Kasetsart J. (Nat. Sci.) 46 : 200 - 216 (2012)

Genetic Diversity of Feral Populations of Nile Tilapia (*Oreochromis niloticus*) in Thailand and Evidence of Genetic Introgression

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ABSTRACT

The study aimed to understand the genetic diversity of Thai feral populations of Nile tilapia, *Oreochromis niloticus*, which make a substantial contribution to freshwater fish production in the country. The possible introgression from other tilapia populations/species was also investigated. Fourteen microsatellite primers were used to study three feral *O. niloticus* populations collected from reservoirs representing three types of habitats: 1) a wet-land (Sam Roi Yot, ON-SY); 2) a small reservoir (Bang Phra reservoir, ON-BP); and 3) a large reservoir (Bueng Boraphet, ON-BB). The results showed genetic variation that was high in ON-BP (A_r = 7.99, H_e = 0.75) and relatively low in ON-BB (A_r = 5.71, H_e = 0.62) and ON-SY (A_r = 5.78, H_e = 0.71). Genetic data were imported from two reference populations of *O. niloticus* [one population each from the Chitralada Villa Royal Residence, Thailand (ON-CD) and the genetically improved GIFT strain (ON-GIFT)] and one *O. mossambicus* population from South Africa for the model-based clustering analyses. ON-CD contributed to substantial portions of the gene pool of the feral populations. Genetic differentiation among the feral populations was significant ($P < 0.017$, Bonferroni corrected). Introgression at the intraspecific level (from ON-GIFT to ON-BP and ON-BB) was observed while interspecific introgression between *O. niloticus* and *O. mossambicus* was apparent in ON-SY.

Keywords: feral populations, *Oreochromis niloticus,* genetic difference, introgression, Thailand

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* has been transferred beyond its native range in Africa to natural and manmade water bodies worldwide (Trewavas, 1983). Consequently, non-native feral populations are now established in at least 74 countries (Fishbase, 2011). Feral populations have substantially contributed to freshwater fisheries in many countries, exemplified by Thailand, Indonesia and El Salvador (FAO, 2011). However, the production of feral Nile tilapia in some countries was considered to be likely in decline, for example, in Sri Lanka (Amarasinghe and De Silva, 1996), El Salvador, Ecuador, Fiji and Thailand (FAO, 2011). In fact, the reasons for the decline in production are complicated and include habitat destruction (for example, urbanization

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Received date : 06/10/11 Accepted date : 30/01/12

and dam construction) and/or over exploitation (Mitchell and Braun, 2003; Froese and Pauly, 2005; Hussain, 2010). However, genetic factors were believed to account for such reductions, for example genetic introgression by *O. mossambicus* (Amarasinghe and De Silva, 1996), despite the potential advantage resulting from increased genetic variation (Sukmanomon *et al.*, 2012). Furthermore, the feral populations each originated most likely from a small number of founders and hence, may have low genetic variation. Thus, they may not adapt to fluctuating environments. Due to the importance of the feral tilapia production to low income, rural communities, it is worthwhile to evaluate the genetic variation of the feral Nile tilapia populations in Thailand.

O. niloticus in Thailand originated from 50 individuals brought to Thailand by Crown Prince Akihito of Japan as a present for King Bhumipol Aduljadej in 1965 (Damrongratana and Kessanchai, 1966). The stock was said to have originated from 200 fish being transferred from Egypt (Pullin and Capili, 1988). Eventually, only 38 introduced individuals survived and were reared in the Chitralada Villa Royal Residence. The successive generations of these founders were given to the Department of Fisheries, Thailand and have been used for aquaculture and stock enhancement (Tangtrongpiros *et al.*, 1993). Consequently, *O. niloticus* has established populations in natural waters around Thailand through escapees from aquaculture and regular intentional stocking (Asian Development Bank, 2005).

The objectives of this study were to: 1) assess genetic diversity of feral populations of Nile tilapia in Thailand, 2) elucidate the contribution of the newly introduced strain of Nile tilapia to the feral populations and 3) detect interspecific introgression from *Oreochromis mossambicus* of which feral populations were established in Thailand (Karnasuta *et al.*, 1999). The results of this study should benefit the management and utilization of these feral populations.

MATERIALS AND METHODS

Sample collection

The geographical origins and sample sizes of all examined populations are given in Figure 1 and Table 1. Samples of feral populations of *O. niloticus* were collected during March 2004–May 2006, from three reservoirs representing different habitats; 1) a wetland (Sam Roi Yot wetland in Prachuap Khiri Khan province, southern Thailand); 2) a small reservoir (Bang Phra reservoir in Chon Buri province, central Thailand); and 3) a large reservoir (Bueng Boraphet in Nakhon Sawan province, northern Thailand).

The feral tilapia population in the Sam Roi Yot reservoir originated from individuals of *O. niloticus* (Chitralada strain) and *O. mossambicus* that had been cultured around this site with at least some evidence of escapees due to flooding (Rajitparinya *et al.*, 1973). Additionally, feral tilapias could have originated from fish stocked in the adjacent Pranburi Dam (Chittapalapong *et al.*, 1997) which connects to this reservoir. The Bang Phra reservoir was stocked with tilapia fingerlings (Chitralada strain) in the early period of introduction (Chookajorn *et al.*, 1991) and has been stocked recently with fingerlings of the GIFT strain (Sangduen Naksuwan, pers. comm.). While there are no formal records on the stocking of Nile tilapia in the Bueng Boraphet reservoir, the feral tilapia population established here could have come from aquaculture escapees into the Nan River which runs into this reservoir (Srichareondham *et al.*, 1993).

