Summary of microsatellite loci details for *Pseudobagrus vachellii*.

Locus	Primer sequence (5′→3′)	Motif	Size range [bp]	Reference
PV1	TAATGCATTTTCTGCTGCCA CACACGGGGGATGAATTAAG	AGATG	127–152	Wang et al. 2021
PV2	GAAACCCGACTCTGTCAAGG TGAGGGCTAGAAAGGGACAA	TGA	226–283	Wang et al. 2021
PV4	CAGAGGCATTTCTCAGAGGC CAGGTTGCAGGTACTGTCCA	CAAT	168–208	Wang et al. 2021
PV6	TTGCCGTAGTATCGGCTACC TAAGGGGTTCGGATGTGAAG	ATTG	160–192	Wang et al. 2021
PV7	TCGACTGCTGTTTATCCGTCT CGATAAACTTTCGCAGACCC	AAC	248–275	Wang et al. 2021
PV9	AGTCAGGTTGTATGCCCACC ACAGGGAAAGAGACGTGCAT	GAAT	183–215	Wang et al. 2021
PV12	TAATGCATTTTCTGCTGCCA CACACGGGGGATGAATTAAG	AGATG	127–152	Wang et al. 2021
Y73	GCTTTCTTGATGCAACCCAG TGGATATTGACGAGTTCCATGT	CATA	118–138	Zheng et al. 2020

Data analysis. The microsatellite data were analyzed using web-based Genepop software (http://genepop.curtin.edu.au/), with Markov chain parameters of 10 000 dememorizations, 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 diversity index (I), and Nei's genetic distance (D_a) values were calculated using Popgene 1.32, respectively (Nei 1972; Yeh et al. 1997). The genetic differentiation coefficient (F_{st}) is one of the most widely used descriptive statistics for evaluating genetic differentiation between and among populations and can provide important insights into the evolutionary processes that influence genetic variation among populations (Holsinger and Weir 2009). According to the rule by Wright (1965), an F_{st} value of 0.000–0.049 represents low differentiation, values of 0.05–0.25 indicate moderate differentiation and values higher than 0.25 indicate high differentiation among populations. The F_{st} and genetic variation were analyzed using analysis of molecular vari-

ance (AMOVA) with Arlequin 3.5 software (Excoffier and Lischer 2010). An unweighted pair group method with arithmetic mean (UPGMA) phylogenetic tree based on Nei's genetic distance was constructed using MEGA software (version 7.0) (Kumar et al. 2016). The polymorphism information content (PIC) of each locus and population was calculated using Cervus 3.0 (Kalinowski et al. 2007). The genotypes determined using COANCESTRY (V1.0.1.1; Wang 2011) were used to measure relatedness estimates (R) between generations and within-generation genotypes as described by Wang (2002), and the inbreeding coefficient (F) was determined as described by Ritland (1996). Microsatellite data were also analyzed using the STRUCTURE 2.3.3 program and the admixture model was used to estimate population genetic structure among and within species (Evanno et al. 2005). We conducted an analysis with ten iterations for each population size (K) of one–eight, and with the Markov chain Monte Carlo running for 500 000 iterations and an initial burn-in of 100 000 iterations. The K values described by Evanno et al. (2005) were calculated to identify the most reasonable K using the Structure Harvester program (Earl and vonHoldt 2012). The runs were averaged using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007), and the results were visualized using DISTRUCT version 1.1 (Rosenberg 2004).

Results

Summary statistics. No evidence of allelic stutter or large allele dropouts was found in the dataset, and no null alleles were detected at any of the eight loci. Almost all eight loci were highly polymorphic (PIC > 0.5) (Botstein et al. 1980). In this study, microsatellite markers were used for the genetic analysis of three consecutive *P. vachellii* selective breeding generations. The genetic indicators of the eight microsatellite loci are listed in Table 2. The mean observed H_o was 0.80, the mean expected H_e was 0.7506, the mean N_a per locus was 8.625, the effective N_e was 4.448, and I was 1.6396. A comparison of the genetic information of three consecutive selective breeding generations of *P. vachellii* is shown in Table 3. A relatively high level of overall genetic diversity was observed (H_e = 0.6323–0.7663, PIC = 0.5754–0.7183), whereas the number of microsatellite alleles (N_a, N_e), heterozygosity (H_o, H_e), and PIC decreased slightly in the mass selection lines.

