Genetic diversity of the North African catfish, *Clarias gariepinus* (Burchell, 1822) hatchery stocks in Thailand

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**ABSTRACT:** Information on levels of genetic variation is a prerequisite for successful natural resource management and genetic improvement programs. In this study we aimed to identify genetically distinct stocks of North African catfish, *Clarias gariepinus* (Burchell, 1822) in Thailand which could be used as germplasm sources for a selective breeding program. Four hatchery stocks (\(n = 50\) fish/population) were sampled, three from northeastern provinces (Sakon Nakhon, SN; Nong Khai, NK; and Nakhon Ratchasima, NR), and one from central Thailand (Nakhon Nayok, NY). Six microsatellite loci were scored using primers developed for this species. The results revealed significant genetic differentiation among stocks (\(F_{ST} = 0.096; CI = 0.045–0.166\)) with all but one pair of stocks (NK vs SN) being significantly different, as shown by pair-wise \(F_{ST}\). Three stocks showed homozygote excess. Further analyses showed no recent bottlenecks, but some evidence of genotype disequilibrium. Allele diversity was low (\(A_r = 6.00–7.00; A_e = 3.43–4.59\)) while heterozygosity was moderate (\(H_o = 0.52–0.72; H_e = 0.67–0.77\)). The effective population sizes (\(N_e\)) based on linkage disequilibrium method were between 22.2 and 133 individuals. The neighbor-joining (NJ) tree was robust and revealed the closest genetic relationship between SN and NK, which were clearly separated from NR and NY. Two groups of stocks (northeastern, NK+NR+SN vs NY) showed highest variation among groups (13%) as revealed by AMOVA. The results apparently revealed genetically distinct stocks of North African catfish in Thailand which are useful for establishing a base population for a genetic improvement program.

**KEYWORDS:** African catfish, germplasm, base population, microsatellite

**INTRODUCTION**

Aquaculture plays a significant role in producing seafood to fulfill a booming global protein demand. However, while more production is required, available resources for aquaculture, especially land and water, are limited\(^1\). This situation has led to a favoring of species that can tolerate high stocking densities and poor water quality. Among these is the North African catfish, *Clarias gariepinus* (Burchell 1822) (Cg), which has been widely adopted for aquaculture within and outside its native ranges\(^2,3\) and with a global production of 231 094 mt in 2016\(^4\). Cg has been intensively translocated, mainly within Africa and Asia and, to a lesser extent, to Europe and Latin America\(^3\). There has been a concern that the introduced stocks, after a few generations in hatcheries, would show a decline of genetic variation, as has been documented for introduced/hatchery stocks of Cg\(^5–7\) with a few exceptions\(^8,9\). This eventually compromised the species' performance in aquaculture.

Thailand is an important producer of *Clarias* catfish (e.g. 122 418 mt in 2016, ranked second after Nigeria\(^4\)), and 90% of the production is comprised of the hybrid between female native bighead catfish, *Clarias macrocephalus* Günther, 1864 and male Cg introduced from Vietnam via Laos in 1987\(^10\). However, the culture of Cg is rapidly expanding due to its superior growth and disease resistance and increasing consumer acceptance. It is believed that more than one stock of Cg has been introduced to Thailand\(^11\) although the historical records of the introductions are lacking.

Despite their importance as a potential source of germplasm for genetic improvement, Cg stocks in Thailand have been rarely explored for their
genetic diversity. In 2008, four Cg stocks were identified (genetic distance, \( d = 0.036-0.144 \)) using microsatellite data (6 loci)\(^{12}\) and two of them were used in a cross-breeding experiment. Recently, Koolboon et al\(^{13}\), using five microsatellite loci, observed genetic differences among three Cg stocks (\( d = 0.031-0.066 \)) with significantly different growth performance. Owing to the fact that genetic diversity changes over time due to broodstock management regimes, the present study aimed to verify genetically different Cg stocks present in Thailand, and to quantify genetic variation within populations. The information obtained is useful for improving broodstock management and genetic improvement programs for which genetically distinct stocks are required.

