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Genetic changes, intra- and inter-specific introgression in farmed Nile tilapia (*Oreochromis niloticus*) in Thailand

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ABSTRACT

Fourteen microsatellite loci were used to examine genetic changes of four strains in Nile tilapia (*Oreochromis niloticus*) derived from genetically improved farmed tilapia (GIFT) and two strains derived from a local Chitralada strain of Nile tilapia in Thailand. Reference populations, including the ninth generation of GIFT strain, the original Chitralada strain, two conspecific reference populations from Ivory Coast and Uganda, and one population each of *Oreochromis mossambicus* and *Oreochromis aureus*, were also examined. Despite minor genetic changes, three of the four GIFT-derived populations retained their purity as GIFT while genetic variation did not decline. One of the GIFT-derived populations showed high levels of introgression from the Chitralada strain. Likewise, introgression from GIFT to the Chitralada-derived populations was seen. Inter-specific introgression from *O. mossambicus* was observed in the GIFT reference population and one of the Chitralada-derived strains. Introgression from *O. aureus* was detected in one of the GIFT-derived populations with a history of intensive inter-strain crossing. However, the introgression resulted in elevated genetic variation relative to the Chitralada original strains.

1. Introduction

World Nile tilapia (*Oreochromis niloticus*) production has been dramatically increasing recently (e.g. from 970,756 MT in 2000 to 2,334,432 MT in 2008; FAO, 2010). Before 1998, cultured stocks of Nile tilapia faced problems of genetic deterioration due to small numbers of founders and successive bottleneck effects (reviewed by Pullin and Capili, 1988; Eknath and Hulata, 2009). Introgression from the congeneric *Oreochromis mossambicus* (Macaranas et al., 1986; 1995) also presumably accounted for the decline in culture performance in some stocks (Amarasinghe and De Silva, 1996). Since 1997, the GIFT (Genetically Improved Farmed Tilapia) strain has been disseminated and has shown remarkable impacts in enhancing production of Nile tilapia (ADB, 2005; Ponzoni et al., 2010a). The GIFT and GIFT-derived strains have made significant contributions to major producer countries of Nile tilapia; e.g., they have accounted for 80% of the total tilapia seed production in China, 75% in Thailand, and 40% in the Philippines (Ponzoni et al., 2010a).

Thailand is among the world's top five producers of Nile tilapia (FAO, 2011), with annual production averaging 155,000 MT from 2000 to 2009 (FAO, 2011). Amongst freshwater commodities, Nile tilapia contributed more than 40% to total aquaculture production of the country (Department of Fisheries, 2010) and it is a major export commodity, contributing 12,956 MT, valued at more than US$ 22.3 million in 2010 (Customs Department, 2011). Before 1998, aquaculture of Nile tilapia in Thailand relied on the Chitralada strain, which originated from an Egyptian stock that reached Thailand in 1965 *via* Japan (Damrongratana and Kessanchai, 1966). Despite a lack of scientific evidence, the Chitralada-derived stocks were believed to suffer genetic deterioration and poor culture performance caused by poor broodstock management and introgression with *O. mossambicus* as was reported in other Asian strains of Nile tilapia (Gupta and Acosta, 2004; Macaranas et al., 1986). Therefore, when the GIFT strain was introduced to Thailand in 1998 (ADB, 2005), it was well accepted by major hatcheries across the country.

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It is of concern that genetic change may have occurred in the GIFT-derived strains in Thailand. Generally, genetic change in hatcheries is common and usually is accompanied by loss of alleles due to genetic drift, especially when effective population size (N_e) is small (Aho et al., 2006; Allendorf and Phelps, 1980; Coughlan et al., 1998; McKinna et al., 2010; Romana-Eguia et al., 2005). Subsequently, if N_e is continually low, inbreeding will accumulate (Falconer and Mackay, 1996; Romana-Eguia et al., 2005). Moreover, reduction of genetic variation may be triggered by selection (e.g. Appleyard and Ward, 2006), which has been practiced in some tilapia hatcheries (Prayad Soda, pers. comm.). Despite an inconclusive relationship between genetic variation based on molecular markers and performance (e.g. Borrell et al., 2004; Heath et al., 2002; Overturf et al., 2003; Shikano and Taniguchi, 2002), loss of genetic variation was assumed to cause the decline of Nile tilapia production in Fiji (McKinna et al., 2010). Introgression of genes from other species may occur and may also have adverse impacts on production (Amarasinghe and De Silva, 1996).

The critical questions of the present study are: 1) whether genetic change, especially loss of genetic diversity, has occurred in the GIFT derived strains in Thailand; and 2) did introgression occur from the local Chitralada strain and/or *O. mossambicus* into the GIFT derived strains and *vice versa*?

This study provided a scenario which is useful for broodstock management of other tilapia broodstocks in that: 1) broodstock management of the GIFT-derived strains in Thailand followed good broodstock management regimes, mainly by using large number of brooders and equal contribution by family; 2) intra-specific introgression resulted in increasing genetic variation of populations with low genetic variation; 3) genetic differentiation among the GIFT derived strains occurred after a few generations and may eventually result in valuable genetic resources for further genetic improvement; and 4) inter-specific introgression was frequently observed and the impact was still inconclusive.

2. Materials and methods

2.1. Fish samples: origins, collection and DNA extraction

Four GIFT-derived strains, i.e. strains that were intentionally founded with the GIFT strain and/or incorporated the GIFT strain as a resource strain, were collected from two private and two government hatcheries during February to September 2006; two Chitralada-derived strains were collected from a private and a government hatchery (Table 1). The sampling was limited to hatcheries that maintain their own broodstock and have been producing more than 10 million tilapia fingerlings per year for at least 10 years.

Six categories of reference populations were included (Table 1): a GIFT population, a Chitralada population, pure cultured and wild *O. niloticus* from Africa and a population each for *O. mossambicus* and *Oreochromis aureus*. *O. aureus* was included in this study because it was introduced to Thailand in 1980 for production of all-male tilapia (Tangtrongpiros, 1980) through interspecific hybridization with *O. niloticus*.

A piece of caudal fin (about 50 mg) was collected from each individual broodfish, stored in 95% ethyl alcohol and delivered to the Fish Genetics Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok. DNA extraction was performed using the standard protocol of Taggart et al. (1992) with a slight modification: the DNA pellets were resuspended in TE buffer (10 mM Tris–HCl pH 7.5; 1 mM EDTA pH 8.0) and stored at −20 °C until use. The quantity and quality of the extracted DNA were determined by spectrophotometry and agarose gel electrophoresis.

2.2. Microsatellite primers and PCR conditions

Fourteen microsatellite primers developed from DNA of *O. niloticus* by Lee and Kocher (1996) were used (Table 2). A single-locus PCR for each of six microsatellite primers (*UNH172*, *UNH211*, *UNH216*, *UNH222*,

Table 1
Sources of six Nile tilapia hatchery populations in Thailand; four conspecific reference populations from Chitralada Villa Royal Residence Thailand (ON-CD), Uganda (ON-U), Ivory Coast (ON-I) and genetically improved GIFT strain (ON-GIFT); and one population each of *Oreochromis mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E); N = sample size.