Genetic variation and differentiation among generations. AMOVA revealed that the variation among populations was only 6.33%, whereas the variation within populations was 93.67%. Wright (1965) proposed that $F_{st} < 0.05$ indicated low differentiation, $0.05 < F_{st} <$

Table 2. Genetic information of three consecutive *Pseudobagrus vachellii* selective breeding generations based on microsatellite markers.

Locus	Generation	H_o	H_e	PIC	N_a	N_e	I	P_{HW}
PV1	F1	0.8667	0.7701	0.7196	6	4.1190	1.5634	0.8594
	F2	0.8846	0.7006	0.6389	5	3.1962	1.3135	0.2015
	F3	0.5172	0.4398	0.5753	6	1.7613	0.9377	1.0000
	Total	0.7529	0.6788	0.6340	6	3.0751	1.3707	0.7430
PV2	F1	0.8667	0.8582	0.8274	12	6.4057	2.0987	0.0453^{P1}
	F2	0.7692	0.8303	0.7901	10	5.3865	1.8958	0.4104
	F3	1.0000	0.8234	0.4121	8	5.2399	1.7889	0.0817
	Total	0.8824	0.8940	0.8790	15	8.9863	2.3666	0.0433^{P1}
PV4	F1	0.8000	0.7989	0.7573	9	4.6632	1.7798	0.6263
	F2	0.6923	0.7504	0.7061	8	3.7871	1.6194	0.2022
	F3	0.7586	0.6479	0.7827	4	2.7529	1.1643	0.2692
	Total	0.7529	0.7513	0.7130	9	3.9513	1.6352	0.3439
PV6	F1	0.8000	0.7797	0.7340	8	4.2857	1.6859	0.2693
	F2	0.9615	0.8499	0.8138	9	6.0089	1.9566	0.9566
	F3	0.9655	0.8088	0.5814	7	4.8754	1.7118	0.0036^{P2}
	Total	0.9059	0.8315	0.8040	10	5.7662	1.9079	0.0303^{P1}
PV7	F1	0.9667	0.7073	0.6456	7	3.2847	1.4120	0.0170^{P1}
	F2	1.0000	0.6350	0.5457	4	2.6510	1.0729	0.0000^{P3}
	F3	1.0000	0.5420	0.7647	3	2.1399	0.8192	0.0000^{P3}
	Total	0.9882	0.7477	0.7000	8	3.8959	1.5027	0.0000^{P3}
PV9	F1	0.7333	0.7062	0.6587	8	3.2727	1.5158	0.4576
	F2	0.6154	0.6719	0.6279	8	2.9328	1.4405	0.2814
	F3	0.7931	0.7060	0.4230	6	3.2660	1.4314	0.8142
	Total	0.7176	0.7009	0.6690	9	3.2983	1.5740	0.6080
PV12	F1	0.8667	0.7701	0.7196	6	4.1190	1.5634	0.8605
	F2	0.8846	0.7014	0.6399	5	3.2038	1.3152	0.1813
	F3	0.5172	0.4398	0.6566	6	1.7613	0.9377	1.0000
	Total	0.7529	0.6797	0.6360	6	3.0837	1.3733	0.7151
Y73	F1	0.7000	0.7401	0.6842	6	3.6735	1.4767	0.8230
	F2	0.6538	0.6900	0.6187	5	3.0938	1.2538	0.6961
	F3	0.5862	0.6503	0.4121	5	2.7710	1.1687	0.5595
	Total	0.6471	0.7208	0.6640	6	3.5278	1.3864	0.8927
Mean ± SD		0.8000 ± 0.1062	0.7506 ± 0.0713	0.7124 ± 0.0810	8.625 ± 2.826	4.4481 ± 1.8974	1.6396 ± 0.3223	/

H_o = observed heterozygosity, H_e = expected heterozygosity, PIC = mean polymorphism information content per locus, N_a = number of alleles, N_e = effective allele number, I = Shannon diversity index, P_{HW} = Hardy–Weinberg probability test; SD = standard deviation; ^{P1} = $P < 0.05$, ^{P2} = $P < 0.01$, ^{P3} = $P < 0.001$.

Table 3. Comparison of genetic information of three consecutive *Pseudobagrus vachellii* selective breeding generations.