**MATERIALS AND METHODS**

**Fish samples and DNA extraction**

Fin-clips were collected from catfish at four commercial hatcheries (\( n = 50 \) fish/hatchery) in Thailand located in three northeastern provinces, Sakon Nakhon (SN), Nong Khai (NK), and Nakhon Ratchasima (NR), and a central province, Nakhon Nayok (NY) (Fig. 1). These hatcheries were selected according to history of the stock (>5 generations of rearing at each farm, and had potentially originated from different founder stocks). The samples were individually preserved in 95% ethanol until use. DNA was extracted using the standard phenol-chloroform protocol modified from Taggart et al\(^ {14}\) and preserved in TE buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA pH 8.0) at 4 °C until use. All of the protocols were approved by the Kasetsart University Animal Ethics Committee (ID ACKU 61-FIS-004).

**Microsatellite primers and PCR profile**

Six microsatellite primers, Cga01, Cga02, Cga03, Cga06, Cga09, and Cga10, developed by Galbusera et al\(^ {15}\) (Table 1) were used. Each 10 µl of PCR reactions comprised 10 ng DNA template, 0.25 pmol each of forward and reverse primer, 1X PCR buffer, 1.5 mM MgCl\(_2\), 100 µM dNTPs, and 0.2 unit Taq DNA polymerase (Fermentas). The PCR profile (PTC-100TM Programmable Thermal Controller; MJ Research, Inc.) was as follows: 5 min initial denaturation at 95 °C; 35 cycles of 30 sec of denaturation at 95 °C; 30 sec at annealing temperature (52–56 °C); and 1 min of extension at 72 °C, and 5 min at 72 °C. The PCR products were then electrophoresed onto 4.5–6% denaturing polyacrylamide gel and visualized by silver staining. M13 ladder was used as size marker. To minimize scoring errors within and across gels, the marker was loaded into the first, middle and last lanes, and two PCR products of known allele size were included for all gels.

**Statistical analyses**

The individual multilocus genotypes were used for the following statistical analyses. Firstly, each population was tested for Hardy-Weinberg Equilibrium (HWE) and genotypic disequilibrium using exact p-value Markov chain\(^ {16}\), facilitated by the computer package GENEPOP V3.4\(^ {17}\), and adjusted multiple test with Bonferroni correction\(^ {18,19}\). Then the populations showing homozygote excess were tested for the presence of null alleles (alleles that were not amplified), and the genotypic data were adjusted according to the suggestion from the MICROCHECKER program\(^ {20}\). Then the populations were tested against HWE again. Subsequently, the genetic variation within populations, allele frequency, average number of alleles per locus (\( A \)), average effective number of alleles per locus (\( A_e \)), and observed and expected heterozygosity per individual (\( H_o \) and \( H_e \), respectively) were calculated using the GENEPOP
Table 1  Detail of six microsatellite loci developed for North African catfish by Galbusera et al.\textsuperscript{15} and allele size ranges found in four populations used in this study.

| Locus | GenBank | Motif | Primer sequence 5′–3′ | \(T_a\)\(^{(°C)}\) | Size (bp) |
|-------|---------|-------|-----------------------|-----------------|------------|
| Cga01 | U30862  | (GT)\(_{15}\) | GGCTAAAAGACCCCTGTCTG TACACGGTGATGAAAGCAGG | 52              | 85–201     |
| Cga02 | U30863  | (GT)\(_n\), (GT)\(_g\) | GCTAGTGGAAGCCAAGGC ACCCTGTGATGATTTCTTG | 54              | 96–118     |
| Cga03 | U30864  | (GT)\(_{21}\) | CACTTCTACATTTGTGCC ACCTGTATTGATTTCTTG | 56              | 136–176    |
| Cga06 | U30867  | (GT)\(_n\), (GT)\(_g\) | CAGCTCGTGTTTAATTTGGC TTGTACGAGAACCGTGCCAGG | 54              | 134–152    |
| Cga09 | U30871  | (GA), (GT), (GT)\(_{11}\), (N(GT))\(_{n}\), (GT)\(_{4}\) | CGTCACCTCCCTAGAGCG CACGCTGATTCCACATACATGG | 56              | 174–192    |
| Cga10 | U30870  | (GT)\(_{2}\), (GT)\(_{15}\) | GCTGTAGCAAAAATGCAGATGC TCTCCAGAGATCTAGGCTGTCC | 54              | 107–117    |

\(^{1}\) \(T_a\) = annealing temperature.

