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THESIS

GENETIC IMPACTS OF THE DISSEMINATION OF GIFT NILE TILAPIA TO THAILAND

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the Requirements for the Degree of
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Nile tilapia, *Oreochromis niloticus,* contributes the largest portion of Thailand's annual production of freshwater fish. The GIFT strain of Nile tilapia has been introduced to Thailand and contributes to at least 75% of the total seed production. The present study comprised two parts. First, genetic alteration that may have occurred in four GIFT-derived strains (ON-PT, ON-UT, ON-PB and ON-CP) was studied using 14 microsatellite primers. Two Chitralada-derived strains (ON-AIT and ON-AY) were included in the study to evaluate possible introgression from the GIFT strain. Four conspecific reference populations, the 9th generation GIFT, the Chitralada, a culture population from Ivory Coast and a wild population from Uganda were included as well as the congeneric *O. mossambicus* and *O. aureus*. The overall F_{ST}, pairwise F_{ST}, and genetic differentiation tests showed significant differences between all population/species pairs. Genetic variation of the GIFT-derived, Chitralada-derived, and the reference GIFT was relatively high while that of the Chitralada strain was low. Model-based clustering revealed six clusters wherein ≥95% of the GIFT and three GIFT-derived strains (except ON-CP) were assigned to the same cluster. The Chitralada strain was pure (98% assigned to a single cluster) while the two Chitralada-derived strains showed small to high introgression from the GIFT strain. Likewise, introgression from Chitralada to GIFT occurred in two (ON-PT and ON-CP) of four GIFT-derived strains. Interspecific introgression was revealed in three populations including the GIFT strain, one of the GIFT-derived strain (ON-CP), and Chitralada derived-strains (ON-AY) as shown by individual bar plot. No introgression was observed in two GIFT-derived strains, ON-PB and ON-UT. The second part of the study emphasized genetic diversity of the feral populations of Nile tilapia collected from reservoirs locating in three parts of in Thailand, Sam Roi Yot reservoir in southern Thailand (ON-SY), Bang Phra reservoir in central Thailand (ON-BP) and Bueng Boraphet in northern Thailand (ON-BB). The reference populations were the same as part I. The results showed that ON-CD contributed to significant portions of the genepool of the three feral populations. Genetic differentiation among the feral populations was significant; differentiation was also observed between the feral populations and the newly introduced ON-GIFT. 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 observed in ON-SY.

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Srijanya Sukmanomon
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LIST OF ABBREVIATIONS

AAGRDI = Aquatic Animal Genetics Research and Development Institute
ADB = Asian Development Bank
AKVAFORSK = Institute of Aquaculture Research, Norway
CLSU = Central Luzon State University, Philippines
DEGITA = Dissemination and Evaluation of Genetically Improved Tilapia Species in Asia
DGIP = Division for Global and Interregional Program
DOF = Department of Fisheries
FAO = Food and Agriculture Organization
GFII = Genetic Improvement of Farmed Tilapia Foundation International Incorporated
GIFT = Genetic Improvement of Farmed Tilapia
GMT = Genetically Male Tilapia
ICLARM = International Center for Living Aquatic Resources Management
INGA = International Network on Genetics in Aquaculture
NAGRI = National Aquaculture Genetics Research Institute
Pathum FTRC = Pathum Thani Fisheries Test and Research Center
UNDP = United Nations Development Program
Uttaradit FTRC = Uttaradit Fisheries Test and Research Center

Genetic and Statistic Parameters

A = average number of alleles per locus
A_e = effective number of alleles per locus
A_r = allelic richness
bp = base pair
CI = Confidence interval
D = disequilibrium coefficient

LIST OF ABBREVIATIONS (Continued)

F_{IS} = Fixation index

F_{ST} = Fixation index

F_{IT} = Fixation index

H_e = expected heterozygosity

H_o = observed heterozygosity

HWE = Hardy-Weinberg Equilibrium

LD = Linkage disequilibrium

LG = Linkage group

N = Sample size

N_e = Effective population size

P = number of polymorphic loci

P = Probability

SE = Standard error

GENETIC IMPACTS OF THE DISSEMINATION OF GIFT NILE TILAPIA TO THAILAND

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of important freshwater fish species in aquaculture due to their production potential in recent years. Currently, world aquaculture production of Nile tilapia exceeded 2.5 million metric tonnes (mt), valued more than US$ 3.8 billion. Totally, 72 countries practiced tilapia aquaculture in 2011 but five countries, China, Egypt, Indonesia, Thailand and Philippines are dominating, contributing more than 90% of the world tilapia aquaculture production (Food and Agriculture Organization [FAO], 2011).

According to Fisheries Statistics of Thailand, the total production of Nile tilapia in Thailand increased from 42,200 mt in 1989 to 269,500 mt in 2008, with an average annual growth rate of about 11%. Values of Nile tilapia production during 1989 to 2008 ranged from 554.9 to 9,754.7 million Baht. At present, Nile tilapia aquaculture production is the highest among freshwater fish cultured in Thailand, contributing to more than 40% to total freshwater aquaculture production (Department of Fisheries [DOF], 2010). In recent years, Nile tilapia productions are among major export commodities, e.g. 12,956 mt, valued more than US$ 22.3 million in 2010 (Customs Department, 2011).

Besides, the feral populations of Nile tilapia have been established in natural and manmade water bodies around the world at least 77 countries (Fishbase, 2011), e.g. Bangladesh, Philippines, Thailand (Asian Development Bank [ADB], 2005), Indonesia (Abery *et al*., 2005), Hong Kong, Malaysia (De Silva *et al*., 2004), Viet Nam (Phan and De Silva, 2000), Sri Lanka (De Silva, 1997), USA (Costa-Pierce, 2003; Zambrano *et al*., 2006), Madagascar (Benstead *et al*., 2003), Nicaragua (McCrary *et al*., 2001) and Mexico (Zambrano and Marcias-Garcia, 1999). These feral populations have had significant contributions to freshwater fisheries of many countries, e.g. Thailand, Indonesia and El Salvador (FAO, 2011). Capture fisheries production of Nile tilapia in Thailand, mostly from the stocked reservoirs, comprised about 34% of the total Nile tilapia production (e.g. 52,300 mt, valued about 1,882.8 million Baht in 2008; DOF, 2010). At present, Nile tilapia ranks second to silver barb (*Barbonymus gonionotus*) as the most captured fish species from natural reservoirs in Thailand.

In the past, the aquaculture was often based upon poor breeds derived from introductions of very small founder stocks (Pullin and Capili, 1988). As such, most of the stocks were prone to inbreeding. Moreover, there has been widespread introgression of genes from feral populations of the less desirable tilapia species, *O. mossambicus* (Macaranas *et al*., 1986) which may eventually result in decline of yield.

In the above context, the Genetic Improvement of Farmed Tilapia (GIFT) project was started in April 1988 developed by the International Center for Living Aquatic Resources Management (ICLARM; now the WorldFish Center) and co-partners. Under the GIFT project, family and within family selection has been performed for five generations base on a base population with broad genetic background (Eknath *et al*., 1993). The improved strain is currently known as GIFT strain.

The GIFT strain was first introduced to Thailand in 1994 through the project Dissemination and Evaluation of Genetically Improved Tilapia Species in Asia (DEGITA). After three further generations of individual selection since 1998, the GIFT strain have been regularly disseminated to public and private hatcheries and farmed nationwide (ADB, 2005). The GIFT strain was about 38% superior over the local strains in terms of growth. Thus the variable cost of production per unit of fish produced is 28% lower than that of the other Nile tilapia strains (Dey *et al*., 2000). In 2004, based on a survey of 116 tilapia hatcheries in Thailand, the GIFT strain contributed about 75% of the market share whereby 576 million tilapia seed were sold by these hatcheries in 2003, increased from 36% in 2001 (ADB, 2005).

Despite the rapid expansion of the culture of the GIFT strain, there has been limited information on how the farmers managed their strains. It is possible that hatchery stocks could have been managed without genetic concern. Hence, the stocks are prone to genetic deterioration caused by bottleneck effect, inbreeding and negative selection (Hulata *et al*., 1986; Pullin and Capili, 1988; McKinna *et al*., 2010). Moreover, the GIFT strain that has been disseminated throughout the country may have impacts on the Chitralada strain which is a local strain widely used in Thailand before the introduction of the GIFT strain. These necessitate the studies on genetic diversity of the Nile tilapia broodstocks including the Chitralada strains.

In addition, genetic assessment of the feral populations of Nile tilapia in Thailand were also conducted in this study in order to assess the contribution of the GIFT strain to the feral populations which are important for freshwater fisheries production.

OBJECTIVES

The present study has four specific objectives.

1. To quantify the genetic difference between populations and change of genetic variation that may occur in hatchery populations of Nile tilapia in Thailand after dissemination of the GIFT strain,

2. To elucidate genetic introgression at intra- and inter-specific levels in Nile tilapia broodstock in Thailand,

3. To evaluate the genetic contribution of the GIFT strain to feral tilapia populations in Thailand, and

4. To examine genetic introgression at intra- and inter-specific levels of feral tilapia populations in Thailand.

LITERATURE REVIEW

The development of the GIFT strain

The Genetic Improvement of Farmed Tilapia (GIFT) project was started in April 1988. The project was developed by the International Center for Living Aquatic Resources Management (ICLARM; now WorldFish Center), in collaboration with Philippine institutions (the Bureau of Fisheries and Aquatic Resources, the Central Luzon State University and the University of the Philippines) and the Norwegian Institute of Aquaculture Research (AKVAFORSK), with a funding support from the United Nations Development Program/Division for Global and Interregional Program (UNDP/DGIP) and the Asian Development Bank (ADB). The GIFT project was based in the Philippines, using crosses of four wild African strains (Egypt, Ghana, Kenya and Senegal) and four farmed Asian strains (Israel, Singapore, Taiwan and Thailand) existing in the Philippines (Eknath *et al*., 1993). The 25 best performing purebred and crossbred groups out of a complete 8x8 diallel cross were selected to build a genetically mixed base population. Subsequently, combined family and within family selection has been performed to improve growth performance (Eknath, 1995; Eknath and Acosta, 1998). The improved strain has currently known as the GIFT strain. After one generation of selection, the GIFT strain grew 20% faster than the base population and was about 60% heavier at harvest in on-station trials than the widely farmed strain in the Philippines (Eknath, 1995). The average genetic gain in growth per generation across the first five generations of selection was about 12-17%, and the cumulative genetic gain in relation to the base population was 85% (Eknath and Acosta, 1998).

Furthermore, the GIFT strain which performed well or better than the existing farmed tilapia has also been proven to be a source of good genetic material for continued selective breeding (Ponzoni *et al*., 2005). The recent research has confirmed high genetic diversity of GIFT strain that is a good basis for selection (Romana-Eguia *et al*., 2004; Rutten *et al*., 2004a).

The GIFT strain was also superior to other tilapia strains in many performance traits that were not selected for. For example, Rutten *et al.* (2004b) found that the average percentage of fillet yield of the GIFT strain (about 38%) was higher than that of the Chitralada strain (about 35%), while the average egg diameter (3.2 mm), hatching percentage (68%) and a fecundity of 96.9 eggs/g BW of the GIFT strain were higher than those of the Chitralada strain (egg diameter of 3.0 mm, hatching percentage of 41% and a fecundity of 127.5 eggs/g BW/female) (ADB, 2005 cited Hans Komen, personal communication).

The origin of Nile tilapia in Thailand

Major introductions of Nile tilapia to Thailand were summarized in Table 1. Nile tilapia, *O. niloticus* was introduced to Thailand for the first time on 25 March 1965 by His Imperial Highness Crown Prince Akihito of Japan (presently the Emperor of Japan) who presented 50 individuals of Egyptian origin to His Majesty the King Bhumibol Adulyadej of Thailand. Only 19 males and 19 females survived and became the founder stock of the Chitralada strain. The Chitralada strain has been maintaining in ponds at the Chitralada Villa Royal Residence, Bangkok (Damrongratana and Kessanchai, 1966) until present. It has already confirmed as pure *O. niloticus* based on allozyme electrophoresis analysis (McAndrew, 1981). Since 1966 when His Majesty the King presented Nile tilapia fingerlings to the Department of Fisheries, the fish derived from the Chitralada strain were distributed for stocking and aquaculture purposes throughout the country. The fish has since become the most important cultured species in Thailand, and the Chitralada strain is known throughout the world for its remarkable growth performance (Chinnabut *et al*., 2007).