Parameter	Generation		
	F1	F2	F3
n	30	26	29
N_a	7.750 ± 2.053	6.750 ± 2.25	5.625 ± 1.598
N_e	4.2279 ± 1.0049	3.7825 ± 1.2355	3.0709 ± 1.3356
I	1.6370 ± 0.2197	1.4835 ± 0.3146	1.2449 ± 0.3647
H_o	0.8250 ± 0.085	0.8077 ± 0.1454	0.7672 ± 0.2091
H_e	0.7663 ± 0.05	0.7287 ± 0.0761	0.6323 ± 0.1492
PIC	0.7183 ± 0.0542	0.6728 ± 0.0851	0.5754 ± 0.1425

n = number of fish specimens studied, N_a = number of alleles, N_e = effective allele number, I = Shannon diversity index, H_o = observed heterozygosity, H_e = expected heterozygosity, PIC = mean polymorphism information content per locus.

0.15 indicated moderate differentiation and $F_{st} > 0.15$ indicated high differentiation. The overall F_{st} value was 0.06329, which indicated a moderately differentiated degree ($0.05 < F_{st} < 0.15$) (Table 4). The F_{st} values for the three generations ranged from 0.0484–0.1286. The lowest genetic differentiation was observed between F2 and F3 ($F_{st} = 0.0484$) with the smallest genetic distance ($D_a = 0.142$), whereas the highest genetic differentiation was observed between F1 and F3 ($F_{st} = 0.1286$) with the largest genetic distance ($D_a = 0.4373$) (Table 5). The UPGMA phylogenetic tree based on Nei's genetic distance (Nei 1972) indicated that the three generations could divide the population into two clades. The analysis revealed that F2 and F3 formed sister relations and were clustered with F1 (Fig. 3). Structural analysis showed that $K = 2$ was the ideal number of subtypes; that is, three consecutive *Pseudobagrus vachellii* selective breeding generations were most likely to be divided into two different genetic clusters (Fig. 4). Different colors represent different genetic clusters in the figure, and the degree of gene purification increased with the development of breeding.

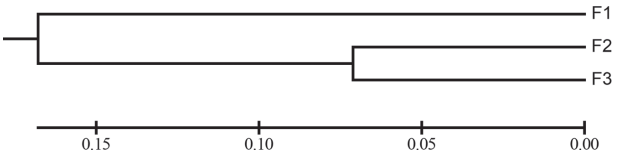

Figure 3. Nei's unweighted pair group method with arithmetic mean (UPGMA) tree of three consecutive *Pseudobagrus vachellii* selective breeding generations of based on microsatellites. Note: Scale bar denotes genetic distance.

Table 4. Analysis of molecular variance (AMOVA) results for three consecutive *Pseudobagrus vachellii* selective breeding generations using eight microsatellite loci.

Source of variation	DF	Sum of squares	Variance component	Percentage
Among populations	2	33.741	0.1934	8.26
Within populations	167	646.792	2.8619	91.74
Total	169	688.700	3.0553	100.00

Genetic differentiation (F_{st}) = 0.06329, DF = degrees of freedom.

Table 5. Genetic differentiation (F_{st}) values and Nei's genetic distance among three consecutive *Pseudobagrus vachellii* selective breeding generations.

Generation	F1	F2	F3
F1		0.05372	0.12864
F2	0.2336		0.04840
F3	0.4373	0.14210	

Note: Nei's genetic distance (below diagonal) and F_{st} (above diagonal).

Partner relatedness and inbreeding coefficient analysis. The results showed that the relation (R) and inbreeding coefficient (F) within each generation had positive values and were the largest in generation F3; the relatedness increased in succeeding generations (Table 6). The R value between generations was negative, except for in F2 and F3, which were positive. Although the F value among all three generations was negative compared to the relatedness between F1 and F2 (−0.08417), the relatedness between F1 and F3 decreased (−0.13296) (Table 7).

Table 6. Relation (R) and inbreeding coefficients (F) within each *Pseudobagrus vachellii* generation.