V3.4\textsuperscript{17}. Effective population size \((N_e)\) of each population was estimated based on linkage disequilibrium using the program NeEstimator (V2)\textsuperscript{21}.

Genetic diversity between populations was assessed as follows. \(F\)-coefficient was calculated using the computer package FSTAT\textsuperscript{22} to test if the studied populations were genetically different. Then pairwise population differentiation was tested using ARLEQUIN V3.01\textsuperscript{23}. Cavalli-Sforza and Edwards genetic distance was calculated using the software PHYLIP V3.63\textsuperscript{24}. Then the genetic distance was used to reconstruct a neighbor-joining tree and bootstrapping using the same software. Bonferroni correction was applied to adjust critical probability for multiple tests.

To clearly distinguish population groups, AMOVA (analysis of molecular variance) was performed between northeastern populations (SN+NK+NR) and NY; SN+NK and NR and NY; SN+NK and NY+NR using ARLEQUIN V3.01\textsuperscript{23}.

RESULTS

HWE and linkage disequilibrium

Three stocks (SN, NK, and NY) departed from HWE towards homozygote excess \((p < 0.001)\) at three (Cga03, Cga06 and Cga10), two (Cga01 and Cga03), and one loci (Cga10), respectively \((p < 0.0016, \text{Bonferroni correction})\) and the test revealed the presence of null alleles at three loci. However, even after the genotypes were adjusted according to the suggestion provided by the program, the tests still showed departure from HWE in these populations. Recent bottleneck test showed normal L-shaped distribution, which implied no evidence for recent inbreeding in these populations. Genotype disequilibrium, non-random segregation of different loci in a gamete, was detected at only three pairs of loci (Cga01-Cga06, Cga01-Cga10, and Cga02-Cga03) \((p < 0.00067 \text{ after Bonferroni correction})\), with the highest incidence in NY (3 loci pairs) and one each in SN and NK. No genotype disequilibrium was detected for NR.

Genetic variation within populations

Among the populations studied, NY possessed the largest number of private alleles (2 alleles of Cga01, 5 alleles of Cga03, 3 alleles of Cga09, and 1 allele at Cga10) while NR and NK had only three (1 allele of Cga01 and 2 alleles of Cga06), and one private allele (of Cga06), respectively (Table 2). Genetic variation within the four populations was characterized by low average number of alleles per locus and average effective number of alleles per locus \((A_e)\) weighted with allele frequencies. Heterozygosity (observed and expected) was moderate for SN and NK, and high in NR and NY (Table 3). It was noteworthy that no statistical differences were observed among populations for each parameter \((p > 0.05)\). The overall \(A\) and \(A_e\) across populations were slightly higher \((A = 9.5; A_e = 5.08)\) than those of individual stocks.

Effective population size \((N_e)\)

\(N_e\) (lowest allele frequency = 0) based on linkage disequilibrium (LD) was lowest for NY \((N_e = 22.2;\)
Table 2 Allele frequency distribution of six microsatellite loci in each population of North African catfish in Thailand.

| Locus  | Size | SN  | NK  | NR  | NY  | PA† |
|--------|------|-----|-----|-----|-----|-----|
| Cga01  | N    | 50  | 50  | 49  | 50  |     |
|        | (N)  |     |     |     |     |     |
| 85     | 0.02 | 0.01| 0.00| 0.00| 0.00|     |
| 87     | 0.10 | 0.12| 0.19| 0.02|     |     |
| 93     | 0.00 | 0.08| 0.20| 0.17|     |     |
| 97     | 0.31 | 0.23| 0.00| 0.00|     |     |
| 101    | 0.13 | 0.09| 0.06| 0.02|     |     |
| 103    | 0.13 | 0.07| 0.05| 0.11|     |     |
| 109    | 0.00 | 0.00| 0.00| 0.01| NY  |     |
| 111    | 0.06 | 0.05| 0.18| 0.25|     |     |
|        |      |     |     |     |     |     |
| 113    | 0.01 | 0.12| 0.08| 0.14|     |     |
| 115    | 0.02 | 0.05| 0.00| 0.00|     |     |
| 119    | 0.00 | 0.00| 0.03| 0.08|     |     |
| 121    | 0.10 | 0.05| 0.07| 0.00|     |     |
| 127    | 0.00 | 0.00| 0.00| 0.01| NY  |     |
| 129    | 0.04 | 0.10| 0.11| 0.05|     |     |
| 133    | 0.03 | 0.03| 0.00| 0.06|     |     |
| 135    | 0.05 | 0.00| 0.00| 0.08|     |     |
| 201    | 0.00 | 0.00| 0.01| 0.00| NR  |     |