In 1983, Israeli strain of Nile tilapia was introduced to Thailand in order to compare growth performance with the Chitralada strain and the hybrid between these two strains was made. The result showed that growth performance of Chitralada strain was better than that of the Israeli strain and their hybrid (Uraiwan and Phanitchai, 1986).

Then, the Swansea strain (originated from the Egypt Manzala strain) was introduced to Thailand in 1992, for the development of YY-male (supermale) (Mair *et al*., 1992). Genetically male tilapia (GMT) was all male offspring of the cross between the YY males and the normal XX females.

The GIFT strain was disseminated by DEGITA during 1994-1995 and Thailand received it through International Network on Genetics in Aquaculture (INGA). Further introductions of GIFT from the Philippines were in 1996, 1998 and 2000 for research and breeding purpose. The results of on-station and on-farm trials in ponds showed that GIFT was 38% superior in growth over Chitralada and GMT strains. From 1998, the GIFT strain has been regularly disseminated to public and private hatcheries and farmers countrywide (ADB, 2005).

In general, the GIFT strain or its derivatives are employed by most of the commercial tilapia farms while the original Chitralada strain and its derivatives are used by small scale farmers. It is also possible that the GIFT and the Chitralada strain are mixed in some farms. However, there has been no clear information on the overall status of the Nile tilapia broodstock in Thailand. It is likely that the broodstock management was performed without or with minimal genetic concerns hence may cause genetic deterioration.

Table 1 Major introductions of Nile tilapia to Thailand during 1965-2000; Note: CLSU = Central Luzon State University, Philippines; ICLARM = International Center for Living Aquatic Resources Management; GIFT = Genetically Improved Farmed Tilapia; NAGRI = National Aquaculture Genetics Research Institute, Thailand; GFII = Genetic Improvement of Farmed Tilapia Foundation International Incorporated; N/A = Not Available

Strain	**Year**	**Source**	**Number of fingerlings**	**Recipients**	**Purpose**
Chitralada	1965	Japan	50	His Majesty the King	First introduction
Israel	1983	Israel	N/A	NIFI	Research
Swansea	1992	CLSU, Philippines and University of Stirling, UK	N/A	NAGRI	Research
GIFT	1994	ICLARM, Philippines	2,000	NAGRI	Research
GIFT	1995	ICLARM, Philippines	3,000	NAGRI	Research
GIFT	1996	ICLARM, Philippines	8,000	NAGRI	Research
GIFT	1998	ICLARM, Philippines	N/A	NAGRI	Breeding program
GIFT	2000	GFII	2,500	NAGRI	Breeding program

Status of Nile tilapia production in Thailand

Aquaculture production of Nile tilapia in Thailand rapidly increased (from 21,115 mt in 1989 to 217,200 mt worth approximately 7,871.9 million Baht in 2008) and presently is the highest (40% of the total production) among cultured freshwater fishes in Thailand. The aquaculture production was mainly from ponds (82%) with only 18% contribution from other types of culture e.g. 3% from paddy cum fish, 1% from ditches, and 14% from cages (DOF, 2010).

Likewise, the production of Nile tilapia from capture fisheries in Thailand, is increasing (e.g. increased from 21,085 mt in 1989 to 52,300 mt with values of 1,882.8 million Baht in 2008, DOF, 2010). At present, Nile tilapia which comprised about 18.2% of the total freshwater fisheries production in 2008 (Fishery Information Technology Center, 2010), ranks second to silver barb (*Barbonymus gonionotus*) as the most captured freshwater fish species in Thailand.

A majority (90%) of Nile tilapia production in Thailand is for domestic consumption, and the rest is exported. It is marketed in various forms, e.g. live, fresh, chilled, frozen whole fish; fresh or chilled, frozen fillets; frozen minced fish meat (surimi); and dried fish. In 2010, the total exported Nile tilapia productions exceeded 12,956 mt, valued more than US$ 22.3 million. The main importers are USA, the Middle East and EU (Customs Department, 2011).

Genetically improved strains of Nile tilapia in Thailand

Genetic improvement of Nile tilapia in Thailand has been carried out since 1993 by the Aquatic Animal Genetics Research and Development Institute (AAGRDI), Department of Fisheries (Chinnabut *et al*., 2007). At present there are three genetically improved strains.

1. the Chitralada I strain

The Chitralada I strain was selected from the original Chitralada strain by a within family selection for five generations. This strain increased growth rate by 22% over its original population. It has been disseminated to public and private sectors since 1993.

2. the Chitralada II strain

The so-called Chitralada II strain was actually the offspring of the cross between the YY-male (supermale) derived from the Egypt Manzala strain and the ordinary XX-female (Mair *et al*., 1992). This all male stock increased yields by 45% over the ancestor. It has been disseminated to public and private sectors since 1997.

3. the Chitralada III strain

The Chitralada III strain of tilapia was selected from the GIFT tilapia by individual selection for three generations. This strain increased yields by 38% over the GIFT strain. It has been disseminated to public and private sectors since 1998.

Genetic diversity

Genetic diversity refers to variations at any level of hereditary units (nucleotides, genes and chromosomes). It is defined by the variation within and among populations which was consequences of recombination, mutation, natural selection, genetic drift and gene flow (Hartl and Clark, 1997; Frankham *et al*., 2002). The differences are the result of evolutionary process that reflects adaptations of individuals or populations (Ayala, 1982). Therefore, genetic diversity is a valuable resource that enables sustainability of the species, and moreover, it is a basic need for successful genetic improvement program.

Genetic variation within populations

Genetic variation within populations determines the ability of a species to survive in a changing environment. A population size is the most important factor to sustain a high level of genetic variation within a population (Frankel and Soule, 1981). Reduction in a population size at the founding event and/or in later generations resulted in decline of genetic variation. In addition genetic variation is also losing as a consequence of inbreeding (Allendorf and Luikart, 2007).

Genetic variation within population is indicated by parameters indicating allele diversity (average number of alleles per locus, average effective number of alleles per locus, allelic richness) and the observed and expected heterozygosity (Allendorf and Luikart, 2007). However, the average number of alleles per locus is affected by sample sizes. Therefore, allelic richness which is average number of allele per locus that takes into account a sample size was introduced (Allendorf and Luikart, 2007 cited El Mousadik and Petit, 1996). This measure uses a rare faction method to estimate allelic richness at a locus for a fixed sample size, usually the smallest sample size (Allendorf and Luikart, 2007). Effective number of allele (A_e) is a measure of allelic evenness which is estimated by the formula, $1/\Sigma p_i^2$, where p is the frequency of the i^{th} allele (Hedrick, 2005).

Observed heterozygosity (H_o) is a proportion of heterozygotes averaged across loci and individuals. Expected heterozygosity (H_e) is estimated from observed frequencies of alleles assuming the population is in Hardy-Weinberg equilibrium. The level of expected heterozygosity (H_e) for a particular locus is calculated by $H_e = 1 - \sum_{i=1}^{k} p_i^2$ where p, is the frequency of the i^{th} allele in a population and k is the number of allele (Hedrick, 2005).

Hardy-Weinberg Equilibrium

The Hardy-Weinberg Equilibrium (HWE) refers to a situation that the allele frequencies and the genotype frequencies of a population are constant from generation to generation if the population is large and the mating occurs randomly, with no selection, mutation or migration, and that, furthermore, there is a simple relationship between the gene frequencies and the genotype frequencies (Falconer and Mackay, 1996 cited Hardy, 1908 and Weinberg, 1908). The relationship is this: if the gene frequencies of two alleles (A_1 and A_2) among the parents are p and q then the genotype frequencies among the progeny are $p^2A_1A_1$, $2pqA_1A_2$ and $q^2A_2A_2$ (Falconer and Mackay, 1996). A deviation from Hardy-Weinberg proportions is caused by any one or combinations of the following mechanisms, selection, mixing of populations in a sample, non-random mating, introduction, and being a small population. The detection of the disequilibrium is one of the first steps in the study of population structure (Raymond and Rousset, 1995a).

Inbreeding and Wahlund effect

Inbreeding refers to mating between related individuals which results in increase homozygosity. Average inbreeding coefficient (F) of a population is determined by effective population size ($\Delta F = 1/2N_e$). Inbreeding may cause deterioration of phenotypes due to accumulation of homozygote recessive of unfavorable recessive alleles. Inbreeding results in excess of homozygotes relative to Hardy-Weinberg expectations. Inbred populations exhibit decline in growth rate, survival rate, and fecundity, as well as smaller adult size and depressed production. In hatchery stocks of fish, reductions of survival and reproduction are highly associated with inbreeding (Vrijenhoek, 1998).

The mixing of two populations with different allele frequencies also results in an excess of homozygotes or a deficiency of heterozygotes, even if Hardy-Weinberg proportions exist within each population. This is known as Wahlund effect (Hedrick, 2005). The reduction in heterozygosity can be measured using F-statistics (Wright, 1965). When there is no deficit of heterozygosity, $F_{IS} = 0$.

Founder effect and population bottlenecks

Founder effect is a phenomenon in which a population originates from a small number of individuals. A bottleneck refers to a sudden restriction in population size (Falconer and Mackay, 1996). It easily occurs in hatcheries when a small number of brooders are used to create next generation broodstock. Both founder effect and bottleneck lead to genetic drift which causes changing in allele frequencies without direction in successive generations. Genetic drift causes reduction of allele diversity while effect on heterozygosity is not obvious (Allendorf and Phelps, 1980). In addition, if population size is continually small inbreeding will accumulate (Shikano and Taniguchi, 2003).

Linkage disequilibrium

Linkage disequilibrium describes association of alleles within gametes (Hedrick, 2005). It corresponds to the difference between the expected and the observed multilocus genotypic frequencies. Various factors can cause linkage disequilibrium including genetic drift, mutation, inbreeding and gene flow or hybridization between two populations. Linkage disequilibrium is strong in small populations as a result of random genetic drift. The range of linkage disequilibrium is 0 to 1. The result is zero if no other processes are acting except recombination. This can result in changes in allele frequency and loss of alleles from populations (Hedrick, 2005).

Genetic differentiation between populations

Genetic differentiation between populations initially occurs when a population is divided unevenly and results in subpopulations with different allele frequencies. Then natural selection will gradually modify the genetic constitution of each subpopulation. In hatchery populations, artificial selection, genetic drift and introduction of genetically diverse stock are main mechanisms enhancing genetic difference between the subpopulations. Genetic differentiation can be quantified based on genetic distance and *F*-statistics (Allendorf and Luikart, 2007).

Genetic distance

Genetic distance is intended to estimate the average number of gene differences per locus between populations (Nei, 1972; Nei, 1976). Cavalli-Sforza and Edwards (1967) proposed angular transformation of gene frequencies to calculate chord distance between two populations or more. Chord distance was calculated by $D_c = (2\sqrt{2}/\pi)\ (\sqrt{1 - \cos\theta})$, since $\cos\theta = \sum_{i=1}^{n} \sqrt{p_{ix} p_{iy}}$, where p_{ix} and p_{iy} are the frequencies of the i^{th} allele in population *x* and *y*, respectively, therefore chord distance becomes: $D_c = 2/\pi\ [2(1 - \sum_{i=1}^{n} \sqrt{p_{ix} p_{iy}})]^{1/2}$.

