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

Anyalak Wachirachaikarn[a,b], **Uthairat Na-Nakorn**[c,*]

[a] Division of Genetics, Department of Sciences, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140 Thailand
[b] Research Unit of Genetic Technology and Applications, Department of Science, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140 Thailand
[c] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900 Thailand

*Corresponding author, e-mail: ffisurn@ku.ac.th

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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 ranged from 6.00–7.00; A_e ranged from 3.43–4.59) while heterozygosity was moderate (H_o ranged from 0.52–0.72; H_e ranged from 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

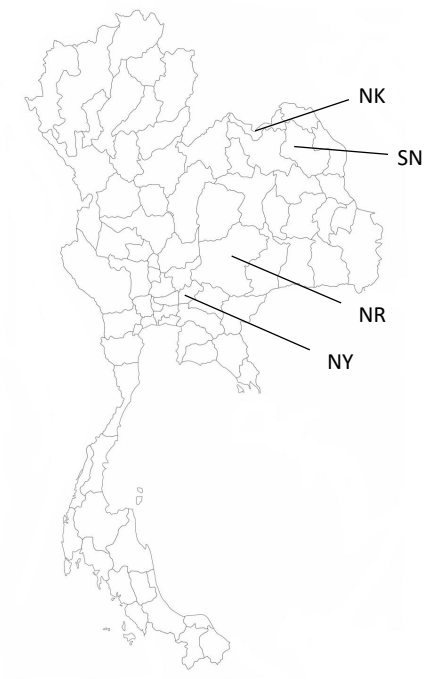

Fig. 1 A map of Thailand showing locations of the North African catfish hatcheries where samples were collected for this study.

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)[15]; 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[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	$(GT)_{15}$	GGCTAAAAGAACCCTGTCTG TACAGCGTCGATAAGCCAGG	52	85–201
Cga02	U30863	$(GT)_{10}N_2(GT)_8$	GCTAGTGTGAACGCAAGGC ACCTCTGAGATAAAACACAGC	54	96–118
Cga03	U30864	$(GT)_{21}$	CACTTCTTACATTTGTGCCC ACCTGTATTGATTTCTTGCC	56	136–176
Cga06	U30867	$(GT)_5N_2(GT)_9$	CAGCTCGTGTTTAATTTGGC TTGTACGAGAACCGTGCCAGG	54	134–152
Cga09	U30871	$(GA)_3N_3(GT)_{11}$ $N(GT)_6N_2(GT)_4$	CGTCCACTTCCCCTAGAGCG CCAGCTGCATTACCATACATGG	56	174–192
Cga10	U30870	$(GT)_2N_2(GT)_{15}$	GCTGTAGCAAAAATGCAGATGC TCTCCAGAGATCTAGGCTGTCC	54	107–117

† T_A = annealing temperature.

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

Genetic diversity between populations was assessed as follows. *F*-coefficient was calculated using the computer package FSTAT[22] to test if the studied populations were genetically different. Then pairwise population differentiation was tested using ARLEQUIN V3.01[23]. Cavalli-Sforza and Edwards genetic distance was calculated using the software PHYLIP V3.63[24]. Then the genetic distance[25] 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[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 ($p < 0.0016$, 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) ($p < 0.00067$ 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* 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†	Locus	Size	SN	NK	NR	NY	PA†
Cga01	(N)	(50)	(50)	(49)	(50)		Cga06	(N)	(49)	(50)	(49)	(50)	
	85	0.02	0.01	0.00	0.00			134	0.00	0.00	0.10	0.00	NR
	87	0.10	0.12	0.19	0.02			136	0.27	0.45	0.29	0.20	
	95	0.00	0.08	0.20	0.17			138	0.28	0.12	0.32	0.14	
	97	0.31	0.23	0.00	0.00			140	0.28	0.16	0.08	0.25	
	101	0.13	0.09	0.06	0.02			142	0.18	0.22	0.11	0.35	
	103	0.13	0.07	0.05	0.11			148	0.00	0.00	0.01	0.00	NR
	109	0.00	0.00	0.00	0.01	NY		150	0.00	0.01	0.09	0.06	
	111	0.06	0.05	0.18	0.25			152	0.00	0.04	0.00	0.00	NK
	113	0.01	0.12	0.08	0.14		Cga09	(N)	(50)	(50)	(49)	(50)	
	115	0.02	0.05	0.00	0.00			174	0.00	0.00	0.00	0.16	NY
	119	0.00	0.00	0.03	0.08			176	0.00	0.00	0.00	0.02	NY
	121	0.10	0.05	0.07	0.00			178	0.14	0.07	0.10	0.00	
	127	0.00	0.00	0.00	0.01	NY		180	0.00	0.01	0.07	0.10	
	129	0.04	0.10	0.11	0.05			182	0.04	0.10	0.21	0.26	
	133	0.03	0.03	0.00	0.06			184	0.17	0.20	0.17	0.19	
	135	0.05	0.00	0.00	0.08			186	0.09	0.13	0.05	0.07	
	201	0.00	0.00	0.01	0.00	NR		188	0.05	0.05	0.02	0.00	
Cga02	(N)	(49)	(50)	(48)	(50)			190	0.00	0.00	0.00	0.01	NY
	96	0.00	0.00	0.05	0.02			192	0.51	0.44	0.37	0.19	
	106	0.47	0.40	0.26	0.29		Cga10	(N)	(50)	(50)	(49)	(50)	
	108	0.02	0.04	0.00	0.01			107	0.00	0.02	0.02	0.12	
	110	0.13	0.19	0.12	0.33			109	0.02	0.06	0.09	0.16	
	114	0.11	0.08	0.13	0.07			111	0.13	0.20	0.26	0.55	
	116	0.02	0.04	0.02	0.12			113	0.65	0.55	0.45	0.00	
	118	0.25	0.25	0.43	0.16			115	0.00	0.00	0.00	0.01	NY
Cga03	(N)	(49)	(49)	(47)	(50)			117	0.20	0.17	0.18	0.16	
	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	NY							
	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								