| Cga02  | N    | 49  | 50  | 48  | 50  |     |
|--------|------|-----|-----|-----|-----|-----|
|        | (N)  |     |     |     |     |     |
| 106    | 0.47 | 0.40| 0.26| 0.29|     |     |
| 108    | 0.02 | 0.04| 0.00| 0.01|     |     |
| 110    | 0.13 | 0.19| 0.12| 0.33|     |     |
| 114    | 0.11 | 0.08| 0.13| 0.07|     |     |
| 116    | 0.02 | 0.04| 0.02| 0.12|     |     |
| 118    | 0.25 | 0.25| 0.43| 0.16|     |     |

| Cga03  | N    | 49  | 49  | 47  | 50  |     |
|--------|------|-----|-----|-----|-----|-----|
|        | (N)  |     |     |     |     |     |
| 136    | 0.00 | 0.00| 0.00| 0.20| NY  |     |
| 138    | 0.00 | 0.00| 0.00| 0.14| NY  |     |
| 140    | 0.00 | 0.00| 0.00| 0.23| NY  |     |
| 142    | 0.00 | 0.00| 0.00| 0.36|     |     |
| 150    | 0.00 | 0.00| 0.00| 0.07| NY  |     |
| 152    | 0.22 | 0.18| 0.23| 0.00|     |     |
| 154    | 0.07 | 0.05| 0.10| 0.00|     |     |
| 156    | 0.67 | 0.76| 0.61| 0.00|     |     |
| 176    | 0.03 | 0.01| 0.06| 0.00|     |     |

| Locus  | Size | SN  | NK  | NR  | NY  | PA† |
|--------|------|-----|-----|-----|-----|-----|
| Cga06  | (N)  | 49  | 50  | 49  | 50  |     |
|        | (N)  |     |     |     |     |     |
| 134    | 0.00 | 0.00| 0.10| 0.00| NR  |     |
| 136    | 0.27 | 0.45| 0.29| 0.20|     |     |
| 138    | 0.28 | 0.12| 0.32| 0.14|     |     |
| 140    | 0.28 | 0.16| 0.08| 0.25|     |     |
| 142    | 0.18 | 0.22| 0.11| 0.35|     |     |
| 148    | 0.00 | 0.00| 0.01| 0.00| NR  |     |
| 150    | 0.00 | 0.01| 0.09| 0.06|     |     |
| 152    | 0.00 | 0.04| 0.00| 0.00| NK  |     |

| Cga09  | (N)  | 50  | 50  | 49  | 50  |     |
|--------|------|-----|-----|-----|-----|-----|
|        | (N)  |     |     |     |     |     |
| 174    | 0.00 | 0.00| 0.00| 0.16| NY  |     |
| 176    | 0.00 | 0.00| 0.00| 0.02| NY  |     |
| 178    | 0.14 | 0.07| 0.10| 0.00|     |     |
| 180    | 0.00 | 0.01| 0.07| 0.10|     |     |
| 182    | 0.04 | 0.10| 0.21| 0.26|     |     |
| 184    | 0.17 | 0.20| 0.17| 0.19|     |     |
| 186    | 0.09 | 0.13| 0.05| 0.07|     |     |
| 188    | 0.05 | 0.05| 0.02| 0.00|     |     |
|        |      |     |     |     |     |     |
| Cga10  | (N)  | 50  | 50  | 49  | 50  |     |
|        | (N)  |     |     |     |     |     |
| 107    | 0.00 | 0.02| 0.02| 0.12|     |     |
| 109    | 0.02 | 0.06| 0.09| 0.16|     |     |
| 111    | 0.13 | 0.20| 0.26| 0.55|     |     |
| 113    | 0.65 | 0.55| 0.45| 0.00|     |     |
| 115    | 0.00 | 0.00| 0.00| 0.01| NY  |     |
|        |      |     |     |     |     |     |
| 117    | 0.20 | 0.17| 0.18| 0.16|     |     |

† PA = private allele.