Theoretically genetic distance between species is approximately 1.0, around 0.1 for subspecies, and 0.01 for local races (Nei, 1975). However, average genetic distance between species averaged from several studies in fishes showed diverse values, for example, genetic distance between congeneric species from diverse array of fishes was 0.62, 0.40 and 0.30 (Oosthuizen, 1993). The genetic distance between congeneric species of *Clarias* in Thailand was between 0.366 for *C. batrachus* and *C. macrocephalus* to 1.181 between *C. batrachus* and *Clarias* sp. (Na-Nakorn *et al*., 2002). Average genetic distance between subspecies, *O. niloticus vulcani* and *O. niloticus niloticus* was 0.041 (Macaranas *et al*., 1995).

F-statistics

The most important approach for measurement of the amount of population differentiation is *F* coefficients (Wright's *F*-statistic). The *F*-statistics describe the distribution of genetic variation within a species that used a series of inbreeding coefficients: F_{IS}, F_{ST} and F_{IT}. F_{IS} is a measure of departure from Hardy-Weinberg proportions within local demes or subpopulations, F_{ST} is a measure of allele frequency divergence among demes of subpopulations, and F_{IT} is a measure of the overall departure from Hardy-Weinberg proportions in the entire base population (or species) due to both non-random mating within local subpopulations (F_{IS}) and allele frequency divergence among subpopulations (F_{ST}) (Wright, 1965).

Nei (1977) extended *F*-statistic as a function of observed and expected heterozygosities for multiple loci as follows:

F_{IS} is a measure of departure from Hardy-Weinberg proportions within local subpopulations to the reduction of heterozygosity due to non-random mating in subpopulations. $F_{IS} = H_S - H_O/H_S$, where H_O is the average observed heterozygosity within a subpopulation, and H_S is the average expected heterozygosity within subpopulation. F_{IS} will be positive if there is a deficit of heterozygotes and negative is there is an excess of heterozygotes. Inbreeding within populations will cause a deficit of heterozygotes.

F_{ST} is a measure of genetic divergence among subpopulations: $F_{ST} = H_T - H_S/H_T$, where H_T is the average expected heterozygosity in a total population and H_S is the average observed heterozygosity in each subpopulation. F_{ST} ranges from zero, when all subpopulations have equal allele frequencies, to one, when all the subpopulations are fixed for different alleles. F_{ST} is sometimes called the fixation index.

F_{IT} is a measure of the total departure from Hardy-Weinberg proportions that includes departure from Hardy-Weinberg proportions within populations and divergence among populations, and can be estimated from: $F_{IT} = H_T - H_O/H_T$, where H_T is the average expected heterozygosity among populations.

Inference of population structure using a model-based clustering method

A model-based clustering method was described by Pritchard *et al*. (2000). It is implemented in the software STRUCTURE. The program concerns that the population assigned by sampling locations may not represent real populations. Therefore, the program re-assigns each individual base on multilocus genotype data into K populations using a Bayesian approach. Each of the K populations is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned to populations, or jointly to two or more populations if their genotypes indicate that they are admixed. STRUCTURE would attribute a probability Pr(X|K) given the data (X), and the log Pr(X|K) is used to determine the more likely number of cluster. Applications of this method include demonstrating the presence of population structure, assigning individuals to populations, identifying cryptic population structure, studying hybrid zone, detecting migrants and admixture individuals and inferring historical population admixture (Pritchard *et al*., 2000).

Statistic used to selected K

The STRUCTURE program determines the true number of populations (K) using the posterior probability of the data for a given *K*, Pr(X|K) (Pritchard *et al*., 2000). This value was obtained by first computing the log likelihood of the data at each step of the Markov chain Monte Carlo (MCMC). Then the average of these values is computed and half their variance is subtracted to the mean. The STRUCTURE output designates this value as 'Ln P(D)' which it refers as *L*(*K*) afterwards (Evanno *et al*., 2005). True number of population (*K*) is often indentified using the maximal value of *L*(*K*) returned by STRUCTURE. However, Pr(X|K) is computationally difficult to estimate, and Pritchard *et al*. (2000) have proposed an *ad hoc* way to approximate the probability of *K* given the genotyped data.

Later it was found that the distribution of *L*(*K*) did not show a clear mode for the true *K* (Evanno *et al*., 2005). In stead, an *ad hoc* quantity based on the second order rate of change of the likelihood function with respect to *K* (ΔK) did shows clear peak at the true value of *K*. The graphical method for detection of the true *K* consists of the following steps; (1) plotted the mean likelihood *L*(*K*) (± SD) over number of runs for each *K* value, (2) plotted the mean difference between successive likelihood value of *K*, $L'(K) = L(K) - L(K\text{-}1)$, this difference corresponds to the rate of change of the likelihood function with respect to *K*, and is noted $L'(K)$, (3) plotted the difference between successive values of $L'(K)$, $|L''(K)| = |L'(K+1) - L'(K)|$, this corresponds to the second order rate change of $L(K)$ with respect to K, (4) estimated ΔK as the mean of the absolute values of $L''(K)$ averaged over number of runs divided by the standard deviation of $L(K)$, $\Delta K = m(|L''(K)|/s[L(K)]$, which expands to $\Delta K = m(|L''(K+1) - 2\,L(K) + L(K-1)|/s[L(K)]$, the model value of the distribution of ΔK to be located at the real K and the height of this model values as an indicator of the strength of the signal detected by STRUCTURE.

Microsatellites DNA

Microsatellites have been using as markers for assessing genetic variation within and between populations of wild and cultured stocks of aquatic species as well as monitoring of genetic change in selection programs. This wide range of applications is due to their abundance, neutrality, co-dominant expression, high levels of polymorphism, and PCR-based analysis. Moreover, sample collection for microsatellite analysis which requires only a small pieces of fin clip preserved in absolute ethanol, is an additional advantage of microsatellite over protein markers such as allozyme (Wright and Bentzen, 1994; O’Reilly and Wright, 1995).

Microsatellites are non-coding repetitive DNA regions comprising of tandem array of di-, tri- or tetra-nucleotide sequences with repeat units of one to six nucleotides and flanked by regions of unique DNA sequences. These microsatellite arrays seem to be highly susceptible to length mutation during DNA replication. Decreasing and increasing amount of single or multiple number of repeats due to slipped strand mispairing during DNA replication, indicated the variation of microsatellite loci (Litt and Luty, 1989). Most of microsatellites found in fish genome are di-nucleotide repeats such as $(GT)_n$ and $(AC)_n$ (O’Reilly and Wright, 1995).

Microsatellites have been isolated and characterized in a large number of fish species including tilapias; *Oreochromis niloticus* (Lee and Kocher, 1996; Carleton *et al*., 2002), *O. shiranus* (Ambali *et al*., 2000), *O. mossambicus* (Saju *et al*., 2010), catfishes; *Clarias gariepinus* (Galbusera *et al*., 1996), *C. macrocephalus* (Na-Nakorn *et al*., 1999; Sukmanomon *et al*., 2003), *C. batrachus* (Yue *et al*., 2003), *Pangasianodon gigas* (Na-Nakorn *et al*., 2006), *P. hypophthalmus* (Volckaert *et al*., 1999), *Mystus nemurus* (Chan *et al*., 2005; Hoh *et al*., 2007); cyprinids; *Cyprinus carpio* (Crooijmans *et al*., 1997), *Ctenopharyngodon idellus* (Li *et al*., 2007), *Carassius auratus gibelio* (Jia *et al*., 2006), *Hypophthalmichthys molitrix* (Gheyas *et al*., 2006), *Labeo rohita* (Das *et al*., 2005; Patel *et al*., 2009), *Barbodes gonionotus* (Kamonrat *et al*., 2002).

Microsatellites loci are often conserved among closely related species. Therefore, cross-species amplification of microsatellites have been demonstrated in various species of teleost fish, for example, a set of microsatellite primers which was designed for common carp, *Cyprinus carpio* were able to amplify DNA of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) (Tong *et al*., 2002), A total of 25 microsatellite loci developed for river catfish, *Mystus nemurus* were successfully cross-amplified in *Pangasius micronemus* and *Clarias batrachus* which were distantly related catfish species within the suborder Siluroidei, (Chan *et al*., 2005). Eight, six and seven primer pairs of ten polymorphic microsatellite primer pairs developed from DNA of *Pangasianodon gigas* amplified DNA from *P. hypophthalmus*, *P. larnaudii* and *P. sanitwongsei*, respectively (Na-Nakorn *et al*., 2006). Six sets of microsatellite primers developed from *Oreochromis shiranus* cross-amplified DNA of the members of the genera *Sarotherodon* and *Tilapia*, and were polymorphic in most of the species (Ambali *et al*., 2000).

Microsatellites are becoming increasingly popular as genetic markers for a range of applications in a variety of studies in fisheries and aquaculture (O’Reilly and Wright, 1995), including intra- and interspecies variation (Tranah and May, 2006), population differentiation (Hassanien and Gilbey, 2005; Liu *et al*., 2009), stock and individuals identification (Skaala *et al*., 2004; Abaunza *et al*., 2008), parentage and kinship analysis (Sriphairoj *et al*., 2007; Na-Nakorn *et al*., 2010), broodstock management (Na-Nakorn and Moeikum, 2009), linkage mapping (Lee *et al*., 2005), quantitative trait loci mapping (Agresti *et al*., 2000; Cnaani *et al*., 2004). Besides, Allendorf and Ryman (1986) stated that microsatellite loci were also useful for detecting genetic variability of many species of hatchery stocks in which slight genetic change always occurred.

Genetic changes due to broodstock management

The genetic changes that occur in hatcheries are attributed to many reasons. Most frequently, founder effect and genetic bottleneck occur when a small number of broodstock were taken from the wild or from other stocks (Norris *et al*., 1999; Fiumera *et al*., 2000; Aho *et al*., 2006; Qin *et al*., 2007), resulting in genetic divergence from the original stock and losing of genetic variation especially an average number of alleles per locus. If the stock is continuously maintained with small number of brooders, then inbreeding would occur (Taniguchi, 2003; Fessehaye *et al*., 2006) and lead to a reduction of heterozygosity. In addition, unintentional and/or artificial selection may occur and change the genetic composition of the stocks (Ryman *et al*., 1995; Iguchi *et al*., 1999; Norris *et al*., 1999; Romana-Eguia *et al*., 2005).

Genetic diversity of hatchery stocks of Nile tilapia

Genetic diversity of hatchery stocks of Nile tilapia received global attention due to the economically importance of this species. The studies using microsatellite markers by Rutten *et al*. (2004a) and Romana-Eguia *et al*. (2004) showed that a majority of hatchery stocks studied were genetically different (e.g. F_{ST} = 0.178; Rutten *et al*., 2004a) and the genetic variation within populations was characterized by low to moderate number of alleles/locus (A ranged from 5 to 9.2) and moderate heterozygosity (H_e = 0.624 - 0.788). Genetic variation of the GIFT strain was high [A = 7.5±1.1, H_e = 0.704, (Rutten *et al*., 2004a); A = 10, A_e = 6.7, H_e = 0.81 (Romana-Eguia *et al*., 2004)] due to its broad genetic background. Whereas the Chitralada strain had relatively low allelic diversity but moderate H_e [A = 5.4±1.2, H_e = 0.624 (Rutten *et al*., 2004a); A = 6.6, A_e = 3.8, H_e = 0.701 (Romana-Eguia *et al*., 2004)].

Introgression among congeneric species of *Oreochromis*

Hybridization and back crossing easily occur among the members of the genus *Oreochromis*. For examples, the exotic *O. mossambicus* and *O. niloticus* which significantly contributed to reservoir fishery in Sri Lanka showed some degree of introgression from *O. niloticus* into *O. mossambicus* in all but two populations examined, while the introgression of *O. mossambicus* was observed in all *O. niloticus* populations (De Silva, 1997). Genetic introgression from *O. niloticus* was observed in a critically endangered native *O. esculentus* in Kenya and thus increased a risk to extinction of this species (Angienda *et al*., 2011).

In some cases an intensive introgression resulted in a genetically unique population. For instance, a feral population of *O. mossambicus* in California, the so called “distinct hybrid ecotype”, showed significant evolutionary divergence from African parental materials caused by hybridization with *O. urolepis hornorum* (Costa-Pierce, 2003).

