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# Genetic Diversity of Two Successive Generations of Selection in Bighead Catfish (*Clarias macrocephalus*) Populations

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**ABSTRACT**

*Selective programs associated with domestication can improve fish production but also can have an impact on broodstock's genetic diversity. In this study, the genetic diversity of original sources (G0) from wild and cultured bighead catfish populations, as well as the two successive generations (G1 and G2) subjected to selection experiments, was assessed. G0 wild adults were collected in Ca Mau conservation area and G0 cultured fish were from a hatchery in Can Tho. The G1 fish included pure crosses and crossbreeds of G0, while G2 was the offspring of the selected G1. Fin clips from 27 to 29 individuals of each fish group were randomly sampled for genetic analysis. The amplification results using six ISSR primers showed that the genetic diversity was relatively higher in G0 (effective number of alleles Ne from 1.43 to 1.49; heterozygosity He from 0.265 to 0.290) than in G1 (Ne = 1.32±0.04; He = 0.201±0.023) and G2 (Ne = 1.34±0.04; He = 0.216±0.023). Genetic differences increased between the original populations and the descending generations. To reduce the detrimental impacts of low genetic diversity in domesticated bighead catfish in the Mekong Delta, it is recommended that broodstock in later generations should be produced in a larger number and exchanged with other sources of known origin and genetic information.*

## 1. INTRODUCTION

Bighead catfish (*Clarias macrocephalus*) is native to Southeast Asia and a key aquaculture species in Cambodia, Thailand, and Viet Nam. Propagation and culture of this species were successful in the early 1960s in Thailand (Senanan et al., 2004), while the domestication of this species in the Mekong Delta has taken place for about 30 generations (Duong & Scribner, 2018). However, there has been a limitation in genetic improvement programs for bighead catfish in Viet Nam. Genetic improvement programs such as selection and crossbreeding have increased aquaculture productions of various species such as salmon, tilapia, and common carp, as reviewed by Gjedrem et al. (2012) and Janssen et al. (2017).

Domestication and genetic improvement programs, on the other hand, can affect genetic diversity of captive populations. Fish genetic diversity in hatcheries tends to decline due to genetic drift, inbreeding, and imbalanced breeding sex ratios (Tave, 1993, 1999). After five continuous generations of mandarin fish (*Siniperca chuatsi*), levels of genetic diversity of the breeding population decreased by 30% compared to that of the first generation (Yi et al., 2015). In contrast, no substantial changes in intra-population genetic diversity were identified over the course of three to

five generations in Atlantic salmon (*Salmo salar*) (Tessier & Bernatchez, 1999). High genetic diversity among generations and no significant genetic differentiation were also found in Nile tilapia (*Oreochromis niloticus)* in Ghana (Diyie et al., 2021). For bighead catfish, a previous study reported that the genetic diversity of cultured populations was relatively high, but lower compared to wild populations in conservation areas (Duong & Scribner, 2018). In large-scale artificial propagation of bighead catfish, males were dissected to take out their testis, which requires renewing broodstock and helps to minimize inbreeding. However, in small-scale fish farms, a low number of broodstock can decrease genetic diversity after only several generations (Tave, 1993, 1999).

Different DNA markers such as SNP (single nucleotide polymorphism), microsatellite, RADP (Randomly amplified polymorphic DNA), ISSR (inter-simple sequence repeat), etc., have been used to investigate the genetic diversity of various fish species (see reviews Çiftci & Okumuş, 2002; Liu & Cordes, 2004; Okumuú & Çiftci, 2003). In comparison to co-dominant markers like SNP and microsatellite, dominant markers like RADP and ISSR are less expensive and easier to use (Liu & Cordes, 2004). Between these dominant markers, ISSR had better reproducibility because of higher annealing temperatures and longer primers (reviewed by Liu et al., 2006). Previous studies on bighead catfish employed microsatellite markers to quantify the genetic diversity of different wild and cultured populations (Duong & Scribner, 2018; Nazia et al., 2021). However, no work has evaluated the effects of selection programs on the genetic diversity of this species.

In the present study, ISSR (inter-simple sequence repeat) markers were employed to evaluate the genetic diversity of the original broodstock of bighead catfish from a wild and cultured population and the two successive generations under selection experiments. The results of this study will offer information about genetic variation and genetic differentiation through selective generations for future genetic improvement programs for bighead catfish in the Mekong Delta, Viet Nam.