Stock abbreviation	Names of hatcheries/locations	Origin of the stock	N
GIFT derived populations			
ON-PT	Pathum Thani Fisheries Test and Research Center (Pathum Thani FTRC)	Founders comprised fifty families of the 9th generation GIFT from the WorldFish Center, Malaysia in 2000, undergone within family selection for growth for 5 generations	50
ON-UT	Uttaradit Fisheries Test and Research Center (Uttaradit FTRC)	Founders comprised 33 out of 50 families of the 9th generation of GIFT from Pathum Thani FTRC, undergone 3 generations of within family selection for growth	50
ON-PB	Nam Sai Farm, Prachin Buri province	Founders comprised the 5th and 9th generation GIFT from the National Aquaculture Genetics Research Institute (NAGRI), Thailand in 1997 and 2000, respectively; no records on number of families, the stocks were merged and subjected to 2 generations of selection for growth	50
ON-CP	Charoen Pokphand Hatchery, Ayutthaya province.	Founders comprised 250 full-sib families using females from 2 sources, GIFT from Pathum Thani FTRC and GIFT originated in the Philippines, and males from Chitralada and other tilapia strain/species from Africa, undergone 5 generations of within family selection for growth	50
Chitralada derived populations			
ON-AIT	Asian Institute of Technology (AIT), Pathum Thani province	Founders comprised members of Chitralada strain reared in Chitralada Villa Royal Residence, Bangkok, no information on number of founders, undergone at least 10 generations of mass selection for growth	50
ON-AY	Rom Sai Farm, Ayutthaya province.	Founders comprised a combination of Chitralada strain and GIFT [GIFT was un-intentionally introduced due to the confused naming of the GIFT strain as Chitralada III (ADB, 2005; Chinnabut et al., 2007)], undergone 2 generations of mass selection for growth	50
Reference populations			
ON-CD	Chitralada population,	Collected from Chitralada Villa Royal Residence, Bangkok	80
ON-GIFT	GIFT population	The 9th generation GIFT originally collected from the Philippines (obtained from N. Taniguchi, Tohoku University, Japan)	28
ON-I	Ivory Coast population	A cultured population from Ivory Coast (obtained from K. Veverica, Auburn University, USA)	20
ON-U	Uganda population	Wild, collected from Lake Albert in Uganda (obtained from W. Mwanja, Uganda)	20
OM-S	Reference population for *O. mossambicus*	A cultured population from South Africa (obtained from G. Hulata, Agricultural Research Organization, Israel)	40
OA-E	Reference population for *O. aureus*	A cultured population from Egypt, (obtained from Mahmoud Rezk, WorldFish Center, Egypt)	40

Table 2
Primer details for microsatellite loci used in this study.

Locus ID	GenBank accession no.	LG no.[a]	Primer sequence (5′–3′)	Dye[b]	T_a (°C)	Repeat type	Size range (bp)
UNH172	G12324	4	AATGCCTTTAAATGCCTTCA CTTTTATAGTCGCCCTTTGTTA	–	60	$(CA)_{17}$	176–246
UNH211	G12362	19	GGGAGGTGCTAGTCATA CAAGGAAAACAATGGTGATA	–	60	$(CA)_{17}$	106–212
UNH216	G12367	23	GGGAAACTAAAGCTGAAATA TGCAAGGAATATCAGCA	–	55	$(CA)_{11}$	120–186
UNH222	G12373	2	CTCTAGCACACGTGCAT TAACAGGTGGGAACTCA	–	58	$(CA)_{17}$	162–196
UNH212	G12363	Not mapped	ACTGTATTCTATAAATGCATTTT GGAATGTGACATTTTGA	–	50	$(CA)_{13}$	178–228
UNH160	G12312	6	CCATTGGCTCTTACATC GATAGCATTTCTGTAGTTATGG	–	60	$(CA)_{35}$	132–210
UNH213	G12364	1	ACTGCTCCTCTTGTTTT TGTGATAAGGTTAATTAAAGTTAGG	6-FAM	59, 56	$(CA)_{17}$	182–218
UNH153	G12305	18	TCTGCTTTGCTTTTTCTCATTCT TACGGCACACTCCCTCCAT	VIC	59, 56	$(CA)_9$	197–243
UNH132	G12285	9	ATATAAGAAACTGAGTCGGTGAG TGGAAATAGAGGGTGGGTGAG	NED	59, 56	$(GA)_6$ GC $(GA)_7$	125–149
UNH138	G12290	16	TTCAGCTTCATCTCTTG CCATTTTAACCTCTCCATCT	PET	59, 56	$(CA)_{26}$	164–228
UNH192	G12344	11	GGAAATCCATAAGATCAGTTA CTTTTTCAGGATTTACTGCTAAG	6-FAM	56, 53	$(CA)_{10}$	144–190
UNH1004	G68281	22	CATCTGAGTCACGCAGGTTC GCTGAGGTGAGTGTGATGGA	VIC	56, 53	$(GT)_{29}$	180–256
UNH982	G68265	3	TCAATACTGTGGTCCCCTCTTT TCTCAGAGCGCTATCTTCCTG	NED	56, 53	$(GT)_{21}$GC $(GT)_9$	120–168
UNH173	G12325	13	CGTGAGAAAACAATGGT TATTGATTTTATAGCTGTCTGG	PET	56, 53	$(CA)_8$	123–207

[a] See the genetic linkage map of tilapia (*Oreochromis* spp.) (Lee et al., 2005)
[b] Fluorescently labeled forward primer for multiplex PCR

UNH212 and *UNH 160*) was performed in a 10-μl reaction volume containing 2.5 ng of template DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 100 μM of each dNTPs, 0.25 μM of each primer and 0.2 units of *Taq* DNA polymerase (Fermentas). PCR was carried out in a PX2 Thermal Cycler (Thermoscientific, USA) under the following conditions: 3 min at 94 °C; followed by 30 cycles of 30 s at 94 °C, 30 s at T_a °C (Table 2), 1 min at 72 °C; with final extension of 5 min at 72 °C. Then the PCR products were separated on a 4.5% polyacrylamide gel. Visualization of the microsatellite profiles was accomplished by silver staining (Bassam et al., 1991). Allele sizes were designated relative to the M13 DNA ladder.

In addition, eight microsatellite primers consolidated into two multiplex PCR reactions (Multiplex 1: *UNH213*, *UNH153*, *UNH132*, *UNH138*; Multiplex 2: *UNH192*, *UNH1004*, *UNH982*, *UNH173*) were scored for all DNA samples. PCR was performed in a 10-μl total reaction volume consisting of 5 ng of template DNA, 1 μl of 10× PCR reaction buffer with 20 mM $MgCl_2$, 0.3–0.4 μl of each primer (10 μM) depending on relative fluorescence in relation to other primers in the multiplex, 1 μl dNTPs (20 μM) and 0.12 μl FastStrat *Taq* DNA polymerase (Roche, Germany). Forward primers were fluorescently end-labeled with 6-FAM, VIC, NED or PET (Applied Biosystems, USA) for visualization. A two-step PCR program was used for the multiplex amplification with the GeneAmp® PCR System 9700 (Applied Biosystems, USA) involving: 5 min at 94 °C; followed by 28 cycles of 30 s at 94 °C, 45 s at a first T_a °C (Table 2), and 1 min at 72 °C; 10 cycles of 30 s at 94 °C, 45 s at a second T_a °C (Table 2), and 45 s at 72 °C; and a final extension of 10 min at 72 °C. One microliter of diluted multiplex PCR product was run on an ABI 3130xl Genetic Analyzer with a LIZ600 size standard (Applied Biosystems, USA). GeneMapper® Software Version 4.0 (Applied Biosystems, USA) was used to analyze the electropherograms and allele sizes. The facilities for multiplex PCR were provided by the Genomic Variation Laboratory, University of California, Davis, CA, USA.