The impacts of the introgression is critical in the fisheries context. Amarasinghe and De Silva, (1996) reported that an imbalance in sex ratio with male dominance and a decline in fecundity in hybrid forms of *O. mossambicus* and *O. niloticus* might lead to a decline in fish yields in reservoirs fishery of Sri Lanka.

MATERIALS AND METHODS

This study was comprised of two parts. The first part dealing with hatchery populations aimed at examining genetic alteration occurring in the GIFT-derived strains. At the same time the strains derived from the Chitralada strain were also included as well as the reference populations including the GIFT population, Chitralada population, two populations of *O. niloticus* from Uganda and Ivory Cost, and a population of *O. mossambicus* and *O. aureus*

The second part of the study aims at evaluating the contributions of the GIFT strain to feral tilapia populations in Thailand. The samples comprise three feral populations of *O. niloticus* in Thailand. The reference populations including in part I was also included.

1. Survey of major Nile tilapia hatcheries

Prior to sample collections a survey on Nile tilapia hatcheries was conducted. In 2006 when the sampling was planned, there were a total of 114 Nile tilapia hatcheries in Thailand (drawn from the database of the DOF, 2006). Fifteen hatcheries which contributed approximate 75% of the total fry production (an estimate of 800 million fry in 2006 base on a production of 205,300 mt, 500 g/fish, survival rate = 50%) were included in the preliminary survey. However, the survey revealed that a majority of the farms did not maintain the broodstock, but usually purchased new brooders every 1.5-2 years. Therefore, the sampling locations were limited to six hatcheries base on two criteria, 1) the hatcheries that continually maintain their own broodstock, and 2) the operation period is at least 10 years.

2. Fish samples: origins and collection

2.1 Nile tilapia hatchery populations

Geographical origins and sample sizes of all examined populations are given in Figure 1 and Table 2. The samples were comprised of fish from six hatcheries which have been annually producing more than 10 million Nile tilapia fingerlings during February to September 2006. The origin of each Nile tilapia broodstock is described below.

Pathum Thani (ON-PT): ON-PT is a broodstock of Pathum Thani Fisheries Test and Research Center (Pathum Thani FTRC; now Pathum Thani Aquatic Animal Genetics Research and Development Center) in Pathum Thani province. The stock originated from 50 families of the ninth generation GIFT from the WorldFish Center, Malaysia in 2000 and has undergone within family selection for growth for five generations.

Uttaradit (ON-UT): This stock is reared in Uttaradit Fisheries Test and Research Center (Uttaradit FTRC; now Uttaradit Aquatic Animal Genetics Research and Development Center) in Uttaradit province. The origin was the 33 families out of 50 families of the ninth generation of GIFT from Pathum Thani FTRC. It has undergone three generations of within family selection for growth.

Prachin Buri (ON-PB): This stock belongs to Nam Sai Farm in Prachin Buri province which has acquired fifth and ninth generation GIFT from the NAGRI (now AAGRDI) in 1997 and 2000 respectively without knowing number of families. Nam Sai Farm has merged these two groups into a single strain and conducted artificial selection for growth for two generations.

CP (ON-CP): This stock is reared in Charoen Pokphand (CP) hatchery in Ayutthaya province. The stock originated from 250 fullsib families using females from two sources, GIFT from Pathum Thani FTRC and GIFT originated in

Philippines, and males from Chitralada and other tilapia strain/species from Africa. The stock has undergone five generations of within family selection for growth.

Ayutthaya (ON-AY): This stock is reared in Rom Sai Farm in Ayutthaya province. It originated from a combination of Chitralada strain and GIFT. The latter strain was unintentionally introduced due to the confused naming of the GIFT strain which underwent three generations of selection in Thailand and was named Chitralada III (ADB, 2005; Chinnabut *et al*., 2007). The stock has undergone two generations of mass selection for growth.

AIT (ON-AIT): This stock belongs to the Asian Institute of Technology (AIT) in Pathum Thani province. It originated from Chitralada brooders reared in Chitralada Villa Royal Resident, Bangkok without information on number of founders but the stock has undergone at least 10 generations for mass selection for growth.

2.2 Feral tilapia populations

Samples of feral populations of *O. niloticus* were collected from three reservoirs representing different geographic regions, Sam Roi Yot reservoir in Prachuap Khiri Khan province, southern Thailand; Bang Phra reservoir in Chon Buri province, central Thailand; and Bueng Boraphet in Nakhon Sawan province, northern Thailand.

Origins of the feral tilapia populations were as follows.

Sam Roi Yot reservoir: *O. niloticus* and *O. mossambicus* have been cultured around this area with evidence that some fish have escaped due to flooding (Rajitparinya *et al*., 1973). Additionally, feral tilapias could originate from fish restocked in the adjacent Pranburi Dam (Chittapalapong *et al*., 1997) which connects to this reservoir.

Bang Phra reservoir: this reservoir has been stocked with tilapia fingerlings (Chitralada strain) in the early period of introduction (Chookajorn *et al*., 1991) and recently stocked with fingerlings of the GIFT strain (Sangduen Naksuwan, personal communication).

Bueng Boraphet reservoir: the feral tilapia population established here could have been aquaculture escapees into the Nan River which runs into this reservoir (Srichareondham *et al*., 1993; Makin *et al*., 2004).

Geographical origins and sample sizes of all examined populations are also given in Figure 1 and Table 2 within Nile tilapia population in hatchery.

2.3 Reference Nile tilapia populations and other relevant tilapiine species

This study also included four *O. niloticus* populations, the Chitralada population collected from Chitralada Villa Royal Residence, Bangkok (n = 80); a population from Lake Albert in Uganda (n = 20; obtained from W. Mwanja, Uganda), a cultured population from Ivory Coast (n = 20; obtained from K. Veverica, Auburn Univeristy, USA) and the ninth generation GIFT originally collected from the Philippines (n = 28; obtained from N. Taniguchi, Tohoku University, Japan). In addition, one cultured population each of *O. mossambicus* (from South Africa, n = 40; obtained from G. Hulata, Agricultural Research Organization) and *O. aureus* (from Egypt, n = 40; obtained from Mahmoud Rezk, WorldFish Center) were included as the reference populations.

3. Sample collection and DNA extraction

A piece of caudal fin (about 50 mg) was collected from each individual, preserved 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. DNA pellet was 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 was determined by spectrophotometry and agarose gel electrophoresis.

4. Microsatellite primers and PCR conditions

Fourteen microsatellite primers developed from DNA of *Oreochromis niloticus* by Lee and Kocher (1996) were used (Table 3). A single-locus PCR each of six microsatellite primers (*UNH172, UNH211, UNH216, UNH222, UNH212* and *UNH 160*) was performed in a 10 µl reaction volume contain 2.5 ng of template DNA, 1X PCR buffer, 1.5mM $MgCl_2$, 100 µM of each dNTPs, 0.25 µM of each primer and 0.2 units of *Taq* DNA polymerase (Fermentas). PCR were carried out in a PX2 Thermal Cycler (Thermoscientific, USA) under the following conditions: 3 min at 94°C followed by 30 cycles of 30 sec at 94°C, 30 sec at T_a°C (Table 3), 1 min at 72°C, with final extension of 5 min at 72°C. Then the PCR products were separated on the 4.5% polyacrylamide gel. Visualization of the microsatellite profiles was accomplished by silver staining (Bassam *et al*., 1991). Allele sizes were designated relatively to the M13 DNA ladder.

In addition, eight microsatellite primers consolidated into two multiplex PCR (Multiplex 1: *UNH213*, *UNH153*, *UNH132*, *UNH138*; Multiplex 2: *UNH192*, *UNH1004*, *UNH982*, *UNH173*) were scored against all DNA samples. PCR was performed in a 10 µl total reaction volume consisting of 5 ng of template DNA, 1 µl of 10X PCR reaction buffer with 20 mM $MgCl_2$, 0.3-0.4 µl of each primer (10 µM) depending on relative fluorescence in relationship 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 labled with 6-FAM, VIC, NED or PET (Applied Biosystems, USA) for visualization. A two-step PCR program was used for the multiplex amplification with GeneAmp® PCR System 9700 (Applied Biosystems, USA) involving: 5 min at 94°C followed by 28 cycles of 30 sec at 94°C, 45 sec at a first T_a°C (Table 3), 1 min at 72°C; 10 cycles of 30 sec at 94°C, 45 sec at a second

T_a°C (Table 3), 45 sec 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 allelic sizes. The facilities for multiplex PCR were provided by the Genomic Variation Laboratory, University of California, Davis CA, USA.

5. Data analyses

Multilocus genotypes were used for analyses. Conformation to Hardy-Weinberg equilibrium (HWE) was tested using a Markov chain approximation of exact test (Guo and Thomson, 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. The test for linkage disequilibrium based on the Chi-square test facilitated by the program GENETIX Version 4.05.2 (Belkhir *et al*., 2004) was also 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 observed departure from HWE towards homozygote excess in some populations, the program Micro-Checker version 2.2.3 (Van Oosterhout *et al*., 2004) was used to explore the evidence of "null-allele" and then the genotypes of populations showing evidences of null alleles were adjusted accordingly, wherever appropriate. 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 independent t-test comparisons in the SPSS statistical package (version

11.5.0) (SPSS Inc, Chicago). CONVERT version 1.31 (Glaubitz, 2004) was used to calculate allele frequencies and identifying private alleles.

The program GENEPOP version 4.0 (Raymond and Rousset, 1995b; Rousset, 2008) was used to test the genetic differentiation between populations based on the allele frequency distribution. Level of genetic differentiation were also assessed using the estimates of pairwise F_{ST} as implemented in ARLEQUIN 3.11 (Excoffier *et al*., 2005) Level of significance of these values were calculated using the same software. To assess the population structure, the F-statistics (F_{ST}, F_{IS} and F_{IT}) were calculated using the program Fstat version 2.9.3.2 (Goudet, 1995). Then the program PHYLIP version 3.67 was employed to calculate the Cavalli-Sforza and Edwards (1967) chord distance (genetic distance) between populations using the adjusted data set with corrected genotypes for null allele. The same program was used for a reconstruction of the phylogenetic tree (Neighbor-joining tree, Felsenstein, 2007) among populations and for estimation of bootstrap-values.

A model-based clustering method for inferring population structure using the program STRUCTURE version 2.3.3 (Pritchard *et al*., 2000; Hubisz, 2009) was also 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), which was calculated using Structure Harvester version 0.6.5 (Earl, 2011). 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 equaling 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 examined population(s).