## 2. MATERIALS AND METHOD

### 2.1. Sample collections

The original populations (G0) of bighead catfish were collected from one cultured population from hatcheries in Can Tho (CT) city and one wild population from U Minh Ha National Park in Ca Mau (CM) province. During 2019 – 2021, two domesticated generations from the original populations were produced. The G1 generation included pure crosses and crossbreeds of G0 (CM, CT, CTxCM, and CMxCT), while the G2 generation was offspring of the G1. About 27 – 29 individuals from each generation were randomly chosen for genetic analysis. Fin clips of these samples were preserved in ethanol 95% until DNA analysis.

### 2.2. ISSR analysis

#### *2.2.1. DNA extraction*

Genomic DNA was extracted using the ammonium acetate method (Saporito-Irwin et al., 1997). About 25 mg of fin clips were used based on the principle of using a salt solution for protein precipitation. DNA was then precipitated by cool absolute ethanol. The precipitated DNA was washed with ethanol two times and then dried at room temperature. Finally, DNA was diluted with TE buffer (Tris-EDTA) and stored at -20°C for further analysis. DNA quality was checked by 1% agarose electrophoresis and scanned under an ultraviolet (UV) transilluminator.

#### *2.2.2. ISSR amplification*

Primer screening and optimization were carried out for 30 primers. Two representatives of each generation (N=6) were randomly selected for this step. Six primers (Table 1) were then chosen based on criteria of polymorphic levels, visibility, and reproducibility. ISSR amplifications were conducted in 10 µL reactions including 5 µL Promega PCR Master Mix (containing Taq DNA polymerase supplied in a reaction buffer (pH 8.5), 400 µM dNTPs, 3 mM $MgCl_2$), 0.4 µL primer (10 µM), 2 µL DNA, and 2.6 µL nuclease-free water. Thermal conditions comprised one cycle of initial denaturation at 95°C for 5 minutes, 38 repeated cycles of denaturation at 95°C for 30 seconds, annealing temperature (Table 1) for 40 seconds and extension at 72°C for 1 minute, and one cycle of final extension at 72°C for 5 minutes.

**Table 1. List of ISSR primers to evaluate genetic diversity of bighead catfish generations**

| No | Primers | Sequence (5' – 3') | Annealing temperature | References |
|---|---|---|---|---|
| 1 | Chiu-SSR1 | $[GGAC]_3A$ | 46°C | Pazza et al., 2007 |
| 2 | HB10 | $[GA]_6CC$ | 46°C | Saad et al., 2012 |
| 3 | ISSR11 | $[CAC]_3GC$ | 44°C | Sharma et al., 2011 |
| 4 | ISSR14 | $[GCT]_6C$ | 46°C | Tanhuanpää et al., 2006 |
| 5 | ISSR15 | $[TCC]_5$ | 46°C | Tiwari et al., 2009 |
| 6 | micro11 | $[GGAC]_4$ | 46°C | Fernandes-Matioli et al., 2000 |

*2.2.3. Electrophoresis and ISSR visualization*

PCR products together with 1kb-DNA ladder (ABM Canada) were loaded into 1.2% agarose gels for electrophoresis. These gels were run in TBE (Tris-borate-EDTA) buffer for 80 minutes at 50 V (Consort EV243) and then immersed in ethidium bromide solution (0.5 µg/mL) for 10 – 15 minutes. ISSR bands were visualized under a UV transilluminator. The gel images were photographed for band scoring. The size bands were estimated based on the size range of the DNA ladder. The presence of the bands was scored 1 and the absence was scored 0 in order to create a binary data matrix as the raw data for genetic diversity analysis.

*2.2.4. Data analysis*

ISSR data was analyzed by GenAlEx 6.5 software (Peakall & Smouse, 2012). Genetic diversity parameters including the percentage of polymorphic loci (P), the number of effective alleles (Ne), Shannon's information index (I), and unbiased expected heterozygosity (uHe) were calculated. Moreover, genetic differentiations among bighead catfish generations were investigated from Nei's unbiased genetic distance. A dendrogram to show the relationship among generations based on UPGMA (unweighted pair-group method with arithmetic average) method was generated using Popgene version (Yeh et al., 1999) and viewed using MEGA 7 (Kumar et al., 2016).