2.3. Data analyses

Conformation to Hardy–Weinberg equilibrium (HWE) was tested using a Markov chain approximation of the exact test (dememorization = 10,000; batches = 1000; iteration per batch = 10,000) (Guo and Thompson, 1992) facilitated by the program GENEPOP version 4.0 (Raymond and Rousset, 1995a; Rousset, 2008). The analyses also provided the locus-wise F_{IS} for each population (Guo and Thompson, 1992). The test for linkage disequilibrium based on the Chi-square test facilitated by the program GENETIX Version 4.05.2 (Belkhir et al., 2004) also was performed, wherein the disequilibrium coefficient (D) was estimated as $D = P_{AB} - p_A p_B$; where P_{AB} is the observed frequency of the *AB* gamete and p_A and p_B are the allele frequencies at loci *A* and *B*, respectively.

Due to the departure from HWE towards excess of homozygotes in some populations, the program Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004) was used to explore the evidence for "null-alleles" and then the genotypes of the populations showing evidence of null alleles were adjusted accordingly. The populations were once again tested for the departure from HWE.

Genetic variation within populations (average number of alleles/locus-*A*, effective number of alleles/locus-A_e, observed and expected heterozygosity-H_o and H_e, respectively) was estimated using the program POPGENE version 1.32 (Yeh and Boyle, 1999). Calculation of allelic richness (A_r), the A_r estimated from the smallest sample size, was performed using the program FSTAT version 2.9.3.2 (Goudet, 1995). The differences between populations were tested for each parameter (A, A_e, A_r, H_o and H_e) using the independent *t*-test comparison in the SPSS statistical package (version 11.5.0) (SPSS Inc., Chicago), followed by post-hoc multiple comparison. CONVERT version 1.31 (Glaubitz, 2004) was used to calculate allele frequencies and identify private alleles.

The population structuring was evaluated based on the *F*-statistics (F_{ST}, F_{IS} and F_{IT}), which were calculated using the program Fstat version 2.9.3.2 (Goudet, 1995). GENEPOP version 4.0 (Raymond and Rousset, 1995b; Rousset, 2008) was used to test the genetic differentiation between populations based on allele frequency distributions. The pairwise F_{ST} and significance tests were calculated using ARLEQUIN 3.11 (Excoffier et al., 2005). Then the program PHYLIP version 3.67 was used to calculate the Cavalli-Sforza and Edwards (1967) chord distance (genetic distance) between populations using the adjusted data set with corrected genotypes for null-alleles. The same program was used for a reconstruction of the phylogenetic tree (Neighbor-joining tree, Felsenstein, 1993) among populations and for estimation of bootstrap-values.

To examine possible genetic introgression, a model-based clustering method for inferring population structure using the program STRUCTURE version 2.3.3 (Pritchard et al., 2000; Hubisz et al., 2009) was employed. Analysis was run using the admixture ancestry model with twenty runs of a burn-in time of 100,000 followed by 100,000 iterations. The best number of clusters, *K*, was decided according to the *ad hoc* statistic ΔK based on the rate of change in the log probability of data between the successive *K* values (Evanno et al., 2005), that was calculated using STRUCTURE HARVESTER version 0.6.5 (Earl, 2011). Averaged proportion of individual membership in each cluster was calculated for each predefined population/species using a Bayesian clustering algorithm in STRUCTURE. The clustering is meaningful when the proportion of membership is highest in a single cluster; the non-contaminated population would have a proportion of membership equaling 1 in one cluster and 0 in the other clusters. The population of origin would be the reference

population that showed the highest proportion of membership in the same cluster with the examined population(s).

3. Results

Despite the typically high polymorphism of microsatellites, the locus *UNH222* was monomorphic in the Ivory Coast samples of *O. niloticus*. Two loci (*UNH172* and *UNH138*) were monomorphic in the *O. mossambicus* samples (fixed for the alleles *UNH172**246 and *UNH138**164). These alleles also were observed in *O. niloticus* populations; both alleles were found in ON-AY ($P=0.030$ for *UNH172**246; 0.070 for *UNH138**164), and ON-AIT ($P=0.020$ for *UNH172**246; 0.070 for *UNH138**164). The allele *UNH138**164 was observed in ON-CP ($P=0.060$), ON-CD ($P=0.013$) and ON-GIFT ($P=0.143$) which may not be identical by descent with those found in *O. mossambicus*.

3.1. Genetic variation within populations of the Nile tilapia hatchery populations

Genetic variation within populations was calculated after adjustment for null alleles (Table 3). The GIFT-derived populations showed relatively high genetic variation ($A_r=6.64\pm2.36$ of ON-PB to 8.54 ± 2.86 of ON-CP; $H_e=0.73\pm0.10$ of ON-PB to 0.79 ± 0.10 of ON-CP), while that of the population of origin (ON-GIFT) was also relatively high ($A_r=7.14\pm2.09$; $H_e=0.74\pm0.13$). On the contrary, the Chitralada strain (ON-CD) showed relatively low genetic variation ($A_r=4.93\pm1.59$; $H_e=0.65\pm0.15$), while the descendant populations had relatively high genetic variation ($A_r=7.14\pm1.78$ of ON-AIT to 8.23 ± 2.74 of ON-AY; $H_e=0.73\pm0.12$ of ON-AIT to 0.76 ± 0.12 of ON-AY). Genetic variation of the genetically improved GIFT strain was significantly higher than that of the Chitralada strain (ON-CD) (Table 3).

The reference Nile tilapia hatchery population from Ivory Coast showed remarkably low A_r and H_e, while those parameters from the wild reference population from Uganda were moderate. Genetic variation of the *O. mossambicus* population from South Africa (OM-S) was very low both for the parameters representing allele diversity and heterozygosity, while *O. aureus* population (OA-E) had moderate genetic variation (Table 3).

3.2. Hardy–Weinberg equilibrium and linkage disequilibrium

The Fisher exact test revealed that ON-CP, ON-AY, ON-CD and ON-I had an excess of homozygotes and did not conform to Hardy–Weinberg expectation, while ON-PT and OM-S had an excess of heterozygotes ($P<0.0036$; Bonferroni correction) (Table 4). However, after the allele frequencies were adjusted for the presence of null alleles [three loci in ON-CP (*UNH172*, *UNH1004* and *UNH982*) and ON-AY (*UNH216*, *UNH212*, and *UNH982*); two loci for ON-I (*UNH172*, and *UNH211*); and one locus each for ON-PT (UNH222), ON-UT (UNH1004), ON-PB (UNH172), ON-AIT (*UNH982*), ON-CD (*UNH222*), ON-U (*UNH172*), ON-GIFT (*UNH211*), OM-S (*UNH173*) and OA-E (*UNH211*)], the re-analyses showed the same results as the Fisher exact test, but the locus-wise F_{IS} was significant at only 1–3 loci of 14 loci in each population.

All populations showed linkage disequilibrium (LD) at least at two locus pairs (ranged from 2–25 loci pairs in a population). Among the Nile tilapia hatchery populations the most pronounced LD, shown at 25 loci pairs, was observed in ON-AY, followed by 14 loci pairs in ON-AIT, 13 loci pairs in ON-CP, 9 loci pairs in ON-PT, 4 loci pairs in ON-UT, 2 loci pairs in ON-PB. The reference *O. niloticus* populations showed LD at 4 loci pairs in ON-CD; 3 loci pairs in ON-GIFT; 2 and 12 loci pairs in ON-U and ON-I, respectively. However, the LD coefficient was relatively low across the Nile tilapia hatchery populations (0.0122 ± 0.0013 to 0.0197 ± 0.0023). The reference Nile tilapia populations showed much lower disequilibrium (D) [$D=0.0103\pm0.0028$ (ON-CD), 0.0129 ± 0.0084 (ON-I)] except for ON-GIFT which showed the highest value of mean D ($D=0.0457\pm0.0075$) (Table 5).