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

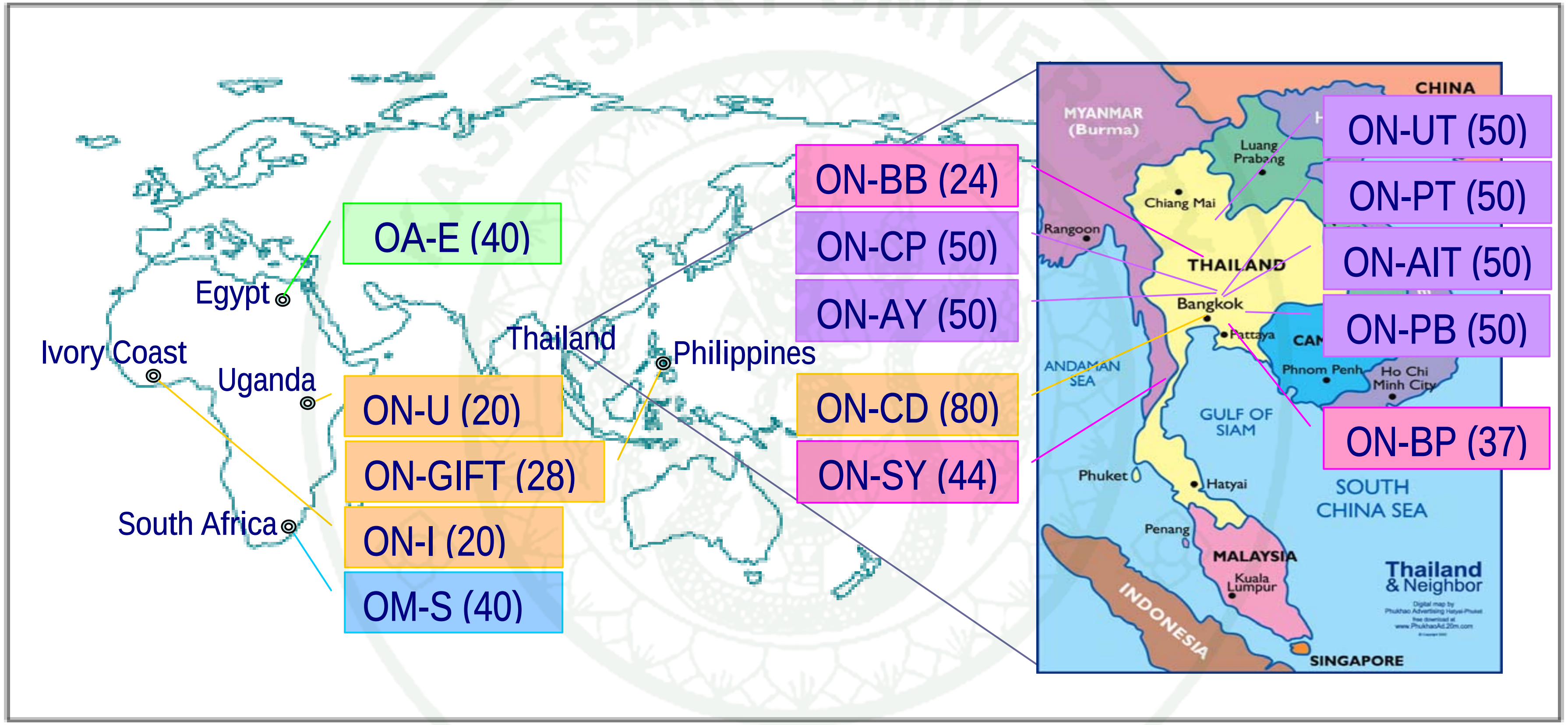

Figure 1 A map showing sampling localities of six Nile tilapia hatchery populations (text box filled with purple), three feral populations of *Oreochromis niloticus* (text box filled with pink) in Thailand, four conspecific reference populations (text box filled with orange) of which abbreviations were as shown in Table 2, and one population each of *O. mossambicus* (OM-S; text box filled with blue) and *O. aureus* (OA-E; text box filled with green).

Table 2 Description of six Nile tilapia hatchery populations and three feral populations of *O. niloticus* 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); A population each of *O. mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E). N = sample size.

Species	**Population Names**	**N**	**Locations**	**Coordinates**
O. niloticus				
	GIFT derived hatchery populations			
	Pathum Thani (ON-PT)	50	Brooder ponds, Pathum Thani Fisheries Test and Research Center, Pathum Thani	14°1′92″N 100°42′62″E
	Uttaradit (ON-UT)	50	Brooder ponds, Uttaradit Fisheries Test and Research Center, Uttaradit	17°28′78″N 100°7′64″E
	Prachin Buri (ON-PB)	50	Brooder ponds, Nam Sai Farm, Prachin Buri	13°58′8″N 101°12′42″E
	CP (ON-CP)	50	Brooder ponds, Charoen Pokphand Farm, Ayutthaya	14°13′86″N 100°38′21″E
	Chitralada derived hatchery populations			
	Ayutthaya (ON-AY)	50	Brooder ponds, Rom Sai Farm, Ayutthaya	14°9′11″N 100°33′53″E
	AIT (ON-AIT)	50	Brooder ponds, Asian Institute of Technology, Phatum Thani	14°4′54″N 100°37′03″E
	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

Table 2 (Continued)

Species	Population Names	N	Locations	Coordinates
Reference populations				
	Chitralada (ON-CD)	80	Brooder ponds, Chitralada Villa Royal Residence, Dusit, Bangkok	13°46′14″N 100°31′18″E
	Uganda (ON-U)	20	Lake Albert, Uganda (provided by W. Mwanja)	1°00′00″N 30°5′00″E
	Ivory Coast (ON-I)	20	Ivory Coast (cultured at Auburn University and provided by K. Veverica)	6°51′00″N 5°18′00″W
	GIFT strain (ON-GIFT)	28	Ninth generation Genetically Improved Farmed Tilapia (G_9 GIFT) strain (collected from the Philippines and provided by Professor N. Taniguchi, Tohoku University, Japan)	14°35′00″N 121°00′00″E
O. mossambicus				
	South Africa (OM-S)	40	Natal, South Africa (cultured at the Agricultural Research Organization and provided by G. Hulata)	28°30′00″S 30°30′00″E
O. aureus				
	Egypt (OA-E)	40	WorldFish Center (provided by Mahmoud Rezk)	30°2′00″N 31°13′00″E

Table 3 Primer details for microsatellite loci used in this study

Locus ID	GenBank Accession no.	LG no.[a]	Primer Sequence (5’-3’)	Dye[b]	Ta (°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

Table 3 (Continued)

Locus ID	GenBank Accession no.	LG no.[a]	Primer Sequence (5’-3’)	Dye[b]	Ta (°C)	Repeat type	Size range (bp)
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 a genetic linkage map of tilapia (*Oreochromis* spp.) (Lee *et al*., 2005)

[b] fluorescently labeled forward primer for multiplex PCR

RESULTS

Genetic change of the GIFT-derived hatchery populations comparing to the GIFT population

Allele frequencies of the fourteen microsatellite loci used in this study are shown in Appendix Table 1. Despite the typical high polymorphism of microsatellites, the locus *UNH222* was monomorphic in the Ivory Coast samples of *O. niloticus* and two loci (*UNH172* and *UNH138*) were monomorphic in the *O. mossambicus* samples. These alleles (*UNH172**246 and *UNH138**164) which are fixed in *O. mossambicus* were also observed in *O. niloticus* populations, namely, both alleles were found in ON-AY ($p = 0.030$ for *UNH172**246; 0.070 for *UNH138**164), ON-AIT ($p = 0.020$ for *UNH172**246; 0.070 for *UNH138**164); *UNH138**164 was observed in ON-CP ($p = 0.060$), ON-CD ($p = 0.013$) and ON-GIFT ($p = 0.143$).

Hardy-Weinberg Equilibrium and Linkage disequilibrium

The Fisher's exact test revealed that six populations, ON-CP, ON-AY, ON-CD and ON-I did not conform to Hardy-Weinberg expectations and displayed homozygote excess while those of ON-PT and OM-S displayed heterozygote excess ($P < 0.0036$; Bonferroni correction) (Table 4). However, when the presence of null alleles was tested using the program Micro-Checker, an indication of null alleles was detected for 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*). Therefore, the genotypes of the loci with null alleles were adjusted according to the Chakraboty algorithm provided by the program. The re-analyses showed the same results of the Fisher's exact test but the locus-wise HWE tests were significant at only 1 - 3 loci of 14 loci in each population (Table 5).

Table 4 Test for conformation to Hardy-Weinberg Equilibrium showing the locus-wise F_{IS}, the probability for overall Fisher's exact test (α = 0.0036 after Bonferroni correction for multiple tests). * denotes statistical significant ($P < 0.0036$)

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	0.1962	-0.0177	0.0623	-0.0324	-0.0390	-0.0138	0.0182	-0.0852	0.1215	-0.0513	-0.0574	-0.0094	0.0002
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	0.1319	0.0381	-0.0064	0.0145	0.0803
Prachin Buri (ON-PB)	0.1455	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	0.0117
CP (ON-CP)	0.1632*	0.0211	0.0565	0.0972	0.1160	0.0082	0.0357	-0.1062	0.1238	0.1061*	0.0683	0.1434	0.2226*	0.0647	0.0835	0.0000
Ayutthaya (ON-AY)	0.1098	-0.0397	0.1707	0.0493*	0.1982*	0.1128	0.1115*	0.0943	0.1075	0.0162	0.0523	0.0152	0.3226*	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	0.2548	0.0392	0.0100	0.0264
Chitralada (ON-CD)	0.0866	0.1105	0.0678	0.2675*	-0.0882	0.0975	0.0007	-0.1112	0.0255	0.0815	0.0936	-0.0033*	-0.0542	-0.0321	0.0479	0.0000
Uganda (ON-U)	0.2739	-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	0.0913
Ivory Coast (ON-I)	0.4290	0.3022*	-0.0270	-	-0.0556	0.0270	-0.4975*	1.0000	-0.3790*	0.3968	0.3596	-0.3194	-0.1875	-0.0629	0.0207	0.0000
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	0.6727
O. mossambicus																
South Afirca (OM-S)	-	-0.0428	-0.2039*	-0.2145	-0.5584*	-0.1380	0.2166	0.0557	-0.1692	-	0.0140*	-0.3929	-0.2381	0.6372*	-0.0952	0.0000
O. aureus																
Egypt (OA-E)	0.0545	0.1932	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	0.0249

Table 5 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 (α = 0.0036 after Bonferroni correction for multiple tests). * denotes statistical significant ($P < 0.0036$)

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	-0.0139	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	0.0106	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	0.0093	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	0.0668	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	0.0752	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	-0.0014	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	0.0300	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	0.0360	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	-0.0421	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 Afirca (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*	-0.1720	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	0.0500	0.0904

Linkage disequilibrium (LD) was discovered wherein all populations showed LD at least at two loci pair (Table 6, Appendix Table 2). Among the Nile tilapia 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 *D* [*D* = 0.0103±0.0028 (ON-CD) to 0.0129±0.0084 (ON-I)] except for ON-GIFT which showed the highest value of mean *D* (*D* = 0.0457±0.0075).

Population structure

The overall F_{ST} calculated across all populations/species was 0.209 and 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) but still significantly higher than zero. This indicated the existence of a population structure among ten populations of *O. niloticus*. The F_{ST} 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, hence implied an existence of population structuring within each group.

Pairwise F_{ST} estimates and level of significance for all population pairs are presented in Table 7. F_{ST} among *O. niloticus* samples ranged from 0.0118 (ON-PT and ON-UT) to 0.3359 (ON-U and ON-I) and that among *O. niloticus* samples and other species; *O. mossambicus* were from 0.3579 (ON-CP) to 0.5857 (ON-I), and *O. aureus* were from 0.3012 (ON-CP) to 0.5263 (ON-I). The pairwise F_{ST} estimates revealed significant differentiation between the GIFT-derived strains and GIFT (pairwise F_{ST} = 0.0156 - 0.0475). Notably, the Chitralada-derived strains were different with the original Chitralada with relatively higher degree (pairwise F_{ST} = 0.0600 - 0.0862). Likewise, relatively high pairwise F_{ST} (0.1753) revealed remarkable genetic difference between the GIFT and Chitralada strain. All of the pairwise F_{ST} values were statistically significant ($P < 0.0008$).