† PA = private allele.

confidence interval, CI = 15.9–32.0), moderate for SN (N_e = 73.2; CI = 35.9–344.1) and NR (N_e = 71.1; CI = 37.3–241.9), and highest for NK (N_e = 133.0; CI = 54.7–infinite).

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	F_{is} [†]
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	50	6	3.42	0.76	0.71	−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	0.73	0.73	−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[25] 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 ($p < 0.001$).

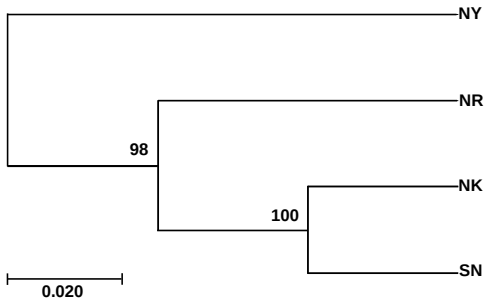

Fig. 2 A neighbor-joining tree of the four hatchery populations of North African catfish in Thailand based on Cavalli-Sforza and Edwards (1967) genetic distance. Bootstrap values are indicated at the nodes.

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 square	Variance	% variation	p-value
Among groups	59.09	0.342	13.45	.00001
Among populations within group	15.90	0.059	2.31	.00001
Within populations	840.73	2.145	84.24	.00196
Total	915.72	2.546		

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) [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) [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 [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) [28], and H_o of four Cg populations from central Thailand (0.50–0.69) [12].

No evidence of inbreeding

Although the three stocks showed significant homozygote excess, which may be a sign of inbreeding, the absence of recent bottlenecks 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) [29]. Our finding was supported by Wachirachaikarn et al [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 [30], $F = 1/2N_e$), thus it is important for sustainability of the populations. Tave [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) [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 [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) [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 [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[11]. 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[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[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[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*[37]; Asian sea bass, *Lates calcarifer*[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*[39], and Pacific white shrimp, *Litopenaeus vannamei*[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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REFERENCES

1. Na-Nakorn U, Koonawootrittriron S (2015) Challenges for genetic improvement of livestock and aquatic animals. *Res & Knowl* **1**, 18–25.
2. Dai W, Wang X, Guo Y, Wang Q, Ma J (2011) Growth performance, hematological and biochemical responses of African catfish (*Clarias gariepinus*) reared at different stocking densities. *African J Agri Res* **6**, 6177–6182.
3. Na-Nakorn U, Brummett RV (2009) Use and exchange of aquatic resources for food and aquaculture: *Clarias* catfish. *Rev Aquacult* **1**, 214–223.
4. FAO (2018) Fishery and Aquaculture Statistics. Global Aquaculture Production 1950–2016 (FishstatJ). In: FAO Fisheries and Aquaculture Department, Rome. [Available at: http://www.fao.org/fishery/statistics/software/fishstatj/en]
5. Van Der Bank FH, Grobler JP, Du Preez HH (1992) A comparative biochemical genetic study of three populations of domesticated and wild African catfish (*Clarias gariepinus*). *Comp Biochem Physiol B* **101**, 387–390.
6. Van Der Walt LD, Van Der Bank FH, Steyn GJ (1993) Allozyme variation in domesticated African catfish (*Clarias gariepinus*) from The Netherlands. *Comp Biochem Physiol B* **104**, 15–18.
7. Galbusera P (1997) The genetic variability of wild and inbred populations of the African catfish *Clarias gariepinus* Burchell, 1822. PhD Thesis, Katholieke Universiteit Leuven, Belgium. [Cited after Volckaert FAM, Hellemans B (1999) Survival, growth and selection in a communally reared multifactorial cross of African catfish *Clarias gariepinus*. *Aquaculture* **171**, 49–64.]
8. Teugels, GG, Guyomard R, Legendre M (1992) Enzymatic variation in African clariid catfishes. *J Fish Biol* **40**, 87–96.
9. Barasa JE, Mdyogolo S, Abila R, Grobler JP, Skilton RA, Bindeman H, Njahira MN, Chemoiwa EJ, et al (2017) Genetic diversity and population structure of the African catfish, *Clarias gariepinus* (Burchell, 1822) in Kenya: implication for conservation and aquaculture. *Belg J Zool* **147**, 105–127.
10. FishBase (2007) A global information system on fishes. [Available at: www.fishbase.org/home.htm]