Genetic diversity between populations
Overall $F_{ST}$ (0.096 ± 0.034) was significantly greater than zero (bootstrapping value 0.045–0.166), and thus revealed significant differences among populations. Pair-wise population differentiation tests showed that all but one population pair (excepted SN vs NK) were significantly different ($p < 0.0083$, Bonferroni correction). Likewise, the pair-wise $F_{ST}$ revealed significant difference between all but one comparison (except for NK and SN).

Genetic distance and a phylogenetic tree
Genetic distance between populations ranged between 0.025 (NK vs SN) and 0.161 (NY vs SN) (Table 4). Notably, NY showed high genetic distance ($d = 0.087–0.161$) against all other populations. The neighbour-joining tree (Fig. 2) was robust with bootstrap values of 98–100%. The tree confirmed...
Table 3  Genetic variation within populations (locus-wise and average across loci) of four hatchery stocks of North African catfish in Thailand.

| Pop/locus | n  | A  | A_e | H_o | H_e | Fis† |
|-----------|----|----|----|-----|-----|------|
| SN        |    |    |    |     |     |      |
| Cga01     | 50 | 12 | 6.27| 0.80| 0.85| 0.058 |
| Cga02     | 49 | 6  | 3.21| 0.73| 0.69|−0.057|
| Cga03     | 49 | 4  | 1.96| 0.20| 0.49| 0.590 |
| Cga06     | 49 | 4  | 3.91| 0.41| 0.75| 0.460 |
| Cga09     | 50 | 6  | 3.12| 0.70| 0.69|−0.021|
| Cga10     | 50 | 4  | 2.08| 0.28| 0.53| 0.470 |
| Average   | 48.5| 6.0| 3.43| 0.52| 0.67| 0.221 |
| NK        |    |    |    |     |     |      |
| Cga01     | 50 | 12 | 8.36| 0.66| 0.89|−0.260|
| Cga02     | 50 | 6  | 3.73| 0.80| 0.74|−0.083|
| Cga03     | 49 | 4  | 1.65| 0.10| 0.40|−0.745|
| Cga06     | 49 | 7  | 4.55| 0.86| 0.79|−0.064|
| Cga09     | 50 | 7  | 3.73| 0.72| 0.74| 0.026 |
| Cga10     | 50 | 5  | 2.66| 0.52| 0.63| 0.177 |
| Average   | 50 | 6.7| 3.93| 0.59| 0.69| 0.135 |
| NR        |    |    |    |     |     |      |
| Cga01     | 49 | 10 | 6.91| 0.75| 0.86| 0.127 |
| Cga02     | 48 | 6  | 3.54| 0.80| 0.74|−0.005 |
| Cga03     | 47 | 4  | 2.29| 0.49| 0.57| 0.143 |
| Cga06     | 49 | 7  | 4.55| 0.86| 0.79|−0.089 |
| Cga09     | 49 | 7  | 4.36| 0.86| 0.78|−0.102 |
| Cga10     | 49 | 5  | 3.23| 0.61| 0.70| 0.124 |
| Average   | 48.5| 6.5| 4.15| 0.72| 0.74| 0.028 |
| NY        |    |    |    |     |     |      |
| Cga01     | 50 | 12 | 6.99| 0.70| 0.87| 0.193 |
| Cga02     | 50 | 7  | 4.19| 0.74| 0.77| 0.039 |
| Cga03     | 50 | 5  | 4.05| 0.72| 0.76| 0.054 |
| Cga06     | 50 | 5  | 4.03| 0.74| 0.76| 0.026 |
| Cga09     | 50 | 8  | 5.53| 0.80| 0.83| 0.034 |
| Cga10     | 50 | 5  | 2.72| 0.50| 0.64| 0.218 |
| Average   | 50 | 7.0| 4.59| 0.70| 0.77| 0.092 |
| Overall   | 49.8| 9.5| 5.08| 0.63| 0.77| 0.116 |

† No statistical difference was observed between populations. \( F_{is} = (H_e - H_o) / H_e \).