Table 3
Genetic variation within Nile tilapia hatchery populations in Thailand and six reference populations; N = sample size, P = number of polymorphic loci, A = number of alleles per locus, A_e = effective number of alleles per locus, A_r = allelic richness, H_o, H_e = observed and expected heterozygosity, respectively; values in parentheses denote SD; Differences in mean values in the same column with different superscripts are statistically significant ($\alpha=0.05$).

species/population	N	Genetic variation					
		P	A	A_e	A_r	H_o	H_e
O. niloticus							
Pathum Thani (ON-PT)	50	14	8.93^{ab} (3.10)	5.07^{a} (2.42)	7.73^{a} (2.74)	0.77^{a} (0.11)	0.76^{a} (0.13)
Uttaradit (ON-UT)	50	14	9.00^{ab} (3.46)	4.76^{ab} (2.08)	7.49^{a} (2.66)	0.75^{a} (0.11)	0.75^{a} (0.12)
Prachin Buri (ON-PB)	50	14	7.57^{bcd} (2.65)	4.17^{abc} (1.91)	6.64^{abc} (2.36)	0.72^{a} (0.11)	0.73^{a} (0.10)
CP (ON-CP)	50	14	10.57^{a} (3.94)	5.42^{a} (2.19)	8.54^{a} (2.86)	0.74^{a} (0.09)	0.79^{a} (0.10)
Ayutthaya (ON-AY)	50	14	10.14^{ab} (3.74)	4.88^{ab} (2.04)	8.23^{a} (2.74)	0.71^{a} (0.12)	0.76^{a} (0.12)
AIT (ON-AIT)	50	14	8.64^{abc} (2.59)	4.16^{abc} (1.68)	7.14^{ab} (1.78)	0.73^{a} (0.11)	0.73^{a} (0.12)
Chitralada (ON-CD)	80	14	6.07^{cd} (1.90)	3.30^{bcd} (1.29)	4.93^{cde} (1.59)	0.63^{ab} (0.14)	0.65^{a} (0.15)
Uganda (ON-U)	20	14	7.50^{bcd} (3.84)	3.99^{abc} (2.66)	7.50^{a} (3.84)	0.67^{a} (0.21)	0.69^{a} (0.17)
Ivory Coast (ON-I)	20	13	3.43^{e} (2.31)	2.05^{d} (1.44)	3.43^{de} (2.31)	0.38^{c} (0.33)	0.36^{b} (0.28)
GIFT strain (ON-GIFT)	28	14	7.64^{bcd} (2.24)	4.37^{abc} (1.87)	7.14^{ab} (2.09)	0.74^{a} (0.10)	0.74^{a} (0.13)
O. mossambicus							
South Africa (OM-S)	40	12	3.14^{e} (1.23)	2.05^{d} (0.81)	2.93^{e} (1.08)	0.51^{bc} (0.33)	0.43^{b} (0.25)
O. aureus							
Egypt (OA-E)	40	14	5.86^{d} (4.47)	2.91^{cd} (2.48)	5.04^{bcd} (3.65)	0.46^{c} (0.24)	0.48^{b} (0.26)

Table 4
Test for conformation to Hardy–Weinberg equilibrium (after the adjustment of allele frequencies due to null allele) showing the locus-wise F_{IS}, the probability for overall Fisher's exact test ($\alpha = 0.0036$ after Bonferroni correction for multiple tests). * denotes statistical significance ($P<0.0036$)

Population	Locus										F_{IS}		Prob. exact test
	UNH 172	UNH 211	UNH 216	UNH 222	UNH 212	UNH 160	UNH 213	UNH 153	UNH 132	UNH 138	UNH 192	UNH 1004	UNH 982	UNH 173	
O. niloticus															
Pathum Thani (ON-PT)	0.0725*	−0.0570	−0.3275	0.1273	−0.0177	0.0623	−0.0324	−0.0390	−0.0138	0.0182	−0.0852	0.1215	−0.0513	−0.0574	−0.0094	0.0005
Uttaradit (ON-UT)	0.0514	0.0921	−0.2273	0.0252	0.0880	−0.0002	−0.1741	−0.0071	0.0913	0.1013	−0.0862	0.0886	0.0381	−0.0064	0.0145	0.1591
Prachin Buri (ON-PB)	0.1323	0.0247	0.0895	−0.0998	−0.0379	0.0283	0.0622	−0.1533	0.0383	−0.0228	0.0473	−0.0515	0.0878	−0.0205	0.0106	0.0184
CP (ON-CP)	0.0785*	0.0211	0.0565	0.0972	0.1160	0.0082	0.0357	−0.1062	0.1238	0.1061*	0.0683	0.1002	0.1485*	0.0647	0.0835	0.0000
Ayutthaya (ON-AY)	0.1098	−0.0397	0.1227	0.0493*	0.0810	0.1128	0.1115*	0.0943	0.1075	0.0162	0.0523	0.0152	0.1772*	0.0683	0.0998	0.0000
AIT (ON-AIT)	0.1332	0.0275	−0.0842	−0.0180	0.0007	0.0033	0.0106	−0.0449	−0.1003	0.0003	−0.1553	0.0020	0.0934	0.0392	0.0100	0.1470
Chitralada (ON-CD)	0.0866	0.1105	0.0678	0.0520	−0.0882	0.0975	0.0007	−0.1112	0.0255	0.0815	0.0936	−0.0033*	−0.0542	−0.0321	0.0479	0.0001
Uganda (ON-U)	0.1795	−0.0314	0.1264	−0.0076	−0.1271	0.0563	−0.1934	0.3968	−0.3412	0.1853	−0.0156	0.1419	0.1584	0.1762	0.0455	0.2330
Ivory Coast (ON-I)	0.1595	0.2623	−0.0270	-	−0.0556	0.0270	−0.4975*	1.0000	−0.3790*	0.3968	0.3596	−0.3194	−0.1875	−0.0629	0.0207	0.0001
GIFT strain (ON-GIFT)	0.1550	0.1637	−0.1670	−0.2990	−0.0970	−0.0239	−0.0904	−0.2213	0.1274	0.1353	0.1196	0.0935	−0.1232	−0.0532	−0.0024	0.5957
O. mossambicus																
South Africa (OM-S)	-	−0.0428	−0.2039*	−0.2145	−0.5584*	−0.1380	0.2166	0.0557	−0.1692	-	0.0140*	−0.3929	−0.2381	−0.2000*	−0.0952	0.0000
O. aureus																
Egypt (OA-E)	0.0545	0.1418	0.0862	0.2320	0.0714	−0.0864	−0.0630	0.1491	−0.2412	0.0640	0.0746	−0.0056	0.0561	−0.0428	0.0573	0.0904

Table 5
Results of linkage disequilibrium (LD) tests based on the Chi-square tests; Population abbreviations are as in Table 1; D = disequilibrium coefficient

Population	No. of loci pairs for LD	D (mean ± SE)
ON-PT	9	0.0194 ± 0.0025
ON-UT	4	0.0122 ± 0.0013
ON-PB	2	0.0190 ± 0.0002
ON-CP	13	0.0171 ± 0.0024
ON-AY	25	0.0197 ± 0.0023
ON-AIT	14	0.0142 ± 0.0012
ON-CD	4	0.0103 ± 0.0028
ON-U	2	0.0125 ± 0.0125
ON-I	12	0.0129 ± 0.0084
ON-GIFT	3	0.0457 ± 0.0075
OM-S	2	−0.0066 ± 0.0010
OA-E	17	0.0121 ± 0.0025

3.3. Population structure and genetic diversity between populations

The overall F_{ST} calculated across all populations/species was 0.209 and was significantly greater than zero ($CI_{99\%} = 0.185–0.240$). When *O. aureus* and *O. mossambicus* samples were removed from the analyses, the F_{ST} was reduced to 0.121 ($CI_{99\%} = 0.097–0.153$). This result indicated the existence of population differentiation among the populations of *O. niloticus*. The F_{ST} values among GIFT and GIFT-derived populations (0.0316; $CI_{99\%} = 0.021–0.044$); among GIFT and GIFT-derived populations except ON-CP (0.0262; $CI_{99\%} = 0.012–0.044$), and Chitralada and Chitralada-derived strains (0.0676; $CI_{99\%} = 0.039–0.115$) were relatively low but statistically significant, and hence implied an existence of population differentiation among populations within each group.