## 3. RESULTS

### 3.1. Amplification of ISSR markers

There were 113 samples from three generations of bighead catfish from four populations, including G0-Culture CT (n=27), G0-Wild CM (n=28), G1 (n=29) and G2 (n=29). A total of 61 bands (alleles) were generated with a size range from 500 bp (HB10 and micro11) to 3,000 bp (ISSR11) (Figure 1). The number of bands from each primer varied from 6 (ISSR15) to 12 (ISSR11, ISSR14, and micro11), while band numbers from each population ranged 53 (G1), 56 (G2), 58 (G0-Culture CT), and 59 (G0-Wild CM). One private allele was only found in G0-Wild CM.

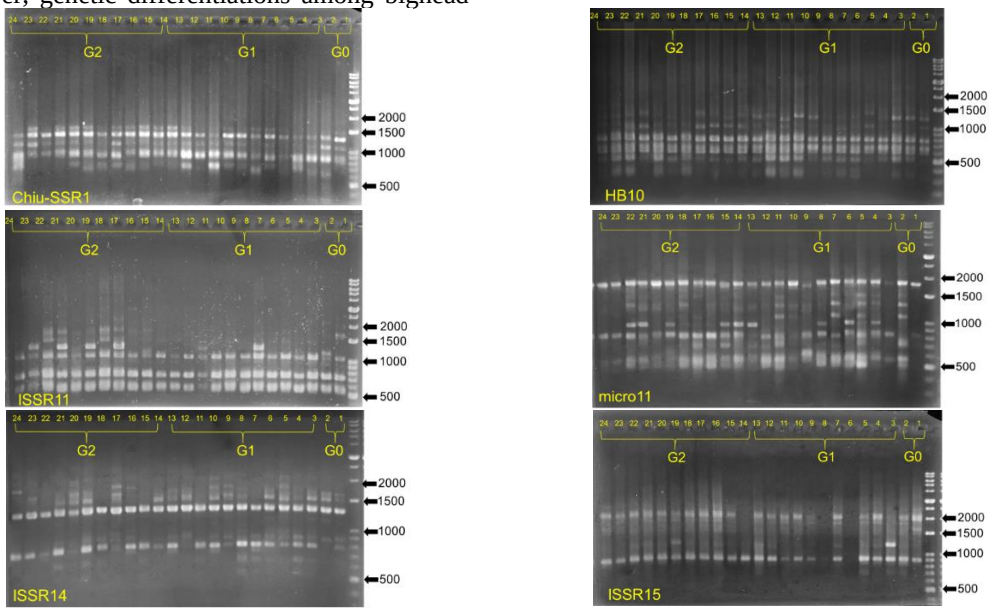


**Figure 1. PCR profile of six ISSR markers used in this study (G0, G1, and G3 are generations of bighead catfish)**

### 3.2. Genetic diversity across domesticated generations of bighead catfish

Genetic diversity parameters of bighead catfish across domesticated generations are shown in Table 2. The percentage of polymorphic loci was from 70.5 to 78.7% with the average value of 75.4±2.0% across the four fish populations. Overall, the number of effective alleles, Shannon index, and unbiased expected heterozygosity reached the average values of 1.39±0.022, 0.364±0.016, and 0.243±0.012, respectively.

When the four fish populations were compared, both two G0 populations had higher levels of genetic diversity than successive G1 and G2 generations. In which, G0-Wild CM showed the highest genetic diversity (Ne=1.49±0.05, I= 0.424±0.033, and uHe=0.290±0.024) and the lowest was G1 (Ne=1.32±0.04, I= 0.332±0.032, and uHe=0.201±0.023).

**Table 2. Genetic diversity (mean±SE) of bighead catfish across domesticated generations**

| Populations | N | P (%) | Ne | I | uHe |
|---|---|---|---|---|---|
| G0-Culture CT | 27 | 78.7 | 1.43±0.04 | 0.394±0.032 | 0.265±0.024 |
| G0-Wild CM | 28 | 78.7 | 1.49±0.05 | 0.424±0.033 | 0.290±0.024 |
| G1 | 29 | 70.5 | 1.32±0.04 | 0.307±0.033 | 0.201±0.023 |
| G2 | 29 | 73.8 | 1.34±0.04 | 0.332±0.032 | 0.216±0.023 |
| Overall | 113 | 75.4±2.0 | 1.39±0.022 | 0.364±0.016 | 0.243±0.012 |