Table 6 Results of linkage disequilibrium tests based on the Chi square test of six Nile tilapia hatchery populations in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. 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

Table 7 A matrix of pairwise F_{ST} value among all of population pairs (below diagonal) and p-value (above diagonal); Every test 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	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-UT	0.0118	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-PB	0.0323	0.0330	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-CP	0.0363	0.0338	0.0476	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-AY	0.0732	0.0712	0.0869	0.0349	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-AIT	0.1403	0.1391	0.1596	0.0753	0.0526	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-CD	0.1750	0.1776	0.1942	0.1031	0.0862	0.0600	***	0.0000	0.0000	0.0000	0.0000	0.0000
ON-U	0.1490	0.1410	0.1700	0.1214	0.1196	0.1451	0.1801	***	0.0000	0.0000	0.0000	0.0000
ON-I	0.2782	0.2856	0.3115	0.2803	0.2965	0.3161	0.3248	0.3359	***	0.0000	0.0000	0.0000
ON-GIFT	0.0156	0.0186	0.0475	0.0369	0.0713	0.1423	0.1753	0.1525	0.3125	***	0.0000	0.0000
OM-S	0.3658	0.3709	0.3830	0.3579	0.3803	0.3991	0.4425	0.4422	0.5857	0.3908	***	0.0000
OA-E	0.3157	0.3217	0.3325	0.3012	0.3365	0.3622	0.3946	0.4113	0.5263	0.3483	0.4986	***

Genetic diversity among populations

The genetic distance, *d*, (Table 8) within species of *O. niloticus* ranged from 0.0151 (ON-PT and ON-UT) to 0.1399 (ON-PB and ON-I), slightly lower than *d* between species: 0.1485 - 0.1926 (*O. aureus* and *O. niloticus*), 0.1914 - 0.2205 (*O. mossambicus* and *O. niloticus*) and d = 0.1978 (*O. mossambicus* and *O. aureus*). The genetic distance between the GIFT-derived strains and GIFT (0.0228 - 0.0395) lower than the Chitralada-derived strains and Chitralada (0.0430 - 0.0497). The genetic distance (0.0908) revealed remarkable genetic difference between the GIFT and Chitralada strain. The allele frequency distribution showed significant differences between all pairs of populations. (Fisher's exact test, $P < 0.0008$, Bonferroni corrected; Appendix Table 3).

A phylogenetic dendrogram

The neighbor-joining tree (Figure 2) revealed that the GIFT-derived hatchery populations clustered well with their presumed origin, ON-GIFT. The hatchery population from Phathum Thani FTRC (ON-PT) was the most similar to the Uttaradit FTRC (ON-UT) (bootstrap = 78.0%), followed by Nam Sai Farm (ON-PB) (bootstrap = 61.8%) and the GIFT reference population (ON-GIFT) (bootstrap = 100.0%). The admixture between GIFT and Chitralada was clearly shown for ON-CP and ON-AY which were placed between the two reference populations of origin with relatively high bootstrap. The Chitralada-derived AIT population was the most similar to the Chitralada population (bootstrap = 96.7%). The *O. niloticus* populations from Uganda (ON-U) and Ivory Coast (ON-I) formed a cluster which was distantly placed between a cluster of the GIFT and its derivative populations and the Chitralada strain. The tree also showed a clear separation of *O. aureus* and *O. mossambicus* from *O. niloticus,* whereby *O. mossambicus* was more distanced from *O. niloticus* than *O. aureus* was.

Table 8 Carvalli-Sforza and Edwards chord distance between six Nile tilapia hatchery populations, four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*.

	ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S
ON-UT	0.0151										
ON-PB	0.0249	0.0253									
ON-CP	0.0340	0.0341	0.0390								
ON-AY	0.0513	0.0534	0.0506	0.0268							
ON-AIT	0.0874	0.0885	0.0875	0.0519	0.0318						
ON-CD	0.0963	0.1030	0.0975	0.0579	0.0497	0.0430					
ON-U	0.1054	0.0991	0.1067	0.0899	0.0909	0.1035	0.1022				
ON-I	0.1286	0.1306	0.1399	0.1256	0.1308	0.1379	0.1170	0.1362			
ON-GIFT	0.0258	0.0228	0.0395	0.0378	0.0512	0.0861	0.0908	0.1016	0.1310		
OM-S	0.1946	0.1973	0.1964	0.1935	0.1920	0.1923	0.2205	0.2039	0.2121	0.1914	
OA-E	0.1531	0.1585	0.1627	0.1485	0.1714	0.1804	0.1769	0.1926	0.1922	0.1651	0.1978

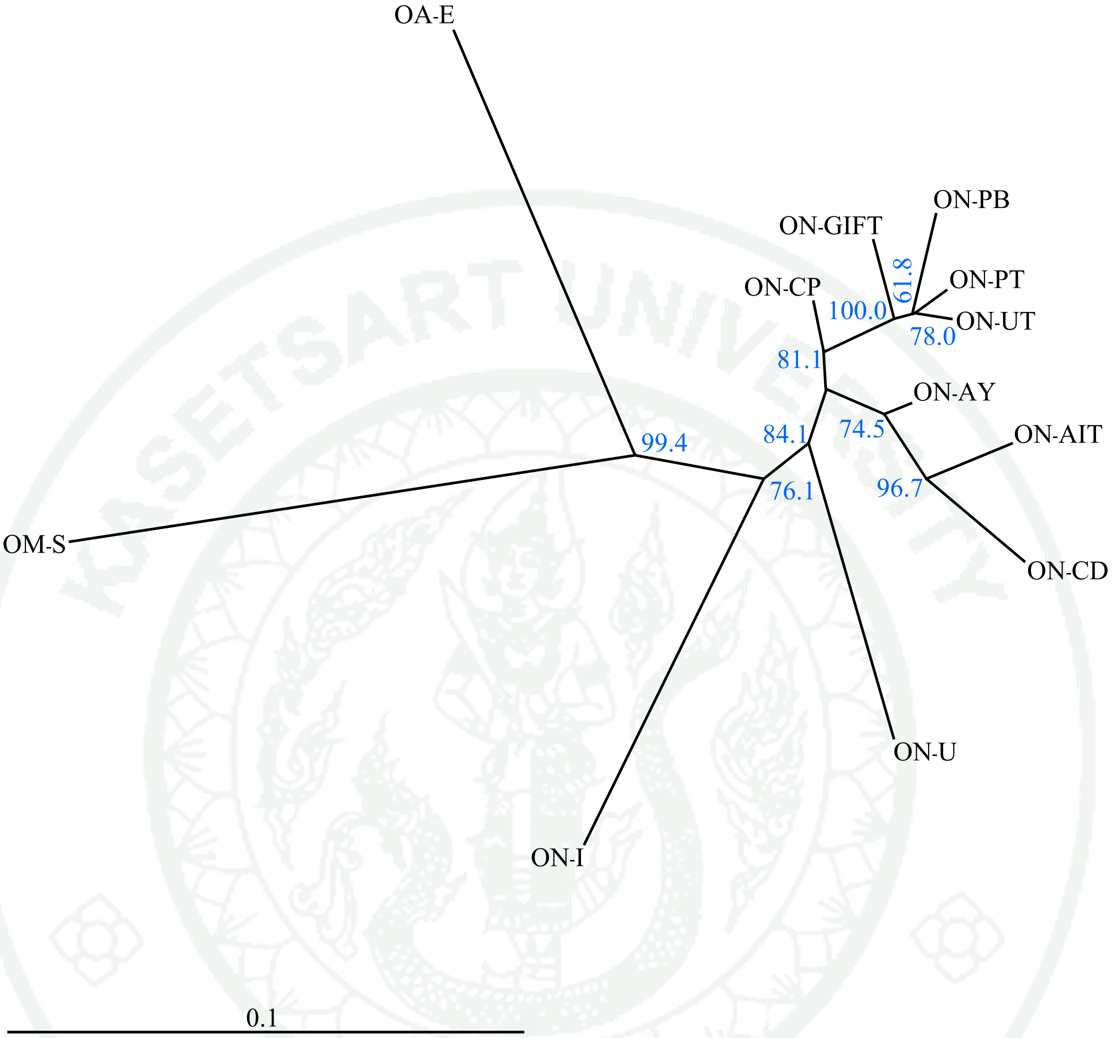

Figure 2 A phylogenetic dendrogram reconstructed from Carvalli-Sforza and Edwards chord distance between six Nile tilapia hatchery populations in Thailand; four conspecific reference populations from Chitralada Villa Royal Residence Thailand (ON-CD), Uganda (ON-U), Ivory Coast (ON-I) and genetically improved GIFT strain (ON-GIFT); A population each of *O. mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E).

Population clustering and population origin

The clustering based on the highest ΔK value (140.08), showed that the best fitted number of populations (K) was six (Figure 3). Cluster 1 comprised Chitralada strain (ON-CD) (an average admixture proportion, q_1 = 0.978) and ON-AIT (q_1 = 0.936). Cluster 2 comprised of 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). Cluster 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 genepool with *O. niloticus* in Thailand, and so did the *O. mossambicus* (OM-S) and *O. aureus* (OA-E) populations (Table 9).

An individual bar plot (Figure 4) shows the probability of assignment of individuals to six genetic groups (K = 6). Admixed individuals determined by $q_{2\text{-}6} > 0.1$ were not found in the reference ON-CD. While 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 contain admixed individuals from ON-CD (q_1 = 0.112 - 0.134) (two individuals). On the contrary, ON-CP contained 20% pure Chitralada (q_1 = 0.874 - 0.984) and 40% pure GIFT (q_2 = 0.899 - 0.990); 36% admixed individual of 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 admixture of ON-CD and ON-GIFT (q_1 = 0.536 - 0.861, q_2 = 0.114 - 0.451). ON-AY comprised of 42% pure ON-CD (q_1 > 0.907), 22% pure GIFT (q_2 > 0.809) and 34% admixed individuals of 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).

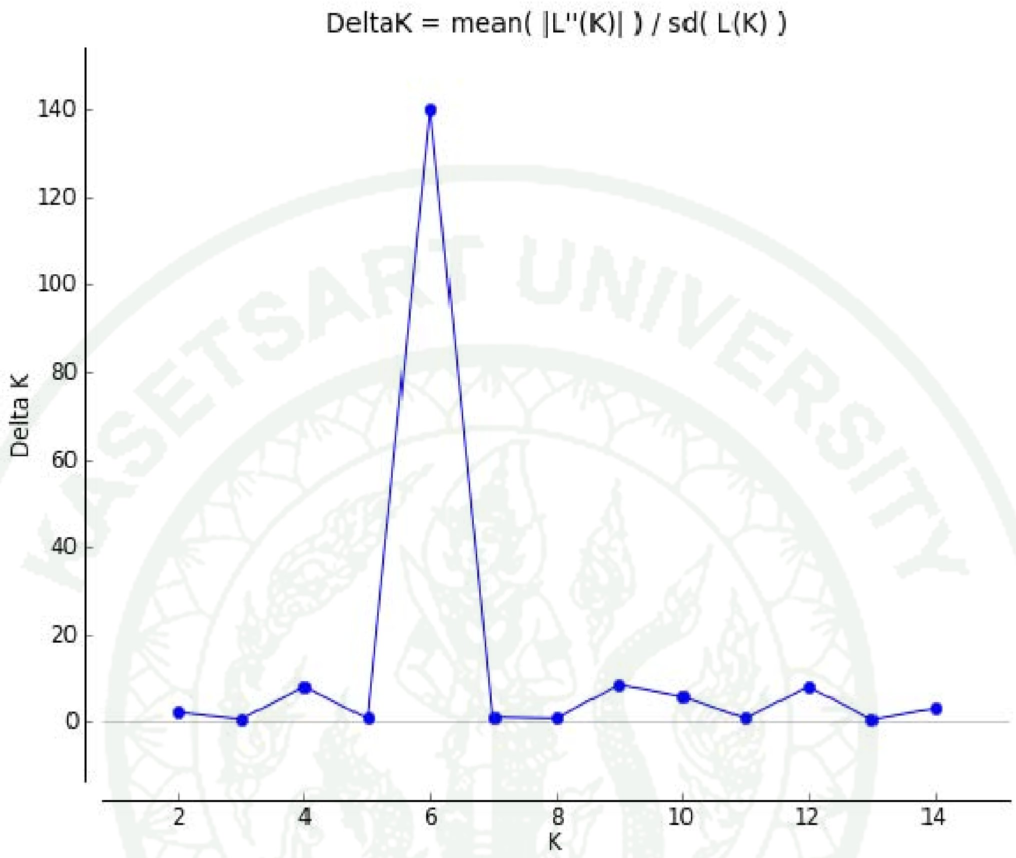

Figure 3 DeltaK (ΔK) value calculated for assuming K = 1 - 15 clusters of six Nile tilapia hatchery populations in Thailand; four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*