Pairwise F_{ST} estimates (Table 6) revealed significant differentiation between the GIFT-derived strains and GIFT (pairwise $F_{ST} = 0.0156–0.0475$; $P<0.0008$, Bonferroni correction) and the range of genetic distance-d was 0.0228–0.0395. Notably, the Chitralada-derived strains were different from the original Chitralada (pairwise $F_{ST} = 0.0600–0.0862$; $P<0.0008$, Bonferroni correction; $d = 0.0430–0.0497$). Likewise, relatively high pairwise F_{ST} (0.1753) and genetic distance (0.0908) revealed genetic differences between the GIFT and Chitralada strains. Genetic differentiation between ON-CD and ON-GIFT with the reference Nile tilapia from Uganda ($F_{ST} = 0.1525–0.1801$) was lower than that between ON-CD, ON-GIFT and ON-I from Ivory Coast ($F_{ST} = 0.3125–0.3248$). In addition, the allele frequency distribution showed significant differences between all pairs of populations (Fisher's exact test, $P<0.0008$, Bonferroni correction).

3.4. A phylogenetic dendrogram

The neighbor-joining tree (bootstrap = 61.8–100%) (Fig. 1) gave a clear picture of genetic relationships among the major Nile tilapia broodstocks in Thailand. A majority of the GIFT-derived populations (ON-PB, ON-PT, ON-UT) clustered with ON-GIFT from which they presumably originated. The Chitralada (ON-CD) clustered with one (ON-AIT) of the two descendant populations. The admixture between Chitralada and GIFT was clearly shown for ON-CP and ON-AY, which were placed between the two reference populations of origin. It is noteworthy that the *O. niloticus* populations from Uganda (ON-U) and Ivory Coast (ON-I) formed a cluster distinct from the clusters of the GIFT and Chitralada reference populations and their derivative populations. The tree also showed a clear separation of *O. aureus* and *O. mossambicus* from *O. niloticus*, whereby *O. mossambicus* was more divergent from *O. niloticus* than *O. aureus*.

3.5. Population clustering and population origin

The assignment test-derived clustering based on the highest ΔK value (140.08), showed that the best-fitted number of populations (K) was 6

Table 6
A matrix of pairwise F_{ST} value (below diagonal) and Carvalli-Sforza and Edwards chord distance (above diagonal) among the populations of Nile tilapia and the reference populations/species; Every test for pairwise F_{ST} was significant different ($P<0.0008$ after Bonferroni correction for multiple tests).

	ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E
ON-PT	**	0.0151	0.0249	0.0340	0.0513	0.0874	0.0963	0.1054	0.1286	0.0258	0.1946	0.1531
ON-UT	0.0118	**	0.0253	0.0341	0.0534	0.0885	0.1030	0.0991	0.1306	0.0228	0.1973	0.1585
ON-PB	0.0323	0.0330	**	0.0390	0.0506	0.0875	0.0975	0.1067	0.1399	0.0395	0.1964	0.1627
ON-CP	0.0363	0.0338	0.0476	**	0.0268	0.0519	0.0579	0.0899	0.1256	0.0378	0.1935	0.1485
ON-AY	0.0732	0.0712	0.0869	0.0349	**	0.0318	0.0497	0.0909	0.1308	0.0512	0.1920	0.1714
ON-AIT	0.1403	0.1391	0.1596	0.0753	0.0526	**	0.0430	0.1035	0.1379	0.0861	0.1923	0.1804
ON-CD	0.1750	0.1776	0.1942	0.1031	0.0862	0.0600	**	0.1022	0.1170	0.0908	0.2205	0.1769
ON-U	0.1490	0.1410	0.1700	0.1214	0.1196	0.1451	0.1801	**	0.1362	0.1016	0.2039	0.1926
ON-I	0.2782	0.2856	0.3115	0.2803	0.2965	0.3161	0.3248	0.3359	**	0.1310	0.2121	0.1922
ON-GIFT	0.0156	0.0186	0.0475	0.0369	0.0713	0.1423	0.1753	0.1525	0.3125	**	0.1914	0.1651
OM-S	0.3658	0.3709	0.3830	0.3579	0.3803	0.3991	0.4425	0.4422	0.5857	0.3908	**	0.1978
OA-E	0.3157	0.3217	0.3325	0.3012	0.3365	0.3622	0.3946	0.4113	0.5263	0.3483	0.4986	**

(Fig. 2). Cluster 1 comprised Chitralada (ON-CD) (an average admixture proportion, $q_1=0.978$) and ON-AIT ($q_1=0.936$). Cluster 2 comprised ON-GIFT (an average admixture proportion, $q_2=0.936$), ON-PT ($q_2=0.972$), ON-UT ($q_2=0.981$), and ON-PB ($q_2=0.980$). Clusters 3, 4, 5 and 6 comprised each population of ON-U ($q_3=0.976$), ON-I ($q_4=0.968$), OM-S ($q_5=0.991$) and OA-E ($q_6=0.977$), respectively. Population admixture comprising ON-CD and ON-GIFT was revealed for ON-CP ($q_1=0.403$, $q_2=0.559$) and ON-AY ($q_1=0.566$, $q_2=0.394$). Notably, the reference populations of *O. niloticus* from Ivory Coast and Uganda shared the least proportion of gene pool with *O. niloticus* in Thailand, as did the *O. mossambicus* (OM-S) and *O. aureus* (OA-E) populations (Table 7).

Fig. 3 shows the probability of assignment of individuals to the six inferred genetic groups ($K=6$). Each vertical bar represents an individual and each color represents the probability of which the individual was assigned to each gene pool (admixture proportion, q_i); for example, an individual assigned to two genetic groups at $q_i=0.5$ is a first-generation hybrid. Only the assignments with $q_i>0.1$ were taken into account.

Admixed individuals determined by $q_{2-6}>0.1$ were not found in the reference ON-CD. In ON-GIFT, four admixed individuals were observed comprising three individuals with q_1 (ON-CD) = 0.167–0.455 and an individual with q_5 (OM-S) = 0.162. Among the GIFT-derived strains, only ON-PT contained admixed individuals from ON-CD ($q_1=0.112$–0.134) (2 individuals). In contrast, ON-CP contained 20% pure Chitralada ($q_1=0.874$–0.984) and 40% pure GIFT ($q_2=0.899$–0.990); 36% ON-CD and ON-GIFT ($q_1=0.105$–0.883, $q_2=0.107$–0.857) and 4% interspecific admixture between ON-CD and OA-E ($q_1=0.744$–0.842, $q_6=0.106$–0.136). Among the Chitralada-derived populations, the majority of ON-AIT individuals were pure Chitralada ($q_1=0.876$–0.992), whereas seven individuals were an admixture of ON-CD and ON-GIFT ($q_2=0.114$–0.451). ON-AY comprised 42% pure ON-CD ($q_1>0.907$), 22% pure GIFT ($q_2>0.809$) and 34% admixed individuals (ON-CD and ON-GIFT; $q_1=0.104$–$0.0.826$; $q_2=0.157$–0.779); 2% ON-CD and OM-S ($q_1=0.826$; $q_5=0.141$).