A piece of caudal fin (about 50 mg) was collected from each individual, kept in 95% ethyl alcohol and delivered to the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok. The DNA extraction protocol of Taggart *et al.* (1992) was performed with slight modification (for example, fin tissue

Table 1 Sources of three feral populations of *Oreochromis niloticus* in Thailand, two reference populations of *O. niloticus* from the Chitralada and GIFT strains and one population of *O. mossambicus* from South Africa. (N = sample size).

Population name	N	Location	Coordinates
Feral populations			
Bang Phra (ON-BP)	37	Bang Phra Reservoir, Si Racha, Chon Buri	13°11′60″N 100°58′60″E
Bueng Boraphet (ON-BB)	24	Bueng Boraphet Reservoir, Nakhon Sawan	15°41′00″N 100°15′00″E
Sam Roi Yot (ON-SY)	44	Sam Roi Yot Reservoir, Kui Buri, Prachuap Khiri Khan	12°11′00″N 99°57′00″E
Reference populations			
Chitralada strain (ON-CD)	80	Brooder ponds, Chitralada Villa Royal Residence, Dusit, Bangkok	13°46′14″N 100°31′18″E
GIFT strain (ON-GIFT)	28	The ninth generation, genetically improved farmed tilapia (G_9 GIFT) originally collected from the Philippines (provided by N. Taniguchi, Tohoku University, Japan)	14°35′00″N 121°00′00″E
O. mossambicus (OM-S)	40	A culture population from South Africa (provided by G. Hulata, the Agricultural Research Organization, Israel)	28°30′00″S 30°30′00″E

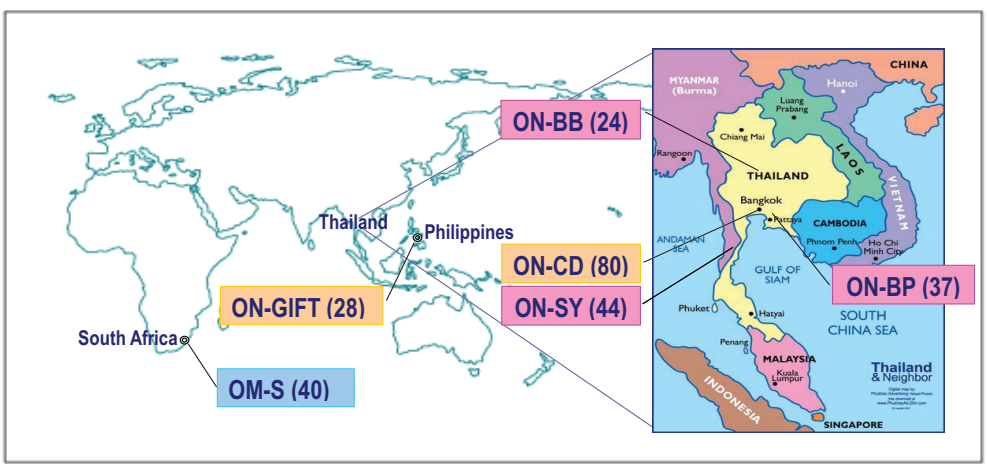

Figure 1 Map showing sampling localities of three feral populations of *Oreochromis niloticus* in Thailand (ON-BP = Bang Phra population; ON-BB = Bueng Boraphet population; ON-SY = Sam Roi Yot population), two conspecific reference populations (ON-CD = Chitralada Villa Royal Residence, Thailand; ON-GIFT = ninth generation GIFT strain) and one population of *O. mossambicus* (OM-S). Values in parentheses represent sample sizes.

was used instead of blood; DNA precipitation was done using ethanol instead of propanol), and the DNA was stored at -20 °C until used.

The model-based clustering analyses included genetic data based on the same sets of microsatellite primers—namely, the two *O. niloticus* populations (Sukmanomon *et al.*, 2012) comprising the Chitralada strain and ninth generation GIFT strain (Table 1). One population of *O. mossambicus* from South Africa was also included to detect possible interspecific introgression.

Microsatellite primers and polymerase chain reaction conditions

Fourteen microsatellite primers developed from DNA samples of *Oreochromis niloticus* by Lee and Kocher (1996) were used as detailed in Sukmanomon *et al.* (2012). Each of the six primer pairs (*UNH172, UNH211, UNH216, UNH222, UNH212* and *UNH 160*) was run in a single locus PCR performed in a 10 μL reaction volume containing 2.5 ng of template DNA, 1× PCR buffer, 1.5mM $MgCl_2$, 100 μM of each dNTP, 0.25 μM of each primer and 0.2 units of *Taq* DNA polymerase (Fermentas). Polymerase chain reaction (PCR) was carried out in a PX2 thermal cycler (Thermo Fisher Scientific, Waltham, MA,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 (see Sukmanomon *et al.*, 2012), 1 min at 72 °C, with final extension of 5 min at 72 °C. Separation of the PCR products was carried out on 4.5% polyacrylamide gel and visualized by silver staining (Bassam *et al.*, 1991). Allele sizes were designated relative to the M13 DNA ladder.