Coefficient	Generation		
	F1	F2	F3
R	0.01879	0.10225	0.28897
F	0.06303	0.01196	0.07394

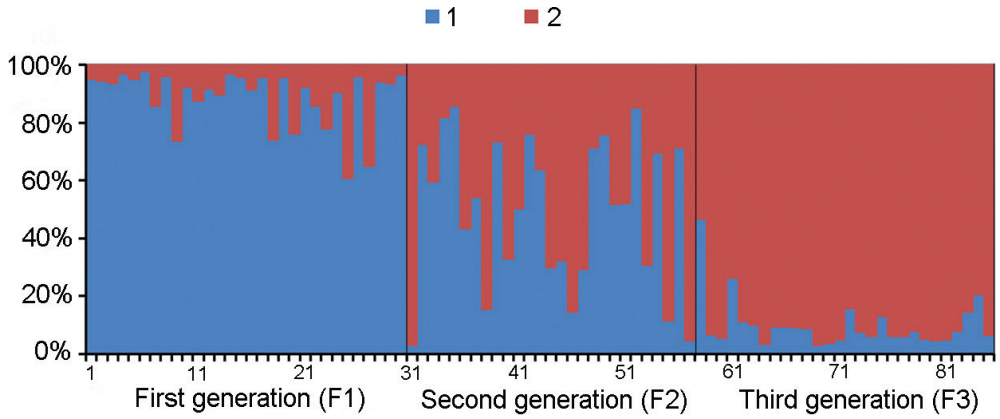

Figure 4. Population genetic structure of the three consecutive *Pseudobagrus vachellii* generations. The assignment results show that $K = 2$ (parameter introduced by Evanno et al. 2005). The two colors represent two different genetic clusters. The Y-axis denotes the proportion of ancestral components in an individual relative to other populations.

Table 7. Relation (*R*) and inbreeding coefficient (*F*) among three *Pseudobagrus vachellii* generations.

Generation	F1	F2	F3
F1		–0.08417	–0.13296
F2	–0.03138		0.09238
F3	–0.07943	–0.01061	

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

Discussion

Selection quickly improves certain traits, but the genetic diversity is usually lower than that of founder populations (Zhang et al. 2010). Many fish species have high fecundity and require relatively few parents to produce offspring; therefore, maintaining genetic diversity over successive generations is a recognized challenge for aquaculture breeding programs (Zhang et al. 2010; Ortega-Villaizan et al. 2011; Liu et al. 2018; Varney and Wilbur 2020). The presently reported study revealed that the genetic diversity of *Pseudobagrus vachellii* declined slightly after three generations of breeding. These results are similar to those obtained from other artificial breeding of aquatic animals. For example, a report showed that even when using a relatively large number of banana shrimp (*Penaeus merguiensis* De Man, 1888) broodstocks, a substantial loss of allelic diversity within lines over 14 generations is still observed (Knibb et al. 2014). Li et al. (2018) found a slight decrease in genetic diversity over three successive generations of early- and late-maturing strains of the Chinese mitten crab (*Eriocheir sinensis* Milne Edwards, 1853). In cultured silver-lipped pearl oysters, *Pinctada maxima* (Jameson, 1901), genetic diversity decreased, and the effective population size was reduced (Lind et al. 2009). High genetic diversity was observed among generations of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), in Ghana (Diyie et al. 2021), and Wang et al. (2022) reported that the genetic diversity of cultured *Procambarus clarkii* (Girard, 1852) tends to decline. These studies are important for ensuring the sustainability of the aquaculture industry.

Generally, $0.25 < PIC < 0.50$ meant that the single sequence repeat (SSR) loci were moderately polymorphic, and $PIC > 0.50$ meant that the SSR loci were highly polymorphic (Botstein et al. 1980). In the presently reported study, with an increase in breeding generations, the genetic diversity of the three artificially selected populations gradually decreased, and the PIC values were 0.7183, 0.6728, and 0.5754, respectively, indicating that the SSR loci were highly polymorphic. The overall number of alleles declined from 7.75 to 5.63, and a similar decline in the number of alleles was reported in previous studies (Zhang et al. 2018; Varney and Wilbur 2020). A study of three successive selection lines of Pacific abalone showed that the mean H_o and H_e values decreased from 0.679 to 0.622 and 0.756 to 0.649, respectively (Chen et al. 2017). These results were similar to those of the presently reported study in that after three consecutive *P. vachellii* selective breeding generations, the mean H_o and H_e values from the F1 generation to the F3 generation decreased from 0.8250 to 0.7672 and from 0.7663 to 0.6323, respectively. These results revealed a high level of genetic diversity in the three successive generations of breeding populations.