Table 4  Genetic distances (below diagonal) and pairwise \( F_{ST} \) (upper diagonal) among the four hatchery stocks of North African catfish in Thailand based on Carvalli-Sforza and Edwards (1967) genetic distance.

|        | SN   | NK   | NR   | NY   |
|--------|------|------|------|------|
| SN     | –    | 0.010| 0.043**| 0.178**|
| NK     | 0.025| –    | 0.029**| 0.155**|
| NR     | 0.077| 0.0447| –    | 0.123**|
| NY     | 0.161| 0.118| 0.087| –    |

*" denotes highly significant difference (\( p < 0.001 \)).

Table 5  The results of AMOVA between the northeastern stocks (SN, NK and NR) and NY of North African catfish in Thailand.

| Source of variation | Sum of Variance | % variation | p-value |
|---------------------|-----------------|-------------|---------|
| Among groups        | 59.09           | 13.45       | .00001  |
| Among populations   | 15.90           | 2.31        | .00001  |
| Within populations  | 840.73          | 84.24       | .00196  |
| Total               | 915.72          | 94.00       |         |

Fig. 2  A neighbor-joining tree of the four hatchery populations of North African catfish in Thailand based on Cavalli-Sforza and Edwards (1967) genetic distance. Bootstrap values are indicated at the nodes.

The close genetic relationship between NK and SN, following by NR and NY being the most remote population.

Results of AMOVA analysis

AMOVA analysis revealed statistically significant variation (\( p < 0.0001 \)) among groups in all three group combinations with variance component among groups ranged from 0.089 (SN+NK and NR+NY) and 0.342 (northeastern and NY). The variance among groups of the latter comprised 13.45% (Table 5) of the variation and was higher than those of the rest (~10%). The highest variation occurred within populations (84.24–89.13%) while the variance among populations within groups comprised only 0.84–7.17%.

DISCUSSION

Genetic variation of the stocks

Genetic variation in terms of average number of alleles per locus of hatchery stocks in this study
fall in the same range as previously reported for four hatchery stocks from central Thailand ($A = 4.67–12.17$; $A_e = 3.15–5.80$)\textsuperscript{12}, and slightly lower than those of hatchery stocks in Kenya, based on the same sets of microsatellites ($A = 8.83–10.83$, $A_e = 5.5–5.9$)\textsuperscript{9}. In general, loss of alleles is commonly occurred in hatchery populations and was accounted by founder effects (using a small number of founders) and subsequent genetic drift and inbreeding\textsuperscript{26,27}. However, according to the sufficient estimated $N_e$ in most populations reported herein (73–133, except for 22 in NY) the allele loss was unlikely occurred. The minor discrepancy observed herein might be a result of small sample size ($n = 50$) used in the analyses. Noteworthy, $A$ and $A_e$ averaged across four stocks were relatively high and thus implied that the allele diversity could be increased by crossing these stocks. Heterozygosities (observed and expected) were moderate to high as compared to the average $H_o$ among freshwater fishes ($H_o = 0.54$)\textsuperscript{28}, and $H_o$ of four Cg populations from central Thailand (0.50–0.69)\textsuperscript{12}.

**No evidence of inbreeding**

Although the three stocks showed significant homozygote excess, which may be a sign of inbreeding, the absence of recent bottleneck effects indicated that no inbreeding occurred. Rather, the genotype disequilibrium present in these stocks revealed that the homozygote excess could have emerged from the mixing of genetically different populations (Wahlund effect)\textsuperscript{29}. Our finding was supported by Wachirachaikarn et al\textsuperscript{12}, wherein both genetic markers and results from the crossing experiment reflected that no inbreeding had occurred in Thai hatchery stocks of Cg included in their study.

**Effective population size ($N_e$)**

$N_e$ directly determines the inbreeding rate of each population (inbreeding coefficient $F = 1/2N_e$), thus it is important for sustainability of the populations. Tave\textsuperscript{31} recommended a minimum $N_e$ of 45–50 individuals for hatchery populations to avoid inbreeding depression. In this study most populations except NY had sufficient $N_e$ and hence support the previous conclusion on inbreeding. This may be a result of using large numbers of broodstock, rather than other good broodstock management practices (e.g., mating with a 1:1 sex ratio, and using equal numbers of offspring from each family for broodstock recruitment)\textsuperscript{30}, which historically were not routine practices in most hatcheries in Thailand.