4. Discussion

4.1. Genetic change of the GIFT-derived hatchery populations compared to the original GIFT population

The small, but statistically significant genetic differences observed among the GIFT-derived Nile tilapia populations and between each population and the GIFT strain implies that genetic changes have occurred since the dissemination of the GIFT strain to Thailand. Generally, genetic change in hatcheries is common and caused by the

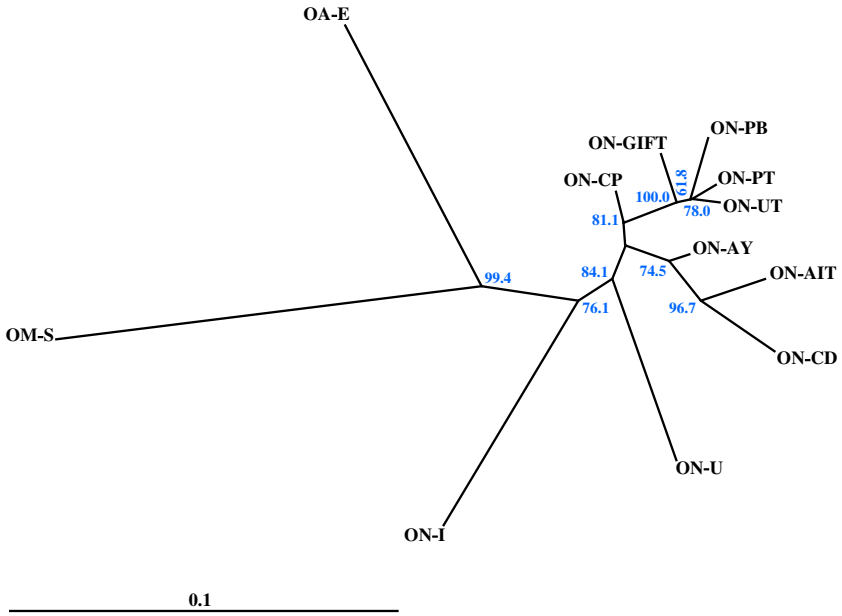

Fig. 1. A phylogenetic dendrogram reconstructed from Carvalli-Sforza and Edwards (1967) chord distance between six Nile tilapia hatchery populations in Thailand (GIFT- derived: Pathum Thani FTRC, ON-PT; Uttaradit FTRC, ON-UT; Nam Sai Farm, ON-PB; Charoen Pokphand Hatchery, ON-CP and Chitralada-derived: Rom Sai Farm, ON-AY; Asian Institute of Technology, ON-AIT); four conspecific reference populations from Chitralada Villa Royal Residence Thailand (ON-CD), Uganda (ON-U), Ivory Coast (ON-I) and genetically improved GIFT strain (ON-GIFT); and a population each of *O. mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E). Significant bootstrap values are shown at each nodes of the dendrogram.

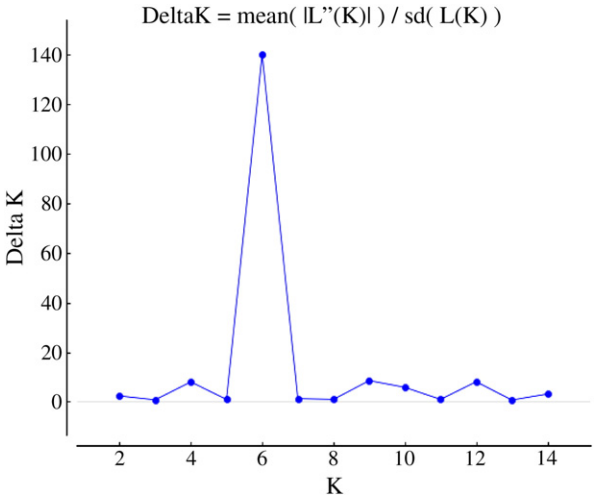

Fig. 2. DeltaK (ΔK) value calculated for assuming K = 1–15 clusters of six Nile tilapia hatchery populations in Thailand, four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*.

following factors: genetic drift due to using small numbers of broodstock during the founding event and in later generations, selection (natural and artificial; intentional and un-intentional), introduction of genetically different populations, and non-random mating (Aho et al., 2006; Fiumera et al., 2000; Norris et al., 1999; Qin et al., 2007; Taniguchi, 2003). Due to the relatively high genetic variation, especially allelic richness, of the GIFT-derived populations, it is assumed that genetic drift which has a pronounced effect on allele loss (Allendorf and Phelps, 1980) may only partly be responsible for the genetic differentiation observed in this study. This inference is supported by the large N_e assumed from the following information: 1) breeding of Nile tilapia requires a large number of females to meet demand for fingerlings due to low fecundity (approximately 96.9 and 127.5 eggs/g BW for GIFT and Chitralada strain, respectively; ADB, 2005 cited Hans Komen, pers. comm.), and 2) short generation time and high survival partly contribute to large N_e (Allendorf and Luikart, 2007). As such, even though the sex ratio of the mating was not always 1:1 (Fessehaye et al., 2006), its impact on reduction of N_e was partly compromised (Falconer and Mackay, 1996). Selection may cause genetic change at neutral markers, and may have had a considerable contribution to genetic change of the GIFT-derived strains, as was reported in Atlantic salmon, *Salmo salar* (Norris et al., 1999), Nile tilapia (Romana-Eguia et al., 2005) and Pacific oyster (Appleyard and Ward, 2006), probably through linkages between the markers and the traits under selection. In this study, the farm records showed that artificial selection was applied to all hatchery populations (e.g. within-family selection for ON-PT, ON-UT and ON-CP). Non-random mating, which is a part of the selection program, probably enhanced the genetic change that occurred in the GIFT-derived strains while gene flow from other strains did not occur according to the model-based clustering.

Table 7
Average admixture proportion (q_i) of each pre-defined population in each of the six clusters ($K = 6$); (STRUCTURE: 100,000 Burn-in period; 100,000 Reps).

Population	Inferred Clusters						Number of Individuals
	1	2	3	4	5	6	
ON-PT	0.011	0.972	0.005	0.004	0.003	0.004	50
ON-UT	0.005	0.981	0.005	0.004	0.002	0.004	50
ON-PB	0.006	0.980	0.005	0.004	0.002	0.003	50
ON-CP	0.403	0.559	0.016	0.008	0.003	0.011	50
ON-AY	0.566	0.394	0.022	0.006	0.007	0.004	50
ON-AIT	0.936	0.042	0.009	0.004	0.005	0.004	50
ON-CD	0.978	0.004	0.005	0.009	0.001	0.004	80
ON-U	0.011	0.005	0.976	0.004	0.002	0.002	20
ON-I	0.012	0.006	0.006	0.968	0.007	0.001	20
ON-GIFT	0.038	0.936	0.005	0.005	0.008	0.007	28
OM-S	0.002	0.002	0.002	0.002	0.991	0.002	40
OA-E	0.003	0.003	0.013	0.002	0.002	0.977	40

4.2. Inter-specific introgression

We inferred interspecific introgression, as suggested by the model-based clustering, from *O. mossambicus* to ON-GIFT and ON-AY, and from *O. aureus* to ON-CP. In the case of ON-GIFT, despite the low proportion of membership sharing with OM-S (0.8%), the bar plot (model-based clustering) showed an individual with admixture probability of 0.162 from OM-S that implied introgression. Introgression of *O. mossambicus* was allowed during the foundation of the GIFT strain, probably through introgressed founding stocks from the Philippines (Macaranas et al., 1995). The presence of *O. mossambicus* mt DNA haplotypes in the sixth generation of GIFT from WorldFish Centre, Malaysia (McKinna et al., 2010) confirmed this information. However, in the present study the relatively high LD coefficient of ON-GIFT (0.0457 compared to less than 0.02 in other populations) suggested that the introgression occurred recently (Allendorf et al., 2001). In fact, the GIFT project prevented any further introgression with *O. mossambicus* because of various assumed negative impacts (Macaranas et al., 1995; Amarasinghe and De Silva, 1996; McKinna et al., 2010). Therefore, the reason for the apparent recent introgression of *O. mossambicus* into the GIFT strain is unknown.