In the presently reported study, the F_{st} among the various generations of *P. vachellii* was 0.06329, indicating a moderate degree of differentiation. Additionally, the lowest genetic differentiation was observed between F2 and F3 (F_{st} = 0.0484), whereas the highest genetic differentiation was observed between F1 and F3 (F_{st} = 0.12864), indicating that the genetic similarity of the selected offspring increased gradually. However, the genetic structure of F3 changed significantly compared to that of F1.

In terms of successive generations of mass selection, strategies to avoid inbreeding are of critical concern (Fu et al. 2017). Inbreeding depression, through the loss of genetic variation, can ultimately limit long-term genetic progress through selective breeding (Evans et al. 2004; Varney and Wilbur 2020). The presently reported study showed that there was no inbreeding among the three generations; however, inbreeding occurred in each generation, and the largest inbreeding occurred in generation F3. These results are consistent with those of previous studies on long-term artificial selection, as genotypes become more homogeneous, leading to inbreeding depression (Zhang et al. 2010; Chen et al. 2017). Therefore, in the subsequent breeding process, pooling of fertilized eggs from multiple crosses to create cohorts and moderate numbers of parents for each generation (at least 30 pairs) can be used to minimize the loss of genetic diversity and inbreeding depression.

In conclusion, the presently reported study revealed that the genetic similarity of the offspring increased gradually by artificial selection, and the genetic diversity of *P. vachellii* declined slightly after three generations of breeding. However, the level of genetic diversity was still high, which has the potential for further breeding. To minimize the negative influence of inbreeding, new strains can be bred by appropriately increasing the number of breeding parents in the subsequent breeding process, thereby reducing the probability of inbreeding and adopting high selection pressure.

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References

Botstein D, White RL, Skolnick MH, Davis R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32(3): 314–331. https://doi.org/10.1017/S0016672300034467

Chen N, Luo X, Lu C, Ke C, You W (2017) Effects of artificial selection practices on loss of genetic diversity in the Pacific abalone, *Haliotis discus hannai*. Aquaculture Research 48(9): 4923–4933. https://doi.org/10.1111/are.13311

Diyie RL, Agyarkwa SK, Armah E, Amonoo NA, Owusu-Frimpong I, Osei-Atweneboana MY (2021) Genetic variations among different generations and cultured populations of Nile tilapia (*Oreochromis niloticus*) in Ghana: Application of microsatellite markers. Aquaculture 544: 737070. https://doi.org/10.1016/j.aquaculture.2021.737070

Duan G, Zhou H, Wang H, Ling J, Hu Y, Pan T, Yang M, Wu L, Jiang H (2023) [Analysis of the morphological traits effects on body weight of 60 days old breeding groups of *Pelteobagrus vachelli* [sic].] Anhui Nongye Daxue xuebao—Journal of Anhui Agricultural University 50: 78–85. [In Chinese]

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359–361. https://doi.org/10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14(8): 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Evans F, Matson S, Brake J, Langdon C (2004) The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*). Aquaculture 230(1–4): 89–98. https://doi.org/10.1016/j.aquaculture.2003.09.023

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fu J, Lü W, Li W, Shen M, Luo X, Ke C, You W (2017) Comparative assessment of the genetic variation in selectively bred generations from two geographic populations of ivory shell (*Babylonia areolata*). Aquaculture Research 48(8): 4205–4218. https://doi.org/10.1111/are.13241

Gjedrem T, Robinson N, Rye M (2012) The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350–353: 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008

Holsinger K, Weir B (2009) Genetics in geographically structured populations: Defining, estimating and interpreting FST. Nature Reviews. Genetics 10(9): 639–650. https://doi.org/10.1038/nrg2611

Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14): 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16(5): 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Knibb W, Whatmore P, Lamont R, Quinn J, Powell D, Elizur A, Anderson T, Remilton C, Nguyen NH (2014) Can genetic diversity be maintained in long term mass selected populations without pedigree information?—A case study using banana shrimp *Fenneropenaeus merguiensis*. Aquaculture 428–429: 71–78. https://doi.org/10.1016/j.aquaculture.2014.02.026

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Li Q, Wu X, He J, Liu Q, Cheng Y (2018) Can genetic diversity be maintained during mass selection of the Chinese mitten crab, *Eriocheir sinensis*? Aquaculture Research 49(4): 1606–1615. https://doi.org/10.1111/are.13616