The low $N_e$ but with comparable level of genetic variation with other populations observed in NY was surprising. However, the explanation was beyond the capacity of our data. Although no evidence of inbreeding was detected in NY based on microsatellite data in this study, low $N_e$ of the NY population raises concern of inbreeding depression in the future. Owing to moderate genetic variation of NY, introduction of a new stock may not be a priority. Rather, the broodstock management regime should be improved (e.g., using large numbers of broodstock with 1:1 mating sex ratio, and keeping variance of family size to a minimum when recruiting new generations of brooders).

There might be disagreement regarding the precision of this estimate because the number of microsatellites used was smaller than the 10–20 loci recommended by Luikart et al\textsuperscript{32}, but our sample sizes do fall in the upper range of the recommendation (25–50 individuals). Therefore, we were confident that the precision of the estimates was acceptable.

**Genetically distinct hatchery stocks**

Based on the NJ tree and pairwise $F_{ST}$, there seemed to be three genetically different stocks of Cg, SN+NK, NR, and NY. The AMOVA results confirmed that there were at least two genetic groups existed which comprised northeastern (SN+NK+NR) and NY populations. The results also indicated that genetic variation within populations accounted for a major proportion of the overall variation. Highly significant genetic differences among hatcheries in the NE and the NY population reflected limited anthropologic transfer of genetic resources between different regions. A significant variance component among hatcheries within the NE region seems to support this speculation. This information is useful for decision making when distinct gene-pools are selected for further use, e.g., founding a base population for a selection program.

The genetic distances between the aforementioned stocks were similar to those previously reported for four populations from central Thailand ($d = 0.036–0.144$)\textsuperscript{12}. This indicated the existence of germplasm diversity of Cg which is essential for genetic improvement. In fact, Cg possesses high genetic diversity both in natural habitats and hatcheries in its native countries\textsuperscript{9,33}. However, the genetic diversity among populations of the introduced stocks have depended on acquired genetic diversity of the founder stocks as well as gene-flow among them. The Cg stocks in Thailand were from...
at least two geographic origins, Central African Republic and Egypt. They may have been separately managed so that gene-flow was limited, resulting in genetic differentiation among stocks, as previously reported for other stocks from central Thailand\textsuperscript{12,13}. On the contrary, the SN and NK stocks were not different, suggesting that they might have originated from the same founder stock(s) and/or there was sufficient gene-flow between them.

Potential uses for genetic improvement

There have been attempts to improve Cg strains by crossing different stocks\textsuperscript{12,34,35}, but with limited success. To our knowledge, selective breeding which relies mainly on utilization of additive genetic variance has not been used to improve traits in Cg except for a selection program run by our group\textsuperscript{36}. Due to the great potential of selective breeding to improve traits with moderate to high heritability (proportion of additive genetic variance to phenotypic variance) such as growth (e.g., in striped catfish, Pangasianodon hypophthalmus\textsuperscript{37}; Asian sea bass, Lates calcarifer\textsuperscript{38}), selective breeding is recommended for the genetic improvement of Cg in Thailand. In this regard, the three genetically different Cg stocks, based on pairwise $F_{ST}$ and the NJ tree, (NR, NY, and NK or SN) are useful as sources of germplasm for the establishment of a founder stock for further selection. The importance of founder stocks with large genetic variation has been highlighted in the successful cases of fish/shellfish genetic improvement, such as Nile tilapia, Oreochromis niloticus\textsuperscript{39}, and Pacific white shrimp, Litopenaeus vannamei\textsuperscript{40}.

Conclusions and recommendations

The conclusions and recommendations drawn from this study are as follows. (1) Genetically distinct hatchery stocks of Cg are available in Thailand. To maximize utilization of these valuable resources, their performance should be studied. (2) Genetic variation within stocks was moderate. However, the stock with low $N_{e}$ should be properly managed by strictly following good broodstock management practices. (3) Based on genetic diversity data, pooling these stocks would expand genetic variation of a novel population. Even though this may not guarantee variation in quantitative traits, this novel population with a large genetic background would be favorable as a founder stock for a selective breeding program.

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