In addition, two multiplex PCR products, each comprised of four microsatellite primers (Multiplex 1: *UNH213*, *UNH153*, *UNH132*, *UNH138*; Multiplex 2: *UNH192*, *UNH1004*, *UNH982*, *UNH173*) were scored against all DNA samples. Each 10 μL total reaction volume consisted 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 the relative fluorescence in relationship to the other primers in the multiplex, 1 μL dNTPs (20 μM) and 0.12 μL FastStrat *Taq* DNA polymerase (Roche, Basel, Switzerland). Forward primers were fluorescently end-labeled with 6-FAM, VIC, NED or PET (Applied Biosystems, Carlsbad, CA, USA) for visualization. A two-step PCR program was used for the multiplex amplification with the GeneAmp® PCR System 9700 (Applied Biosystems, Carlsbad, CA, 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 (detailed in Sukmanomon *et al.*, 2012), 1 min at 72 °C; 10 cycles of 30 s at 94 °C, 45 s at a second T_a °C (detailed in Sukmanomon *et al.*, 2012), 45 s at 72 °C and a final extension of 10 min at 72 °C. An amount of 1 μL of diluted multiplex PCR product was run on an ABI 3130xl Genetic Analyzer with a LIZ600 size standard (Applied Biosystems, Carlsbad, CA, USA). Then, the electropherograms and allelic sizes were analyzed using GeneMapper®Software Version 4.0 (Applied Biosystems, Carlsbad, CA, USA). The facilities for multiplex PCR were provided by the Genomic Variation Laboratory, University of California, Davis, CA, USA.

Data analyses

The following analyses were performed on multi-locus genotypes. A test for conformation to the Hardy-Weinberg equilibrium (HWE) by a Markov chain approximation of the exact test (Guo and Thompson, 1992) was 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. The test for linkage disequilibrium based on the chi-square test was performed using the program GENETIX Version 4.05.2 (Belkhir *et al.*, 2004), wherein the disequilibrium coefficient (*D*) was provided.

Evidence of a "null-allele" was tested

when departure from the HWE toward homozygote excess was observed using the program Micro-Checker version 2.2.3 (Van Oosterhout *et al.*, 2004). Then, the genotypes of populations showing evidence of null alleles were adjusted accordingly, wherever appropriate. The populations were once again tested for departure from the HWE.

Genetic variation within populations (average number of alleles/locus-A, effective number of alleles/locus-A_e and observed and expected heterozygosity;–H_o and H_e, respectively) was estimated using the program POPGENE version 1.32 (Yeh and Boyle, 1999). A 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 independent *t*-test comparison in the SPSS statistical package version 11.5.0 (SPSS Inc., Chicago, USA) was employed to test the differences between populations for each parameter (A, A_e, A_r, H_o and H_e), followed by *post hoc* multiple comparison.

To assess the overall population differentiation, *F*-statistics (F_{ST}, F_{IS} and F_{IT}) were estimated as F_{ST} (theta, θ), F_{IS} (f) and F_{IT} (F) according to Weir and Cockerham (1984) using the program FSTAT version 2.9.3.2 (Goudet, 1995). The genetic differentiation between populations based on the allele frequency distribution was tested using GENEPOP version 4.0 (Raymond and Rousset, 1995b; Rousset, 2008). Then, the pairwise F_{ST} between population pairs with significant probability was calculated using ARLEQUIN version 3.11 (Excoffier *et al.*, 2005).

To detect population admixture, a model-based clustering method for inferring population structure was employed using the program STRUCTURE version 2.3.3 (Pritchard *et al.*, 2000; Hubisz *et al.*, 2009). Analysis was run using the admixture ancestry model with twenty runs with 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) calculated using STRUCTURE HARVESTER version 0.6.7 (Earl, 2001). Then, the averaged proportion of membership of each cluster was calculated for each predefined population/species using a Bayesian clustering algorithm in STRUCTURE. The clustering is meaningful when a proportion of membership is apparently highest in a single cluster and the non-contaminated population would have a proportion of membership equal to a value of 1 in one cluster and 0 in the other clusters. The population of origin would be a reference population that showed the highest proportion of membership in the same cluster with the feral population(s).

In addition, the individual bar plot which showed probabilities of assigning an individual to each cluster (admixture proportion, q_i) was applied where it gave better detailed results. An admixed individual assigned to two genetic groups at $q_i = 0.5$ is a first generation hybrid, while the introgression was characterized by q_i higher or less than 0.5; only the assignments with $q_i > 0.1$ were taken into account.