Table 9 Proportions of membership 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

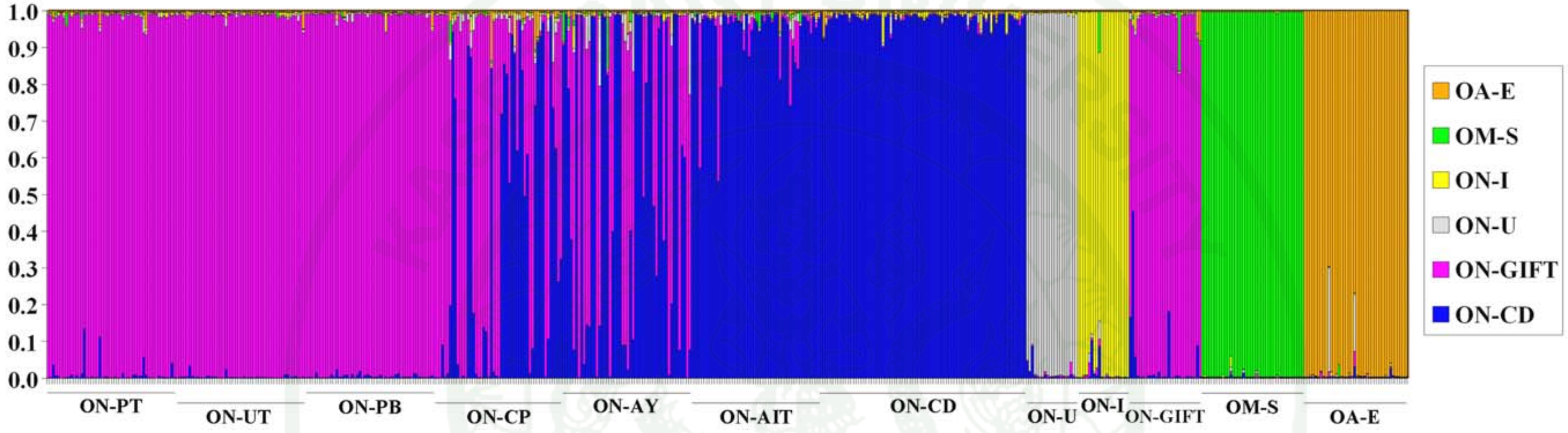

Figure 4 The probability of assignment of individuals to each of the six genetic groups (K = 6) defined in the present study. Each vertical bar represents an individual and each color represents a probability that the individual is assigned to each genepool (admixture proportion, q_i). The values of q_i were provided in the output sheet generated by the program.

Genetic variation within populations of the Nile tilapia hatchery populations

The parameters indicating genetic variation within populations were calculated based on the data after adjustment for null alleles (Table 10). The GIFT-derived populations showed relatively high genetic variation (A_r = 6.64±2.36 of ON-PB to 8.54±2.86 of ON-CP; H_e = 0.73±0.10 of ON-PB to 0.79±0.10 of ON-CP) while that of the GIFT population of origin was also relatively high (A_r = 7.14±2.09; H_e = 0.74±0.13). On the contrary, the Chitralada strain (ON-CD) showed relative low genetic variation (A_r = 4.93±1.59; H_e = 0.65±0.15) while the descendant populations had relatively high genetic variation (A_r = 7.14±1.78 for ON-AIT to 8.23±2.74 for ON-AY; H_e = 0.73±0.12 for ON-AIT to 0.76±0.12 of ON-AY. The genetic variation of the genetically improved GIFT strain was significantly higher than that of the Chitralada strain (ON-CD) (Table 10).

The reference Nile tilapia hatchery population from Ivory Coast showed remarkable low A_r (3.43±2.31) and H_e (0.36±0.28) while those measures from the wild reference population from Uganda were moderate (A_r = 7.50±3.84, H_e = 0.69±0.17). Genetic variation of the *O. mossambicus* population from South Africa (OM-S) was very low both for the parameters representing allele diversity and heterozygosity (A = 3.14±1.23, A_e = 2.05±0.81, A_r = 2.93±1.08, H_o = 0.51±0.33, H_e = 0.43±0.25) while *O. aureus* population (OA-E) had moderate genetic variation (A = 5.86±4.47, A_e = 2.91±2.48, A_r = 5.04±3.65, H_o = 0.46±0.24, H_e = 0.48±0.26) (Table 10).

Table 10 Genetic variation within six 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.

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}	5.07^{a}	7.73^{a}	0.77^{a}	0.76^{a}
				(3.10)	(2.42)	(2.74)	(0.11)	(0.13)
	Uttaradit (ON-UT)	50	14	9.00^{ab}	4.76^{ab}	7.49^{a}	0.75^{a}	0.75^{a}
				(3.46)	(2.08)	(2.66)	(0.11)	(0.12)
	Prachin Buri (ON-PB)	50	14	7.57^{bcd}	4.17^{abc}	6.64^{abc}	0.72^{a}	0.73^{a}
				(2.65)	(1.91)	(2.36)	(0.11)	(0.10)
	CP (ON-CP)	50	14	10.57^{a}	5.24^{a}	8.54^{a}	0.74^{a}	0.79^{a}
				(3.94)	(2.19)	(2.86)	(0.09)	(0.10)
	Ayutthaya (ON-AY)	50	14	10.14^{ab}	4.88^{ab}	8.23^{a}	0.71^{a}	0.76^{a}
				(3.74)	(2.04)	(2.74)	(0.12)	(0.12)
	AIT (ON-AIT)	50	14	8.64^{abc}	4.16^{abc}	7.14^{ab}	0.73^{a}	0.73^{a}
				(2.59)	(1.68)	(1.78)	(0.11)	(0.12)
	Chitralada Palace (ON-CD)	80	14	6.07^{cd}	3.30^{bcd}	4.93^{cde}	0.63^{ab}	0.65^{a}
				(1.90)	(1.29)	(1.59)	(0.14)	(0.15)
	Uganda (ON-U)	20	14	7.50^{bcd}	3.99^{abc}	7.50^{a}	0.67^{a}	0.69^{a}
				(3.84)	(2.66)	(3.84)	(0.21)	(0.17)
	Ivory Coast (ON-I)	20	13	3.43^{e}	2.05^{d}	3.43^{de}	0.38^{c}	0.36^{b}
				(2.31)	(1.44)	(2.31)	(0.33)	(0.28)
	GIFT strain (ON-GIFT)	28	14	7.64^{bcd}	4.37^{abc}	7.14^{ab}	0.74^{a}	0.74^{a}
				(2.24)	(1.87)	(2.09)	(0.10)	(0.13)
O. mossambicus								
	South Africa (OM-S)	40	12	3.14^{e}	2.05^{d}	2.93^{e}	0.51^{bc}	0.43^{b}
				(1.23)	(0.81)	(1.08)	(0.33)	(0.25)
O. aureus								
	Egypt (OA-E)	40	14	5.86^{d}	2.91^{cd}	5.04^{bcd}	0.46^{c}	0.48^{b}
				(4.47)	(2.48)	(3.65)	(0.24)	(0.26)

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

Contribution of GIFT tilapia in feral populations in Thailand

The data set obtained from part I revealed monomorphisms of the locus *UNH222* in the Ivory Coast samples of *O. niloticus* and two loci (at *UNH172*246* and *UNH138*164*) were monomorphic in the *O. mossambicus* samples. Notably, the *UNH172*246* was also detected in ON-BP (p = 0.027) and ON-SY (p = 0.477) while the allele *UNH138*164* was detected in the three feral populations (ON-BP, p = 0.054; ON-BB, p = 0.021 and ON-SY, p = 0.421) and two reference populations (ON-GIFT, p = 0.143; ON-CD, p = 0.013) (Appendix Table 4).

Hardy-Weinberg Equilibrium and Linkage disequilibrium

The Fisher's exact test revealed that four populations, ON-BP, ON-SY, ON-CD and ON-I did not conform to Hardy-Weinberg expectation towards homozygote excess while that of OM-S was towards heterozygote excess ($P < 0.0036$; Bonferroni correction) (Table 11). However when the presence of null alleles was tested using the program Micro-Checker, the sign of null alleles was shown for six loci in ON-BP (*UNH211, UNH222, UNH212, UNH153, UNH192* and *UNH1004*); four loci for ON-SY (*UNH172, UNH222, UNH160* and *UNH1004*); two loci for ON-I (*UNH172,* and *UNH211*); and one locus each for ON-BB (*UNH172*), ON-CD (*UNH222*), ON-U (*UNH172*), ON-GIFT (*UNH211*), OM-S (*UNH173*) and OA-E (*UNH211*). Therefore the genotypes of the loci with null alleles were adjusted according to the Chakraboty algorithm provided by the program. The re-analyses showed the same results of the Fisher's exact test. Whereas the locus-wise HWE tests showed that every locus conformed to HWE in ON-SY and homozygote excess at three loci in ON-BP (Table 12).

Table 11 Test for conformation to Hardy-Weinberg Equilibrium 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 significant ($P < 0.0036$)

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																
Bang Phra (ON-BP)	0.1150	0.2275	0.1064	0.2993*	0.2155	0.0824	-0.0286*	0.4030	-0.1188*	-0.0059	0.3204*	0.1852*	0.0156	0.0052	0.1241	0.0000
Bueng Boraphet (ON-BB)	0.2485	0.0350	0.0873	0.2663	0.0394	-0.1233	-0.0478	-0.1767	-0.1248	0.2114	-0.2063	0.1593	-0.0142	-0.0337	0.0345	0.1948
Sam Roi Yot (ON-SY)	0.1644	0.1348	0.1254	0.4071*	-0.0013	0.2119	-0.1990	-0.0825	-0.2187	-0.0443	0.0484	0.1667	0.1204	-0.0336	0.0655	0.0000
Chitralada (ON-CD)	0.0866	0.1105	0.0678	0.2675*	-0.0882	0.0975	0.0007	-0.1112	0.0255	0.0815	0.0936	-0.0033*	-0.0542	-0.0321	0.0479	0.0000
Uganda (ON-U)	0.2739	-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	0.0762
Ivory Coast (ON-I)	0.4290	0.3022*	-0.0270	-	-0.0556	0.0270	-0.4975*	1.0000	-0.3790*	0.3968	0.3596	-0.3194	-0.1875	-0.0629	0.0207	0.0000
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	0.5976
O. mossambicus																
South Afirca (OM-S)	-	-0.0428	-0.2039*	-0.2145	-0.5584*	-0.1380	0.2166	0.0557	-0.1692	-	0.0140*	-0.3929	-0.2381	0.6372*	-0.0952	0.0000
O. aureus																
Egypt (OA-E)	0.0545	0.1932	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	0.0397

Table 12 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 significant ($P < 0.0036$)

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																
Bang Phra (ON-BP)	0.1150	0.1223	0.1064	0.0962	0.0891	0.0824	-0.0286*	0.1753	-0.1188*	-0.0059	0.1391	0.1026*	0.0156	0.0052	0.0576	0.0000
Bueng Boraphet (ON-BB)	0.1321	0.0350	0.0873	0.2663	0.0394	-0.1233	-0.0478	-0.1767	-0.1248	0.2114	-0.2063	0.1593	-0.0142	-0.0337	0.0227	0.2536
Sam Roi Yot (ON-SY)	0.1370	0.1348	0.1254	0.1025	-0.0013	0.0514	-0.1990	-0.0825	-0.2187	-0.0443	0.0484	0.0790	0.1204	-0.0336	0.0210	0.0001
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	0.0300	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	0.0360	0.2307
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	-0.0421	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	0.5952
O. mossambicus																
South Afirca (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*	-0.1720	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	0.0500	0.0954

Linkage disequilibrium (LD) was discovered wherein all populations showed LD at least at one loci pair (Table 13, Appendix Table 5). The most intensive LD, shown at 45 loci pairs was observed in ON-BP following 10 loci pairs in ON-BB, 9 loci pairs in OA-E, 8 loci pairs in ON-SY, 7 loci pairs in ON-I, 2 loci pairs in ON-CD and ON-GIFT and one loci pair in ON-U and OM-S. However, among the feral *O. niloticus* populations, linkage disequilibrium was more intensive in ON-SY as indicated by high value of mean *D* (disequilibrium coefficient = 0.0668±0.0199) comparing with ON-BB (*D* = 0.0305±0.0033) and ON-BP (*D* = 0.0237±0.0017). The reference populations/species showed much lower *D* [-0.0076±0.0103 (OM-S) to 0.0386±0.0042 (ON-GIFT)].