*Note: N: sample size, P: Percentage of Polymorphic Loci, Ne: Number of Effective Alleles, I: Shannon's Information Index, uHe: Unbiased Expected Heterozygosity*

### 3.3. Genetic differentiation among domesticated generations of bighead catfish

The pairwise Nei's unbiased genetic distance and genetic identity of bighead catfish across the four populations ranged from 0.009 to 0.057, and from 0.991 to 0.944, respectively (Table 3). Two original populations were highly similar to each other with a genetic identity of 0.991. However, the increase in genetic differentiation and the decrease of genetic identity between G0 populations and their descending generations (G1 and G2) were found from 0.048 to 0.057, and from 0.953 to 0.944, respectively. Moreover, the UPGMA dendrogram (Figure 2) which was constructed based on Nei's unbiased genetic distance, revealed the genetic relationship among populations of the three domesticated generations of bighead catfish. The two G0 populations formed a branch, separating from the other group of G1 and G2. This genetic difference between populations was also shown by principal coordinates analysis (PCoA). Coordinator 1 showed mainly the difference between the G0 populations and their descending generations of bighead catfish, explaining 12.72% variation, while coordinator 2 indicated the difference between the two G0 populations with 6.82% variation explained (Figure 3).

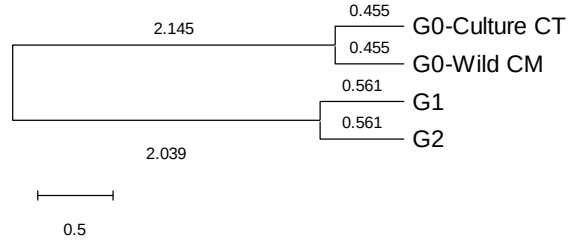


**Figure 2. UPGMA dendrogram from Nei's unbiased genetic distances across three domesticated generations of bighead catfish**

Molecular analysis of variance (AMOVA) showed a larger genetic variation within populations (86.3%) than among populations (13.7%).

**Table 3. Nei's unbiased genetic distance (below diagonal) and genetic identity (above diagonal) of bighead catfish across domesticated generations**

| Populations | G0-Culture CT | G0-Wild CM | G1 | G2 |
|---|---|---|---|---|
| G0-Culture CT | | 0.991 | 0.951 | 0.944 |
| G0-Wild CM | 0.009 | | 0.953 | 0.949 |
| G1 | 0.050 | 0.048 | | 0.989 |
| G2 | 0.057 | 0.053 | 0.011 | |

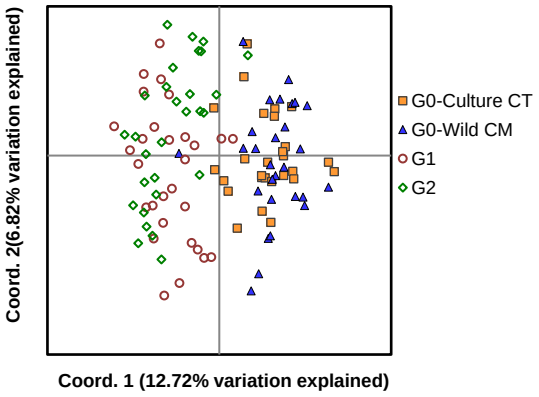


**Figure 3. Principal coordinates analysis (PCoA) of three domesticated generations of bighead catfish**

## 4. DISCUSSION

### 4.1. Genetic diversity across domesticated generations of bighead catfish

The present study revealed a decrease in genetic diversity in two successive generations when compared to their original broodstock populations, although the G2 fish had a slightly higher level of genetic diversity than the G1 generation. This decrease could be the result of the low numbers of breeders used in the experiments producing the G1 and G2 generations. Consequently, genetic drift caused the reduction of all genetic diversity parameters. Particularly, the effective number of alleles and heterozygosity in the G1 generation were 8.3% and 31.8% lower than the G0 cultured CT, and 12.9% and 44.3% lower than the G0 wild CM.