Notably, in the present study, we observed the allele *UNH138*164* which was fixed in OM-S, in *O. niloticus* populations (ON-CD, ON-GIFT, ON-AY, ON-AIT). However, this was not solid evidence of genetic introgression from OM-S because the fixation of a locus of a highly polymorphic microsatellite, although not frequently reported, is not impossible (Antoro et al., 2006). Additionally, the results from the model-based clustering did not support an assumption of introgression from *O. mossambicus*. It is possible that this allele (*UNH138*164*) is naturally shared among *O. niloticus* and *O. mossambicus* and the fixation in OM-S may be a result of small population of OM-S as supported by very low A_r and heterozygosity.

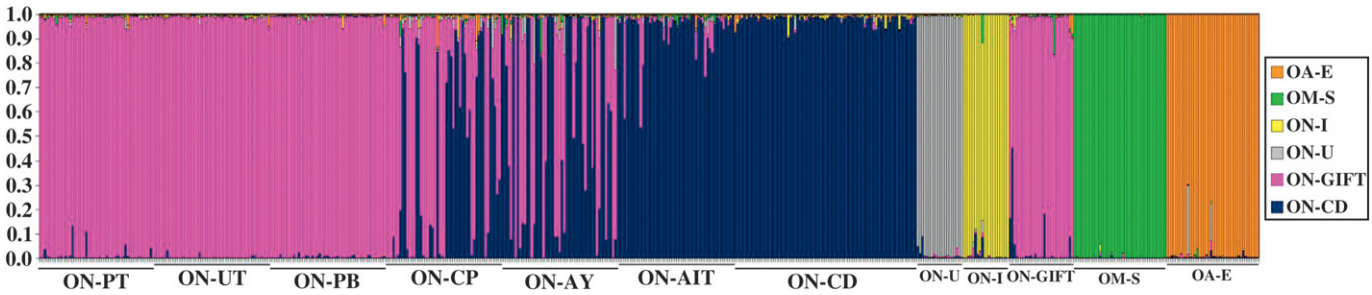

Fig. 3. The probability of assignment of individuals to each of the six genetic groups ($K = 6$) defined in the present study. Each vertical bar represents an individual and each color represents a probability that the individual is assigned to each genepool (admixture proportion, q_i).

The apparent presence of *O. aureus* alleles in ON-CP was unexpected. In fact, *O. aureus* was introduced to Thailand as a male parent for production of all-male tilapia by interspecific hybridization with *O. niloticus* (Tangtrongpiros, 1980). The reason of the introgression was unknown.

4.3. Possible impacts of interspecific introgression

Introgression in fishes is assumed to be generally detrimental. For example, in Nile tilapia, introgression from *O. mossambicus* was assumed to result in reduced growth rate (Macaranas et al., 1995; Micha et al., 1996), decline in fecundity and early maturation (Amarasinghe and De Silva, 1996; McKinna et al., 2010). Introgression from African catfish, *Clarias gariepinus*, was assumed to be responsible for decline of abundance of wild Günther's walking catfish, *C. macrocephalus* (Na-Nakorn et al., 2004; Senanan et al., 2004). Theoretically, interspecific introgression can be beneficial as source of genetic variation in recovery projects for rare species (Allendorf and Luikart, 2007; e.g. the rare Galápagos finch, Grant and Grant, 1998). To date, empirical data for impact of interspecific introgression is limited and seems to vary among species groups. For example, there was reduced fitness of introgressed individuals between bighead, *Hypophthalmichthys nobilis* and silver carp, *H. molitrix* (Lamer and Dolan, 2010), while performance of hybrid and introgressed *Mytilus* relative to parental species varied considerably among environments (Shields et al., 2008). In aquaculture, it is likely that uncontrolled introgression may have adverse impacts; for example introgression from *O. mossambicus* has led to undesired traits in feral Nile tilapia (Amarasinghe and De Silva, 1996). In contrast, the same introgression occurring in the GIFT strain did not result in unwanted traits (e.g. Department of Fisheries, 2006), which may be a consequence of selection which targeted improved growth and appearance (Bentsen et al., 1998; Eknath and Acosta, 1998).

4.4. Intra-specific genetic introgression and possible impacts

High levels of introgression occurred in ON-CP and ON-AY, which are GIFT-derived and Chitralada-derived strains, respectively. Intraspecific introgression occurring between genetically distinct populations or between domesticated and wild populations often leads to adverse impacts due to outbreeding depression (e.g., increased infectious disease susceptibility of introgressed individuals between geographic populations of largemouth bass, Goldberg et al., 2005; coho salmon, *Oncorhynchus kisuth*, Tymchuk et al., 2006). In the case of GIFT and Chitralada strains, such adverse impacts may not exist because the donor and recipient strains are related (ON-CD was one of the grandparents of the founding stock of GIFT, Eknath et al., 2007).

However, crossbreeding of genetically diverse populations was expected to increase genetic variation (e.g. in Adriatic grayling, *Thymallus thymallus* L. 1785; Sušnik et al., 2004). The underlying reason for crossbreeding in the Chitralada-derived ON-AY was to restore genetic variation with concern for maintaining Chitralada integrity. Nevertheless, due to the inaccurate naming of the advanced generations of GIFT as Chitralada III, interstrain crossing occurred (Prayad Soda, pers. comm.). Interstrain crossing was more intensive during the founding of ON-CP in order to broaden the genetic background to enhance selection response. The present results showed that the introgression did not increase genetic variation of the GIFT-derived strains, but did for the Chitralada-derived strains. Detailed discussion on genetic variation is in the later section.

Impacts of introgression on morphology and/or economically important traits may exist. The Chitralada-derived and GIFT strains differ in growth performance; e.g., growth rate and fillet yield of GIFT were higher than in Chitralada-derived strains (Dan and Little, 2000; Dey et al., 2000; Hussain et al., 2000; Rutten et al., 2004). Hence, crossing of these two strains may result in decline of growth performance of the offspring relative to a superior parental strain (the GIFT strain). Moreover, introgression may reduce the superiority of GIFT morphology over the Chitralada strain. For example, the GIFT strain has a smaller proportion of head and a broader trunk than the Chitralada strain (Department of Fisheries, 2006). To date, information on this issue is not available, hence worth closely monitoring.

Introgression observed in ON-PT may be a remnant of the Chitralada strain that was a grandparent of the base population of GIFT rather than recent introgression (inferred on the base of model-based clustering). In contrast, the presence of GIFT alleles in the Chitralada-derived ON-AIT strain may be due to unintentional crossing of ON-AIT with GIFT-derived fish brought in for other purposes (ADB, 2005). Slight introgression may not change the appearance of a strain, and thus possibly causes confusion when the strain is used as founder stock or for genetic improvement programs.