RESULTS

Hardy-Weinberg equilibrium and linkage disequilibrium

The Fisher exact test showed that ON-BB conformed to the HWE while significant homozygote excess was observed in ON-BP and ON-SY ($P < 0.0036$ following the Bonferroni correction). However, the null allele test suggested adjustment of genotypes due to the presence of null alleles of six loci in ON-BP (*UNH211, UNH222, UNH212, UNH153, UNH192* and *UNH1004*); of four loci in ON-SY (*UNH172, UNH222, UNH160* and *UNH1004*); and of one locus in ON-BB (*UNH172*). The re-analyses based on the adjusted genotypes showed the same results where the locus-wise HWE tests showed that every locus

conformed to the HWE in ON-SY and homozygote excess at three loci in ON-BP.

Linkage disequilibrium (LD) was observed in all populations. The most intensive LD, shown at 45 loci pairs, was observed in ON-BP followed by 10 loci pairs in ON-BB and 8 loci pairs in ON-SY. The disequilibrium coefficient (D) indicated that linkage disequilibrium was more recent in ON-SY as indicated by the high value of the mean D (0.0668 ± 0.0199) compared with ON-BB (D = 0.0305 ± 0.0033) and ON-BP (D = 0.0237 ± 0.0017).

Genetic variation within populations of feral populations

Among the three feral populations, ON-BP had significantly ($P < 0.05$) higher allele diversity (A and A_r) than ON-BB and ON-SY (A = 9.50 ± 2.38, A_r = 8.42 ± 1.87; A = 5.79 ± 2.12, A_r = 5.79 ± 2.12; and A = 6.57 ± 1.99, A_r = 6.06 ± 1.71 for ON-BP, ON-BB and ON-SY, respectively). The observed heterozygosity of ON-BP (H_o = 0.74 ± 0.08) was higher ($P < 0.05$) than for ON-BB (0.61 ± 0.18) while it was not different from ON-SY (0.71 ± 0.07), as shown in Table 2.

Genetic diversity among populations

The overall F_{ST} (θ) showed significant population differentiation at least for a pair of populations among the three feral populations (θ = 0.087; $CI_{99\%}$ = 0.066–0.109).

Further testing based on the allele frequency distribution (Fisher's exact test, $P < 0.017$, Bonferroni corrected) and pairwise F_{ST} ($P < 0.017$, Bonferroni corrected) showed significant differences between all pairs of populations. The range in pairwise F_{ST} among the three feral populations was 0.0538–0.1376.

Population clustering and population origin

To identify the genetic composition of the feral populations, the multi-locus data of two populations of Nile tilapia including the Chitralada strain and the GIFT strain (Sukmanomon *et al.*, 2012) and *O. mossambicus* samples were incorporated in the model-based clustering analyses. Based on the highest ΔK value (208.42), the results showed that the best fitted number of populations (K) was 3. The proportion of membership, averaged across individuals in a population (Table 3), clearly assigned the reference populations/species into separate clusters with a high proportion of membership (0.974–0.996), as shown in Table 3. As expected, all of the feral populations shared the highest proportion of membership with ON-CD (0.724, 0.914 and 0.868 for ON-BP, ON-BB and ON-SY, respectively). The contribution of the newly introduced ON-GIFT was relatively low as indicated by a proportion of membership of 0.268 (for ON-BP) whereas the

Table 2 Genetic variation within three feral *O. niloticus* populations in Thailand.

Population	N	Genetic variation					
		P	A	A_e	A_r	H_o	H_e
Bang Phra (ON-BP)	37	14	9.50[a]	4.68[a]	8.42[a]	0.74[a]	0.77[a]
			(2.38)	(1.59)	(1.87)	(0.08)	(0.08)
Bueng Boraphet (ON-BB)	24	14	5.79[b]	2.98[b]	5.79[b]	0.61[b]	0.62[b]
			(2.12)	(1.17)	(2.12)	(0.18)	(0.18)
Sam Roi Yot (ON-SY)	44	14	6.57[b]	3.74[ab]	6.06[b]	0.71[a]	0.72[a]
			(1.99)	(1.00)	(1.71)	(0.07)	(0.09)

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.

Mean values in the same column with different lower-case letter superscripts are statistically significant ($\alpha = 0.05$).

contribution of ON-GIFT was considered as nil in ON-BB and ON-SY (proportions of membership with cluster 2 were 0.082 and 0.012 for ON-BB and ON-SY, respectively). The contribution of *O. mossambicus* (OM-S) was considered as nil (proportion of membership < 0.100) in all but one population whereas 0.121 of ON-SY was assigned to the same cluster as OM-S.

The individual bar plot (Figure 2) supported the model-based clustering, in that ON-BP was comprised mainly of pure ON-CD (23 individuals; q_1 = 0.888–0.996) and small proportions of pure GIFT (7 individuals, q_2 = 0.964–0.993) and 7 admixed individuals between ON-CD and ON-GIFT (q_1 = 0.172–0.880; q_2 = 0.116–0.826). The ON-BB comprised mainly of ON-CD (21 pure ON-CD; q_1 = 0.961–0.997). Nevertheless, despite the lack of any contribution being revealed by the model-based clustering, the individual bar plot showed contributions from ON-GIFT in this populations as indicated by the presence of three admixed individuals between ON-CD and ON-GIFT (q_1 = 0.118–0.620; q_2 = 0.379–0.881). No contribution from ON-GIFT was observed in ON-SY, which comprised 26 pure individuals of ON-CD (q_1 = 0.898–0.995), and 18