A trend of lower genetic diversity in later domesticated generations has been predicted theoretically (Allendorf & Luikart, 2007; Tave, 1999) and supported empirically (Porta et al., 2007; Shikano et al., 2008; Yi et al., 2015). The flatfish *Solea senegalensis* has been domesticated in South Spain and Portugal. After just one generation of domestication, four representative stocks showed a severe loss of genetic diversity compared to wild populations (Porta et al., 2007). In mandarin fish, genetic diversity parameters (effective alleles, the number of alleles, observed and expected heterozygosity) decreased from generation to generation in a selective breeding program, reaching 30% after five generations (Yi et al., 2015). The main cause for the decrease in genetic diversity of mandarin fish was also the small population size. Similarly, in the square-head climbing perch (*Anabas testudineus*) strain, heterozygosity (measured using ISSR and RADP markers) declined 11% and 44% in two large and small populations after three generations, respectively, compared to the original population. A small-scale farm with broodstock sizes of 250 to 500 individuals was shown to have a dramatic loss of genetic diversity, whereas the other population had a population size of 5 to 10 times bigger (Duong & Pham, 2015). A small decrease in genetic diversity estimated based on microsatellite markers (allele loss was 4.7% per generation and heterozygosity fell 1.4% every generation) was reported for Atlantic salmon (Koljonen et al., 2002). However, in other fish, delta smelt (*Hypomesus transpacificus*), an endangered fish species, native to California (USA) was found to maintain genetic diversity after three generations in captivity due to a pedigree-based breeding plan to control inbreeding and supplementing wild fish into each generation to minimize genetic drift (Fisch et al., 2013). Results of the present work and the above studies indicated that genetic diversity has a trend to decrease by generations in captive breeding conditions and the decreased magnitude depends on the control of genetic drift and inbreeding via breeder sizes.

Although the genetic diversity of the G1 and G2 decreased in the present study, the values of genetic diversity parameters in the bighead catfish generations were moderate, which is comparable to the range reported for other fish species in the Mekong region such as kissing gourami *Helostoma temminckii* (Ne: 1.295 to 1.387; uHe: 0.180 to 0.245) (Duong et al., 2018) and *Pangasius krempfi* (Ne: 1.352 to 1.376; uHe: 0.208 to 0.220) (Yen et al., 2019). Genetic diversity of G0 wild CM and G0 cultured CT populations was as high as that of other fish species like black sharkminow *Labeo chrysophekadion* (Mashyaka & Duong, 2021) or endangered snakehead *Channa lucius* (Sawasawa & Duong, 2020).

With the moderate level of genetic diversity observed in the two generations, these broodstocks should be exchanged with other mates from different populations with known origin or genetic information. An example in *Solea senegalensis* showed that broodstock from four domesticated populations had genetic relatedness, indicating that they were from a common origin (Shikano et al., 2008). Therefore, a genetic investigation before broodstock exchange is critical to avoid close genetic mating. In addition, the number of breeders

should be increased to maximize genetic diversity and minimize the negative effects of genetic drift.

### 4.2. Genetic differentiation among domesticated generations of bighead catfish

The results also showed an increase of genetic differentiation between the original populations and the descending generations. This can be explained by the non-random mating (due to intentionally choosing males and females for artificial propagation), dominant effects (due to crossbreeding between wild CM and domesticated CT populations), and genetic drift due to a small number of breeders. Similar results were reported in mandarin fish by Yi et al. (2015), the genetic difference increased between consecutive generations from G1 to G5 (the terms F1 to F5 were used in that study). The higher number of domesticated generations also enlarges the genetic differentiation of hatchery broodstocks of Japanese flounder *Paralichthys olivaceus* from wild populations (Shikano et al., 2008).

A majority of genetic variation (86.3%) existed within populations while 13.7% was among populations (or generations) of bighead catfish. However, this level of among-population genetic variation is high when compared with other fish species. In *Pangasius krempfi* (family Pangasiidae), genetic variation between populations in the Tien and Hau rivers (two main tributaries of the Mekong river in Viet Nam) was 12% (Yen et al., 2019). Meanwhile, *Channa lucius* showed only 4% genetic variation among four wild populations in the Mekong Delta (Sawasawa & Duong, 2020).

## 5. CONCLUSION

The current study revealed that the genetic diversity of the original wild and domesticated populations that were investigated was relatively high, while that of the two successive generations decreased. Genetic differentiation increased between the original populations and the descending generations.

To minimize the possible negative effects of low genetic diversity of bighead catfish in later generations, broodstock should be produced in a larger number and exchanged based on the origin and genetic information.

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