11. Li Y, Helleman B, Pouyaud L, Volkaert FAM (2000) Origin and genetic diversity of aquacultured catfish (*Clarias gariepinus*) in SE Asia. In: *Catfish Asia Workshop*, Bogor, Indonesia.
12. Wachirachaikarn A, Rungsin W, Srisapoome P, Na-Nakorn U (2009) Crossing of African catfish (*Clarias gariepinus*) strains based on strain selection using genetic diversity data. *Aquaculture* **290**, 53–60.
13. Koolboon U, Koonawootrittriron S, Kamolrat W, Na-Nakorn U (2014) Effects of parental strains and heterosis of the hybrid between *Clarias macrocephalus* and *Clarias gariepinus*. *Aquaculture* **424–425**, 131–139.
14. Taggart JB, Hynes RA, Prodöhl PA, Ferguson A (1992) A simplified protocol for routine total DNA isolation from salmonid fishes. *J Fish Biol* **40**, 963–965.
15. Galbusera P, Volckaert FA, Hellemans B, Ollevier F (1996) Isolation and characterization of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822). *Mol Ecol* **5**, 703–705.
16. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.
17. Raymond M, Rousset F (1995) GENEPOP (Version 1.2), population genetics software for exact tests and ecumenicism. *J Hered* **86**, 248–249.
18. Hochberg Y (1988) A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* **75**, 800–802.
19. Rice WR (1989) Analyzing table of statistical tests. *Evolution* **43**, 223–225.
20. Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**, 535–538.
21. Do C, Waples RS, Peel D, Macbeth, GM, Tillet BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour* **14**, 219–214.
22. Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices. [Available at www2.unil.ch/popgen/softwares/fstat.htm]
23. Excoffier LG, Schneider S (2006) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolut Bioinform* **1**, 47–50.
24. Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle.
25. Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution* **21**, 550–570.
26. Allendorf FW, Phelps SR (1980) Loss of genetic variation in a hatchery stock of cutthroat trout. *T Am Fish Soc* **109**, 537–543.
27. Frankham R, Gilligan DM, Morris DR, Briscoe DA (2001) Inbreeding and extinction: effects of purging. *Conserv Genet* **2**, 279–284.
28. De Woody JA, Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *J Fish Biol* **56**, 461–473.
29. Hedrick PW (1985) *Genetics of Populations*, Jones and Bartlett Publishers, Inc., Boston.
30. Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn, Longman, Essex.
31. Tave D (1993) *Genetics for Hatchery Managers*, 2nd edn, Kluwer Academic Publisher, Boston.
32. Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv Genet* **11**, 355–373.
33. Barasa JE, Abila R, Grobler JP, Agaba M, Chemoiwa EJ, Kaunda-Arara B (2016) High genetic diversity and population differentiation in *Clarias gariepinus* of Yala Swamp: evidence from mitochondrial DNA sequences. *J Fish Biol* **89**, 2557–2570.
34. Volckaert FAM, Hellemans B (1999) Survival, growth and selection in a communally reared multifactorial cross of African catfish (*Clarias gariepinus*). *Aquaculture* **171**, 49–64.
35. Sunarma A, Carman O, Zairin Jr M, Alimuddin A (2016) Interpopulation crossbreeding of farmed and wild African catfish *Clarias gariepinus* (Burchell 1822) in Indonesia at the nursing stage. *Aquat Living Resour* **29**, ID 303, 1–8.
36. Srimai W, Koonawootrittriron S, Manee-aphai W, Chatchaiphan S, Koolboon U, Na-Nakorn U (2019) Genetic parameters and genotype-environment interaction for growth traits of North African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture* **501**, 104–110.
37. Sang NV, Klemetsdal G, Ødegård J, Gjøen HM (2012) Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture* **344–349**, 82–89.
38. Domingos J, Smith-Keune C, Robinson N, Loughnan S, Harrison P, Jerry D (2013) Heritability of harvest growth traits and genotype-environment interactions in barramundi, *Lates calcarifer* (Bloch). *Aquaculture* **402–403**, 66–75.
39. Bentsen HB, Eknath AE, Palada-de Vera MS, Danting JC, Bolivar HL, Reyes RA, Dionisio EE, Longalong FM, et al (1998) Genetic improvement of farmed tilapia: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. *Aquaculture* **160**, 145–173.
40. Argue BJ, Arce SM, Lotz AM, Moss SM (2002) Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome virus. *Aquaculture* **204**, 447–460.