Table 3 Proportion of membership of each pre-defined population in each of three clusters (K=3). (STRUCTURE: 100,000 burn-in period; 100,000 replications)

Given population	Inferred cluster			Number of individuals
	1	2	3	
ON-BP	0.724	0.268	0.008	37
ON-BB	0.914	0.082	0.003	24
ON-SY	0.868	0.012	0.121	44
ON-CD	0.993	0.005	0.002	80
ON-GIFT	0.018	0.974	0.008	28
OM-S	0.002	0.002	0.996	40

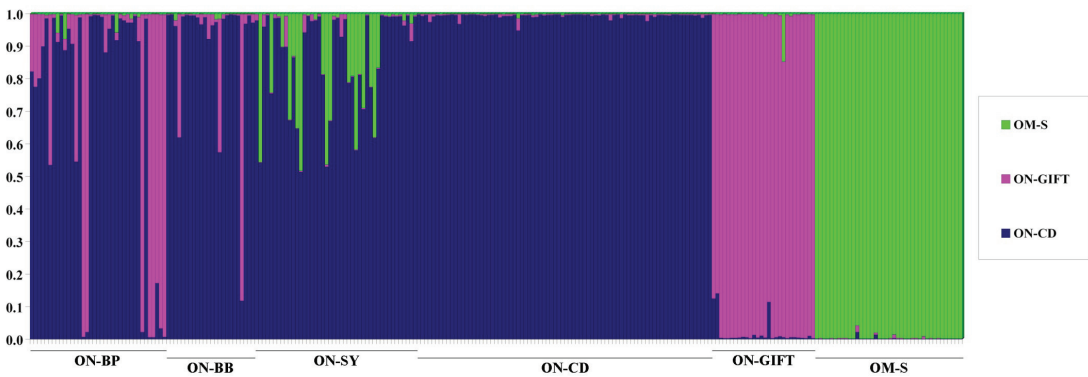

Figure 2 Probability of assignment of individuals to each of three genetic groups (K = 3) defined in the present study. Each vertical bar represents an individual and each color represents a probability that the individual is assigned to each genepool using the admixture proportion, q_i, from output generated by the STRUCTURE program. (ON-BP = Bang Phra population; ON-BB = Bueng Boraphet population; ON-SY = Sam Roi Yot population; ON-CD = Chitralada Villa Royal Residence, Thailand population; ON-GIFT = ninth generation GIFT strain; OM-S = *O. mossambicus* population.)

admixed individuals between ON-CD and OM-S (q_1 = 0.515–0.896; q_3 = 0.102–0.483).

DISCUSSION

Evidence was found that ON-CD (the Chitralada strain) was a major contributor to the gene pools of the three feral populations of *O. niloticus* in Thailand. Contribution from ON-GIFT to the feral populations was observed for ON-BP and ON-BB. Genetic differentiation was apparent among the three feral populations. The feral population from Bang Phra (ON-BP) had relatively higher genetic variation than those of the Bueng Boraphet (ON-BB) and Sam Roi Yot (ON-SY) populations. Interspecific introgression (from *O. mossambicus* to *O. niloticus*) was observed only in the Sam Roi Yot (ON-SY) population.

Evidence of having ON-CD as a population of origin and intra- and inter-specific introgression

All of the feral tilapia populations shared a substantial proportion of membership with a reference population from the Chitralada Villa Royal Residence (ON-CD) as revealed by the model-based clustering. This finding agreed with prior information, which indicated the extensive release of ON-CD into many reservoirs in Thailand (Chookajorn *et al.*, 1991; Tangtrongpiros *et al.*, 1993; Karnasuta *et al.*, 1999). In addition, ON-CD was introduced into the Sam Roi Yot reservoir through aquaculture escapees (Rajitparinya *et al.*, 1973).

Genetic introgression was detected at the intra- and inter-specific levels. The introgressions likely play a significant role in shaping the genetic constitution of the feral populations. Introgression is an important mechanism to increase genetic variation (Grant and Grant, 2002; Almodóvar *et al.*, 2006; Allendorf and Luikart, 2007), whereas adverse impacts are also a concern due to the so-called "out-breeding depression" which refers to the breaking of linkage groups favoring local adaptation (Allendorf *et al.*, 2001; Goldberg *et al.*, 2005; Tymchuk *et al.*, 2006). The model-based clustering showed the remarkable contribution of ON-GIFT to ON-BP. This was in line with the significant homozygote excess and intensive linkage disequilibrium (45 loci pairs) observed for ON-BP which indicated population admixture known as the "Wahlund effect" (Falconer and Mackay, 1996; Hartl and Clark, 1997). Moreover, the individual bar plots clearly supported the population admixture by revealing 18.9% each of relatively pure ON-GIFT and admixed individuals (ON-GIFT and ON-BP) in ON-BP. The LD coefficient suggested that introgression in ON-BP was not recent (Allendorf *et al.*, 2001).

Introgression from ON-GIFT was also observed in ON-BB, but at a lower magnitude (not detected in the model-based clustering; 12.5% admixed individuals revealed by the individual bar plot). There has been no documented release of ON-GIFT into Bueng Boraphet reservoir. Thus, the introgression may have come from escapees from nearby cage cultures (Srichareondham *et al.*, 1993).