4.5. Genetic variation of the hatchery stocks of Nile tilapia in major hatcheries in Thailand

High levels of genetic variation in an aquaculture stock are important to prevent possible negative impacts of inbreeding and can facilitate rapid gains in genetic improvement programs (Dudash and Fenster, 2000; Miller and Kapuscinski, 2003). Due to the fact that genetic variation influences fitness and adaptability of the populations, management of genetic diversity of broodstock is important for the production of high quality seed for aquaculture (Taniguchi, 2003). Genetic variation, especially effective number of alleles per locus, of the hatchery populations included in this study ($A_e = 4.10–5.24$) was lower than that of wild populations from the Nile River (5 populations, 50 fish/population, based on 6 microsatellite loci; $A = 7.22–9.42$; $H_e = 0.493–0.900$) (Hassanien and Gilbey, 2005). Lower genetic variation in hatchery stocks is mainly a consequence of small effective population size (N_e) leading to allele loss due to genetic drift (Allendorf and Phelps, 1980; e.g., in turbot, *Scophthalmus maximus*, Coughlan et al., 1998; Atlantic salmon, Withler et al., 2005; *Litopenaeus vannamei*, Perez-Enriquez et al., 2009). Small N_e, which theoretically reduces heterozygosity in later generations (Crow, 1986), did not have a pronounced effect on reduction of H_e (e.g., in guppy, Shikano et al., 2001; Allendorf and Phelps, 1980). In addition, empirical data also indicated that selection decreased genetic variation based on molecular markers in Atlantic salmon (Norris et al., 1999), and Nile tilapia (Romana-Eguia et al., 2005), despite the neutrality of genetic markers used for the studies.

The genetic variation of the GIFT strain in the present study ($A_r = 7.36 \pm 2.19$, $H_e = 0.75 \pm 0.13$) was slightly lower than that reported by Romana-Eguia et al. (2004) ($A = 10.0$, $H_e = 0.81$) on the same specimens using 5 microsatellite loci. This likely is an artifact of using a small number of loci in the earlier study, while the study by Rutten et al. (2004) which was based on 14 loci supported our result ($A = 7.5$, $H_e = 0.70$). The Chitralada strain was characterized with low allele diversity ($A = 5.4–6.8$, Ambali et al., 2000; Romana-Eguia et al. 2004; Rutten et al., 2004) compared to the average for freshwater fishes ($A = 7.5$, DeWoody and Avise, 2000), while heterozygosity varied considerably ($H_e = 0.624–0.82$, Ambali et al., 2000; Romana-Eguia et al., 2004; Rutten et al., 2004) and was relatively high relative to the average for freshwater fishes (0.46, DeWoody and Avise, 2000).

Among the two major origins of Nile tilapia hatchery populations in Thailand, ON-GIFT has slightly higher molecular genetic variation than the Chitralada population. High genetic variation may be a result of using genetically diverse founder stock (the founder population originated from a combination of eight different Nile tilapia strains) and a relatively high number of brooders (an average of 92 sires and 163 dams/generation during the first five generations) (Bentsen et al., 1998; Eknath and Acosta, 1998; Eknath et al., 1993) and N_e of 88 individuals from generation 6–13 (Ponzoni et al., 2010b). In addition, despite the selection pressure exerted during ten years of the

selection program, good broodstock management, e.g. using a large number of brooders, relatively equal family contributions, and 1:1 mating ratio, may have contributed to retaining the original genetic diversity of the stock. The lower genetic variation of Chitralada strain was not surprising because the strain was founded by a single stock comprising 120 fish transferred from Egypt to Japan (Pullin and Capili, 1988) and the number of brooders was suddenly reduced to 38 fish (Damrongratana and Kessanchai, 1966) when it was introduced from Japan to Thailand. Moreover, it was likely that genetic broodstock management was not systematically applied to this stock.

The introgression from Chitralada to GIFT did not elevate genetic variation as has been reported for other species (Sušnik et al., 2004). This may be because Chitralada was among the founders of GIFT; thus, introgression from Chitralada did not bring in novel alleles to GIFT while heterozygosity may have been elevated in the early generations but declined as random mating proceeded (Falconer and Mackay, 1996). On the contrary, introgression from GIFT increased allelic richness of Chitralada-derived strains, while the result was not pronounced for heterozygosity.

Studies on the relationship between genetic variation and performance have given inconclusive results. For example, heterozygosity was positively related with average tolerance to salinity of guppy (*Poecilia reticulata*) populations (Shikano and Taniguchi, 2002). Heterozygosity of chinook salmon (*Oncorhynchus tshawytscha*) was positively related to fecundity and gonado-somatic index (percentage of gonad weight to body weight), but not with the majority of other reproductive traits (Heath et al., 2002). Immune response to IHNV and mortality rates did not correlate with genetic variation of rainbow trout (*Oncorhynchus mykiss*), while a positive correlation between heterozygosity and feed conversion ratio was observed (Overturf et al., 2003). Allozyme variation of Atlantic salmon (*S. salar*) correlated with body weight, length and fluctuating asymmetry, while microsatellite-based genetic variation did not show such relationships (Borrell et al., 2004). Despite a complicated relationship between genetic variation and performance, the relatively large genetic variation in a majority of hatchery stocks included in the present study suggested that they should be able to retain or improve their performance relative to the original populations. The GIFT strain cultured in Fiji lost approximately 20% of its genetic variation relative to the reference GIFT population. This loss was thought to be responsible in part for the decline of tilapia production in Fiji (McKinna et al., 2010). Production likely could be increased through addition of new genetic resources.

4.6. Genetic variation, broodstock management and implications

If the broodstock for maintaining the commercial strains is not separated from the broodstock used for commercial seed production, undesirable change may occur in the broodstock populations. It is fortunate that the broodstocks included in this study were managed separately from the brooders used for seed production.

According to broodstock management regimes, the hatchery populations used in this study may be divided into four categories; 1) closed populations with within-family selection (comprising ON-PT and ON-UT); 2) closed population with mass selection (ON-AIT and ON-PB); 3) open population (with introduction from other sources) with within-family selection (ON-CP); and 4) open population with mass selection (ON-AY). Although genetic variation of these hatchery populations was not statistically different, a trend was apparent in which the open populations had relatively high allelic diversity (e.g. $A_r = 8.03 \pm 2.74$–8.35 ± 2.69) compared to the "closed" populations (6.61 ± 2.31–7.66 ± 2.76). However, among the open population, the relatively low A_e (effective number of alleles per locus) of ON-AY ($A_e = 4.71 \pm 2.02$) indicated that it is prone to allele loss if N_e is not high enough (recommended $N_e = 50$, FAO, 1980; Tave, 1993). Mass selection which is currently performed on ON-AY can reduce N_e because of high variance of family sizes (Falconer and Mackay, 1996). On the contrary, within-family selection could ensure equal family contributions in an effort to increase or optimize N_e.

Likewise, the low value of A_e in the closed population, ON-AIT put this population at risk of losing alleles. In addition, mass selection currently applied to this population may result in overall decline of genetic variation due to genetic drift and inbreeding (Falconer and Mackay, 1996). Therefore, close monitoring is required (e.g., monitoring of selection response, signs of inbreeding depression, etc.); eventually, introduction of genetically different stocks may be required to mitigate genetic erosion.

It is recommended that the purity of Chitralada should be maintained because this strain has adapted to the Thai environment and thus may have advantageous characters that have not been detected. However, good broodstock management should be practiced to prevent loss of genetic variation. In the future, the Chitralada strain may be a valuable source of genes for mitigation of genetic deterioration in other stocks. Similarly the GIFT-derived strains, including ON-PT, ON-UT and ON-PB, should be managed so that in the future they maintain their genetic differentiation and hence can be used as sources of genetic resources for other populations.

5. Conclusions

The present study provided evidence that: 1) genetic change in GIFT-derived populations has occurred and could eventually result in genetically differentiated strains; 2) the GIFT-derived strains retained high genetic variation probably through using large number of parents; 3) introduction of new genetic material into a population increased genetic variation of populations with low genetic variation; 4) closed populations tended to have lower genetic variation; and 5) unintentional introgression both at intra- and interspecific levels occurred in certain Nile tilapia strains.

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