Lind CE, Evans BS, Knauer J, Taylor JJ, Jerry DR (2009) Decreased genetic diversity and a reduced effective population size in cultured silver lipped pearl oysters (*Pinctada maxima*). Aquaculture 286(1–2): 12–19. https://doi.org/10.1016/j.aquaculture.2008.09.009

Liu D, Zhou Y, Yang K, Zhang X, Chen Y, Li C, Li H, Song Z (2018) Low genetic diversity in broodstocks of endangered Chinese sucker, *Myxocyprinus asiaticus*: Implications for artificial propagation and conservation. ZooKeys 792: 117–132. https://doi.org/10.3897/zookeys.792.23785

Nei M (1972) Genetic distance between populations. American Naturalist 106(949): 283–292. https://doi.org/10.1086/282771

Ortega-Villaizan M, Noguchi D, Taniguchi N (2011) Minimization of genetic diversity loss of endangered fish species captive broodstocks by means of minimal kinship selective crossbreeding. Aquaculture 318(1/2): 239–243. https://doi.org/10.1016/j.aquaculture.2011.04.047

Phuc TH, Vu NT, Nga N, Ky N, Nguyen NH (2021) Assessment of a long-term selective breeding program for giant freshwater prawn *Macrobrachium rosenbergii* since 2007. Aquaculture 541: e736745. https://doi.org/10.1016/j.aquaculture.2021.736745

Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research 67(2): 175–185. https://doi.org/10.1017/S0016672300033620

Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Molecular Ecology Notes 4(1): 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Swain SK, Sahu BP, Das SP, Sahoo L, Das PC, Das P (2022) Population genetic structure of fringe-lipped carp, *Labeo fimbriatus* from the peninsular rivers of India. 3 Biotech 12(11): e300. https://doi.org/10.1007/s13205-022-03369-y.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4(3): 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Varney RL, Wilbur AE (2020) Analysis of genetic variation and inbreeding among three lines of hatchery-reared *Crassostrea virginica* broodstock. Aquaculture 527: e735452. https://doi.org/10.1016/j.aquaculture.2020.735452

Wang Q, Guo W, Cheng W, Deng G, Xu H, Xia R (2021) Isolation of microsatellite markers for *Pelteobagrus vachellii* based on RAD sequencing. Fisheries Science and Technology Information 48(5): 250–254. https://doi.org/10.16446/j.fsti.20200700123

Wang H, Jiang H, Duan G, Song G, Ling J, Pan T, Hu Y, Zhou H, Yang M (2022) The genetic diversity of the rice-crayfish eco-farming *Procambarus clarkii* in Anhui Province, China. Turkish Journal of Fisheries and Aquatic Sciences 22(1): TRJFAS19904. https://doi.org/10.4194/TRJFAS19904

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160(3): 1203–1215. https://doi.org/10.1093/genetics/160.3.1203

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11(1): 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution; International Journal of Organic Evolution 3(3): 395–420. https://doi.org/10.2307/2406450

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.

Zhang T, Kong J, Wang W, Wang Q (2010) Genetic variability assessed by microsatellites in the breeding populations of the shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* in China. Aquaculture 310(1–2): 229–233. https://doi.org/10.1016/j.aquaculture.2010.07.025

Zhang J, Li Q, Wang Q, Cong R, Ge J, Kong L (2018) The impact of successive mass selection on population genetic structure in the Pacific oyster (*Crassostrea gigas*) revealed by microsatellite markers. Aquaculture International 26(1): 113–125. https://doi.org/10.1007/s10499-017-0196-0

Zheng X, Xu J, Zhang J, Wang T, Yin S (2020) [Genetic diversity analysis in four different geographical populations of yellow catfish *Pelteobagrus vachelli* [sic] by microsatellite markers.] Shuichan kexue—Fishery Sciences 39(5): 657–668. [In Chinese] https://doi.org/10.16378/j.cnki.1003-1111.2020.05.003

Zheng X, Fu D, Cheng J, Tang R, Yin S (2021) Effects of hypoxic stress and recovery on oxidative stress, apoptosis, and intestinal microorganisms in *Pelteobagrus vachelli* [sic]. Aquaculture 543: e736945. https://doi.org/10.1016/j.aquaculture.2021.736945