No intra-specific introgression was observed in ON-SY but introgression from *O. mossambicus* was detected. This was not surprising because GIFT has never been released into this reservoir and there was no documented evidence of aquaculture involving GIFT in this area, whereas around the Sam Roi Yot reservoir, *O. mossambicus* has been widely cultured since it was first introduced into Thailand in 1949 (Karnasuta *et al.*, 1999). This species survives well in habitats with low salinity such as the area surrounding the Sam Roi Yot reservoir. As such, when *O. niloticus* was released into this reservoir, introgression may have occurred. The introgression between these species is common and has been previously reported in Thailand (Karnasuta *et al.*, 1999) and worldwide (Macaranas *et al.*, 1986; De Silva and Ranasinghe, 1989; De Silva, 1997).

Genetic differentiation between feral populations of Nile tilapia in Thailand

Despite their common origin, the three feral populations were genetically divergent. This may be explained by a combination of; introgression from other gene pools (for example, ON-GIFT and OM-S); genetic drift after the initial founding event (Ramstad *et al.*, 2004; Allendorf and Luikart, 2007); the cumulative effect of natural selection across at least 30 generations (1 year/ generation); and limited gene flow among the feral populations.

Genetic variation within populations

Genetic variation within populations, especially allele diversity, depends on the genetic constitution of the founding stock and its effective population size since the founding event and in successive generations. There is a tendency for a population under exploitation to reduce its effective population size over time [for example, New Zealand snapper, *Pagrus auratus* in Tasman Bay from 1950 to 1998 (Hauser *et al.*, 2002); plaice, *Pleuronectes platessa* in the North Sea and Iceland from 1924 to 2002 (Hoarau *et al.*, 2004)] which resulted in a decline in genetic variation. However, in the present study, one population (ON-BP) had higher allele diversity than the population of origin (Chitralada; A = 5.4–6.8, and H_e = 0.624–0.82; Ambali *et al.*, 2000; Romana-Eguia *et al.*, 2004; Rutten *et al.*, 2004; A = 6.07 ± 1.90; H_e = 0.65 ± 0.15; Sukmanomon *et al.*, 2012). From this it may be inferred that introgression from GIFT, as discussed in the above section, has brought in novel alleles to ON-BP.

ON-BP had higher genetic variation, especially allele diversity, than that of ON-BB and ON-SY despite the relatively small habitat size (1,856, 21,238 and 9,808 ha for Bang Phra, Bueng Boraphet and Sam Roi Yot, respectively). Generally, a population inhabiting a small area is expected to have lower genetic variation than a population living in a relatively large habitat due to limited gene flow (Allendorf and Luikart, 2007). In this case, ON-BP increased its genetic variation due to gene flow from the released ON-GIFT.

Generally, genetic introgression can increase the genetic variation of the recipient populations (e.g. Sušnik *et al.*, 2004; Sukmanomon *et al.*, 2012). Genetic variation of ON-SY did not increase compared with its presumed ancestor, ON-CD, despite introgression from *O. mossambicus*. This implied that the genetic variation of this population might have been reduced due to other factors, for example, habitat destruction and over-exploitation (Boonthongchuay, 2008), which resulted in a small effective population size. Although the value of A_r for ON-BP was high, the low effective number of alleles per locus observed in all of the feral tilapia populations revealed a high proportion of alleles with low frequencies (see Appendix 1). These alleles are easily lost in later generations and might compromise the adaptive potential of the populations.

One of the feral populations (ON-BP) showed comparable genetic variation (A = 9.50 ± 2.38; H_e = 0.77 ± 0.08) relative to the wild populations of Nile tilapia (A = 7.22– 9.42; H_e = 0.493–0.900, Hassanien and Gilbey, 2005). Whereas, the others (ON-BB and ON-SY) had genetic variation apparently lower than the wild populations. Despite the inconclusive empirical data on the relationship of genetic variation and performance traits (for example, Heath *et al.*, 2002; Shikano and Taniguchi, 2002; Overturf *et al.*, 2003; Borrell *et al.*, 2004), high genetic variation (especially allele diversity) theoretically promotes better adaptability of the populations (Allendorf and Phelps, 1980). As such, it is likely that ON-BP would be able to adapt well and may benefit the yield of artisanal fisheries in this reservoir. However, it is unfortunate that annual production data of the feral populations were available only for the Bueng Boraphet reservoir, so that it was not possible to use these data to contribute to an intensive discussion on the relationship of genetic

variation with the production. In the case of the Bueng Boraphet reservoir, a decline in production (46 t in 1998 reduced to 12 t in 2009; Fisheries Economics Division, 2011), in addition to impacts from other factors (for example, habitat destruction and over-exploitation), might be related with the low genetic variation of ON-BB. Notably, heterozygosities of the feral populations of Nile tilapia in Thailand were in the same range as the values reported for feral Nile tilapia elsewhere, such as in southern California (H = 0.35–0.72; Costa-Pierce, 2003).

Implications on management and utilization of feral populations

The feral tilapia population in the Bang Phra reservoir (ON-BP) had high genetic variation. This implies that it would adapt well with the changing environment. However, the sustainability of this population depends on an effective population size in successive generations. Therefore, management should aim to retain the effective population size. Firstly, there should be monitoring of captive fishery activities and production. Then, measures should be implemented to prevent over-exploitation and improper fishing (for example, poisoning) among other inappropriate activities. In addition, measures to avoid pollution and habitat destruction are required.

Although Nile tilapia is an alien species, the feral populations have been contributing to artisanal fisheries around the country. Therefore, the Thai Department of Fisheries (DoF) allows the release of Nile tilapia into natural waters where feral populations exist (Somying Piamsomboon, DoF regulation issued on 6 January, 2011). This offers a chance to increase the genetic variation of ON-BB and ON-SY by the introduction of other gene pools, probably the GIFT strain. Generally fishery management tends to favor retaining the genetic integrity of a population; thus, translocation is always avoided. In this context, it is likely that the feral populations of Nile tilapia in Thailand are not useful as gene pools for genetic improvement because their gene pools are represented by their ancestors, ON-CD. ON-CD, which is a pure *O. niloticus* population, has been evaluated for its performance and has been widely used for genetic improvement programs (McAndrew, 1981). More importantly the management measures recommended to apply for the Bang Phra reservoir are also recommended for the Sam Roi Yot and Bueng Boraphet reservoirs.

ACKNOWLEDGEMENTS

This study was funded in part from a research contract with the Biodiversity and Biotechnology Interface Program of the United States Agency for International Development (GDG-G-00-01-0010-00, awarded to the University of Minnesota, ARK and WS co-PIs), the Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota (ARK support); the Thailand Research Fund (the Senior Research Scholar Program 2007: project RTA 5080013, awarded to U. Na-Nakorn) and a Royal Golden Jubilee-Ph.D. scholarship (Grant No. PHD/0198/2547) awarded to Srijanya Sukmanomon. The authors appreciate support from Professor Bernie May, Genetic Variation Laboratory, Department of Animal Science, University of California Davis, CA, USA where some of the DNA analyses were performed.

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Appendix 1 Allele frequencies of 14 microsatellite loci in three feral populations of *Oreochromis niloticus* in Thailand. (ON-BP = Bang Phra population; ON-BB = Bueng Boraphet population; ON-SY = Sam Roi Yot population.)

Locus	Allele size	ON-BP	ON-BB	ON-SY	Private alleles
UNH172	184	0.0541	0.0208	0.0000	
	186	0.0270	0.1458	0.0341	
	188	0.2027	0.0625	0.0568	
	190	0.1216	0.2917	0.1023	
	194	0.2432	0.0625	0.1023	
	198	0.0541	0.0000	0.0000	ON-BP
	200	0.0000	0.0625	0.0795	
	204	0.0541	0.0000	0.0114	
	206	0.0000	0.0000	0.0114	ON-SY
	208	0.1757	0.3542	0.1250	
	210	0.0405	0.0000	0.0000	ON-BP
	246	0.0270	0.0000	0.4773	
UNH211	118	0.2162	0.5833	0.1364	
	120	0.1892	0.1042	0.0227	
	122	0.1081	0.0208	0.0000	
	124	0.0135	0.0000	0.0000	ON-BP
	132	0.0541	0.0417	0.0682	
	134	0.0000	0.0208	0.0341	
	142	0.0000	0.0208	0.0000	ON-BB
	144	0.0135	0.0000	0.0000	ON-BP
	146	0.0676	0.0833	0.1705	
	150	0.0000	0.0208	0.0682	
	154	0.0135	0.0000	0.0000	ON-BP
	156	0.0676	0.0208	0.0568	
	162	0.1622	0.0208	0.3977	
	168	0.0811	0.0625	0.0455	
	182	0.0135	0.0000	0.0000	ON-BP
UNH216	120	0.0676	0.0208	0.0568	
	124	0.1216	0.0000	0.0000	ON-BP
	126	0.5676	0.8750	0.4205	
	128	0.1757	0.1042	0.0909	
	138	0.0135	0.0000	0.0000	ON-BP
	156	0.0405	0.0000	0.3295	
	158	0.0135	0.0000	0.1023	
UNH222	164	0.0405	0.0208	0.1023	
	168	0.0405	0.0000	0.4659	
	174	0.0135	0.0000	0.0000	ON-BP
	182	0.0000	0.0625	0.0114	
	184	0.1757	0.0000	0.0114	
	186	0.2297	0.2500	0.0682	
	188	0.3784	0.3750	0.2955	
	190	0.1081	0.2708	0.0341	
	194	0.0135	0.0000	0.0000	ON-BP
	196	0.0000	0.0208	0.0114	
UNH212	186	0.0811	0.0000	0.0000	ON-BP
	188	0.1622	0.2500	0.1591	
	190	0.3514	0.2917	0.1477	
	192	0.2297	0.2292	0.2614	
	194	0.0135	0.0208	0.0000	

Appendix 1 (Cont.)

Locus	Allele size	ON-BP	ON-BB	ON-SY	Private alleles
	200	0.0676	0.0208	0.0000	
	202	0.0811	0.1875	0.0568	
	226	0.0135	0.0000	0.2273	
	228	0.0000	0.0000	0.1477	ON-SY
UNH160	132	0.0270	0.0000	0.0000	ON-BP
	168	0.0135	0.0000	0.0000	ON-BP
	180	0.0946	0.3958	0.4659	
	182	0.0676	0.0000	0.0114	
	184	0.0676	0.0625	0.1705	
	190	0.0270	0.0000	0.0000	ON-BP
	192	0.0000	0.0208	0.0000	ON-BB
	194	0.1622	0.0417	0.0341	
	196	0.0135	0.0000	0.0000	ON-BP
	200	0.0135	0.0000	0.0227	
	202	0.1892	0.1875	0.2614	
	208	0.3243	0.2708	0.0341	
	210	0.0000	0.0208	0.0000	ON-BB
UNH213	182	0.0541	0.1042	0.0682	
	190	0.0135	0.0000	0.0000	ON-BP
	192	0.2838	0.0417	0.5568	
	196	0.0270	0.0000	0.0000	ON-BP
	198	0.0135	0.0000	0.0000	ON-BP
	200	0.0135	0.0000	0.0000	ON-BP
	204	0.1081	0.0417	0.0000	
	206	0.0405	0.0417	0.0000	
	208	0.4189	0.7708	0.3750	
	212	0.0135	0.0000	0.0000	ON-BP
	218	0.0135	0.0000	0.0000	ON-BP
UNH153	205	0.2027	0.0833	0.5000	
	209	0.0135	0.0000	0.0455	
	211	0.7162	0.7708	0.4318	
	215	0.0270	0.1250	0.0227	
	217	0.0135	0.0208	0.0000	
	231	0.0270	0.0000	0.0000	ON-BP
UNH132	125	0.2027	0.3333	0.1023	
	127	0.3784	0.2917	0.4432	
	131	0.0676	0.0417	0.0000	
	133	0.0676	0.0000	0.0000	ON-BP
	135	0.0270	0.0000	0.1477	
	139	0.2568	0.3333	0.2955	
	143	0.0000	0.0000	0.0114	ON-SY
UNH138	164	0.0541	0.0208	0.4205	
	180	0.0270	0.0000	0.0000	ON-BP
	186	0.4730	0.3542	0.3636	
	188	0.0946	0.0417	0.0341	
	190	0.0135	0.0000	0.0000	ON-BP
	192	0.2703	0.5000	0.1477	
	194	0.0270	0.0000	0.0227	
	196	0.0135	0.0000	0.0114	
	204	0.0000	0.0625	0.0000	ON-BB
	208	0.0000	0.0208	0.0000	ON-BB

Appendix 1 (Cont.)

Locus	Allele size	ON-BP	ON-BB	ON-SY	Private alleles
	210	0.0135	0.0000	0.0000	ON-BP
	228	0.0135	0.0000	0.0000	ON-BP
UNH192	144	0.0000	0.0000	0.1023	ON-SY
	146	0.0135	0.0000	0.0227	
	148	0.0405	0.0000	0.0000	ON-BP
	150	0.3514	0.4167	0.3068	
	152	0.3919	0.5625	0.2386	
	154	0.0405	0.0000	0.0000	ON-BP
	156	0.0405	0.0208	0.0455	
	168	0.1216	0.0000	0.2841	
UNH1004	180	0.0135	0.0000	0.0000	ON-BP
	186	0.0135	0.0000	0.0000	ON-BP
	194	0.0000	0.0208	0.0000	ON-BB
	198	0.3108	0.2500	0.1023	
	200	0.0811	0.0000	0.0000	ON-BP
	202	0.1892	0.4792	0.1818	
	206	0.0270	0.0000	0.0000	ON-BP
	220	0.0135	0.0000	0.0000	ON-BP
	222	0.0135	0.0000	0.0000	ON-BP
	228	0.0000	0.0208	0.0000	ON-BB
	230	0.0270	0.0208	0.0000	
	232	0.1081	0.1667	0.0909	
	234	0.0135	0.0000	0.0000	ON-BP
	238	0.1757	0.0417	0.3523	
	246	0.0000	0.0000	0.1932	ON-SY
	256	0.0135	0.0000	0.0795	
UNH982	120	0.0135	0.0000	0.0000	ON-BP
	122	0.0000	0.0000	0.0795	ON-SY
	124	0.0135	0.0000	0.2045	
	126	0.2027	0.5417	0.2841	
	128	0.0405	0.0000	0.0000	ON-BP
	132	0.0135	0.0000	0.0000	ON-BP
	134	0.4054	0.3750	0.2159	
	136	0.0270	0.0208	0.0000	
	140	0.0135	0.0000	0.0000	ON-BP
	156	0.0135	0.0208	0.0114	
	160	0.1216	0.0000	0.1818	
	168	0.1351	0.0417	0.0227	
UNH173	123	0.2297	0.2708	0.2955	
	133	0.0541	0.1250	0.0568	
	135	0.0000	0.0000	0.0114	ON-SY
	187	0.2703	0.1875	0.5227	
	189	0.2838	0.1042	0.0568	
	195	0.0135	0.0000	0.0000	ON-BP
	201	0.0405	0.0417	0.0000	
	205	0.1081	0.2708	0.0455	
	207	0.0000	0.0000	0.0114	ON-SY