Table 13 Results of linkage disequilibrium tests based on the Chi square test of three feral *O. niloticus* populations in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. Population abbreviations are as in Table 1. *D* = disequilibrium coefficient.

Population	**No. of loci pairs for LD**	***D*** **(mean ± SE)**
ON-BP	45	0.0237±0.0017
ON-BB	10	0.0305±0.0033
ON-SY	8	0.0668±0.0199
ON-CD	2	0.0062±0.0001
ON-U	1	0.0299±0.0049
ON-I	7	0.0266±0.0132
ON-GIFT	2	0.0386±0.0042
OM-S	1	-0.0076±0.0103
OA-E	9	0.0093±0.0065

Population structure

The overall F_{ST} calculated across all populations/species was 0.292 and greater than zero ($CI_{99\%}$ = 0.261 - 0.332). When *O. aureus* and *O. mossambicus* samples were removed from the analyses, the F_{ST} was reduced to 0.168 ($CI_{99\%}$ = 0.138 - 0.197) but still significantly higher than zero. This indicated the existence of a population structure among seven populations of *O. niloticus*. The F_{ST} among Thai samples of *O. niloticus* was relatively low but statistically significant (F_{ST} = 0.102; $CI_{99\%}$ = 0.084 - 0.126), implying that at least one population was different from the others.

Pairwise F_{ST} estimates and level of significance for all population pairs are presented in Table 14. F_{ST} among *O. niloticus* samples ranged from 0.0501 (ON-BP and ON-BB) to 0.3907 (ON-I and ON-BB) and that among *O. niloticus* samples and other species; *O. mossambicus* were from 0.3547 (ON-SY) to 0.5857 (ON-I), and *O. aureus* were from 0.3437 (ON-BP) to 0.5263 (ON-I). The pairwise F_{ST} comparisons among Thai feral populations of *O. niloticus* and Chitralada reference population ranged from 0.0537 (ON-BP) to 0.1681 (ON-SY) and that among Thai feral populations of *O. niloticus* and GIFT reference population ranged from 0.1107 (ON-BP) to 0.1715 (ON-SY). All of the pairwise F_{ST} values were statistically significant ($P < 0.0014$).

Table 14 A matrix of pairwise F_{ST} value among all of population pairs (below diagonal) and *p*-value (above diagonal); Every test was significant different ($P < 0.0014$ after Bonferroni correction for multiple tests)

	ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E
ON-BP	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-BB	0.0501	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-SY	0.0809	0.1437	***	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ON-CD	0.0537	0.0620	0.1681	***	0.0000	0.0000	0.0000	0.0000	0.0000
ON-U	0.1154	0.1645	0.2004	0.1801	***	0.0000	0.0000	0.0000	0.0000
ON-I	0.2792	0.3907	0.3196	0.3248	0.3359	***	0.0000	0.0000	0.0000
ON-GIFT	0.1107	0.1688	0.1715	0.1753	0.1525	0.3125	***	0.0000	0.0000
OM-S	0.3935	0.4807	0.3547	0.4452	0.4422	0.5857	0.3908	***	0.0000
OA-E	0.3437	0.4232	0.3579	0.3946	0.4113	0.5263	0.3483	0.4986	***

Genetic diversity among populations

The genetic distance, *d*, (Table 15) within species of *O. niloticus* ranged from 0.0324 (ON-CD and ON-BP) to 0.1526 (ON-I and ON-BB), slightly lower than *d* between species: 0.1773 - 0.2069 (*O. aureus* and *O. niloticus*), 0.1829 - 0.2369 (*O. mossambicus* and *O. niloticus*) and *d* = 0.2126 (*O. mossambicus* and *O. aureus*). The feral populations of *O. niloticus* in Thailand were more genetically similar with the Thai Chitralada population (ON-CD) (*d* = 0.0324 - 0.0749) than with the reference populations from Uganda, Ivory Coast and GIFT strain (*d* = 0.0971 - 0.1526). The allele frequency distribution showed significant differences between all pairs of populations (Fisher's exact test, $P < 0.0014$, Bonferroni corrected; Appendix Table 6).

Phylogenetic dendrogram

The neighbor-joining tree (Figure 5) revealed that the Thai populations clustered well with their presumed ancestor, ON-CD. The population from Bueng Boraphet reservoir (ON-BB) was the most similar to the Chitralada population (ON-CD) (bootstrap = 96.5%), followed by the Bang Phra population (ON-BP) (bootstrap = 60.0%) and Sam Roi Yot (ON-SY) (bootstrap = 98.2%). The *O. niloticus* populations from Uganda (ON-U) and Ivory Coast (ON-I) formed a cluster which was distantly placed between the Thai populations cluster and the GIFT strain. The tree also showed a clear separation of *O. aureus* and *O. mossambicus* from *O. niloticus,* whereby *O. mossambicus* was more distanced from *O. niloticus* than *O. aureus* was.

Table 15 Carvalli-Sforza and Edwards chord distance between three feral *O. niloticus* populations, four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*.

	ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S
ON-BB	0.0344							
ON-SY	0.0476	0.0612						
ON-CD	0.0324	0.0332	0.0749					
ON-U	0.0971	0.0985	0.1328	0.1098				
ON-I	0.1226	0.1526	0.1415	0.1257	0.1464			
ON-GIFT	0.0764	0.0928	0.1200	0.0976	0.1092	0.1408		
OM-S	0.2054	0.2254	0.1829	0.2369	0.2191	0.2279	0.2056	
OA-E	0.1797	0.1939	0.1955	0.1901	0.2069	0.2065	0.1773	0.2126

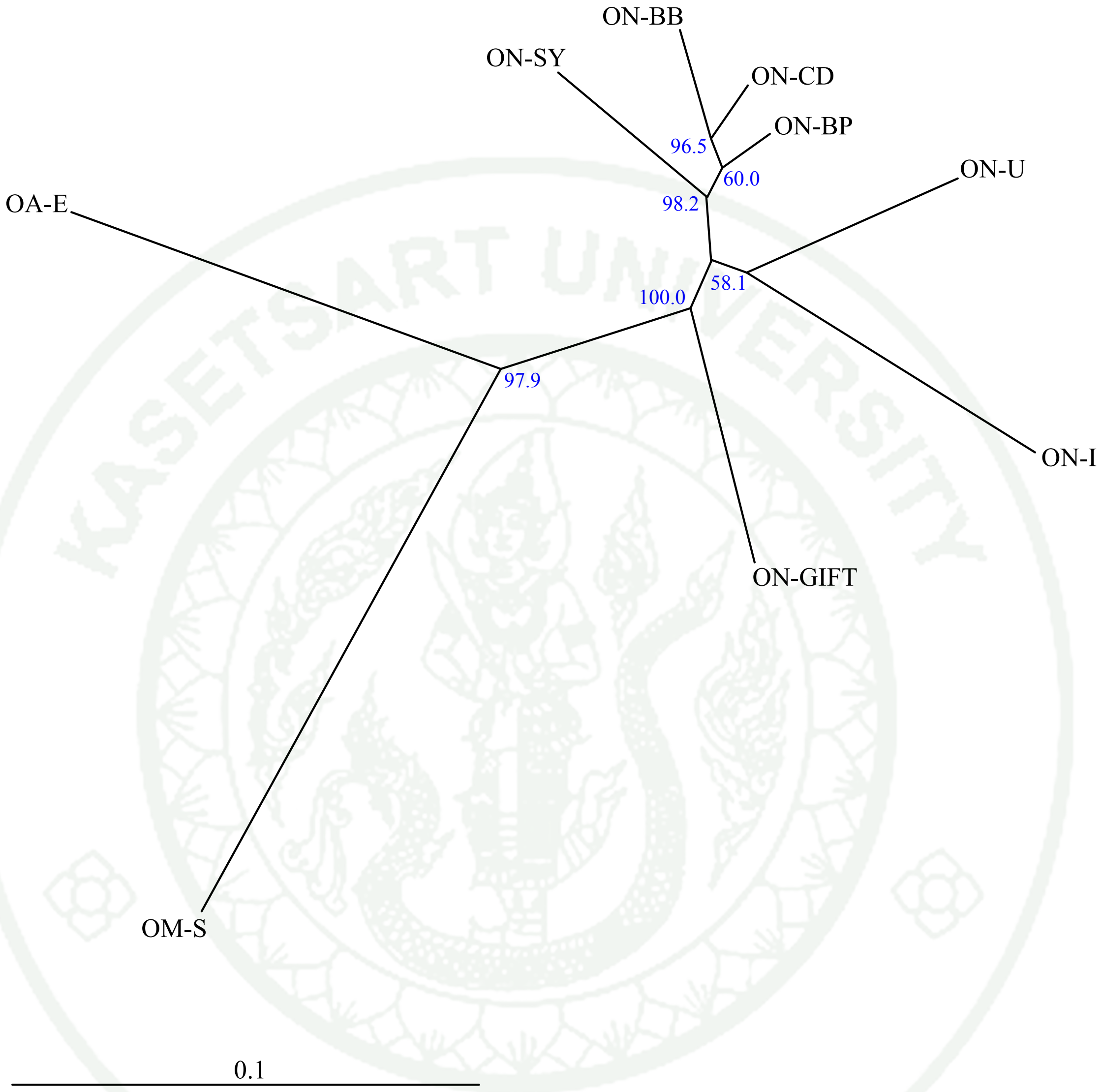

Figure 5 A phylogenetic dendrogram reconstructed from Carvalli-Sforza and Edwards chord distance between three feral populations of *O. niloticus* in Thailand (ON-BB, ON-BP and ON-SY); four conspecific reference populations from Chitralada Villa Royal Residence Thailand (ON-CD), Uganda (ON-U), Ivory Coast (ON-I) and genetically improved GIFT strain (ON-GIFT); A population each of *O. mossambicus* (from South Africa, OM-S) and *O. aureus* (from Egypt, OA-E).

Population clustering and population origin

The clustering using the STRUCTURE program showed, base on the highest ΔK value (469.05), that the best fitted number of populations (K) was seven (Figure 6). However, there was the second peak of ΔK at $K = 4$ (Figure 6) which suggested that there may be sub-structuring in very closely related populations (*O. niloticus* populations) (Pritchard *et al*., 2000). Therefore, the reference populations/species which did not share proportions of membership ($q < 0.1$) (Table 16) with the feral populations (ON-U, ON-I, OA-E) were excluded except for OM-S. OM-S was included because there was a possibility that introgression from *O. mossambicus* may have occurred in some feral populations. Eventually the analysis was repeated with only three reference populations/species (ON-CD, ON-GIFT and OM-S).

The results of the re-analysis showed a single mode (Figure 7) at $K = 3$ base on the highest ΔK value (306.51). This suggested that the best fitted number of populations was three. The proportion of membership, averaged across individuals in a population, clearly assigned the reference populations/species into separate clusters with high proportions of membership (0.974 - 0.996) (Table 17). Among which ON-BP, as expected, shared highest proportion of membership (0.724) with ON-CD following by 0.268 sharing with ON-GIFT. Relative higher proportion of ON-BB genepool (proportion of membership = 0.914) was assigned to cluster 1 with ON-CD, while the assignment of ON-BB to other cluster was considered as *nil* (proportion of membership < 0.1). Notably, a certain proportion of ON-SY genepool (a proportion of membership = 0.121) was assigned to a cluster of *O. mossambicus* while a majority of ON-SY genepool (0.868) was assigned to cluster 1 with ON-CD.

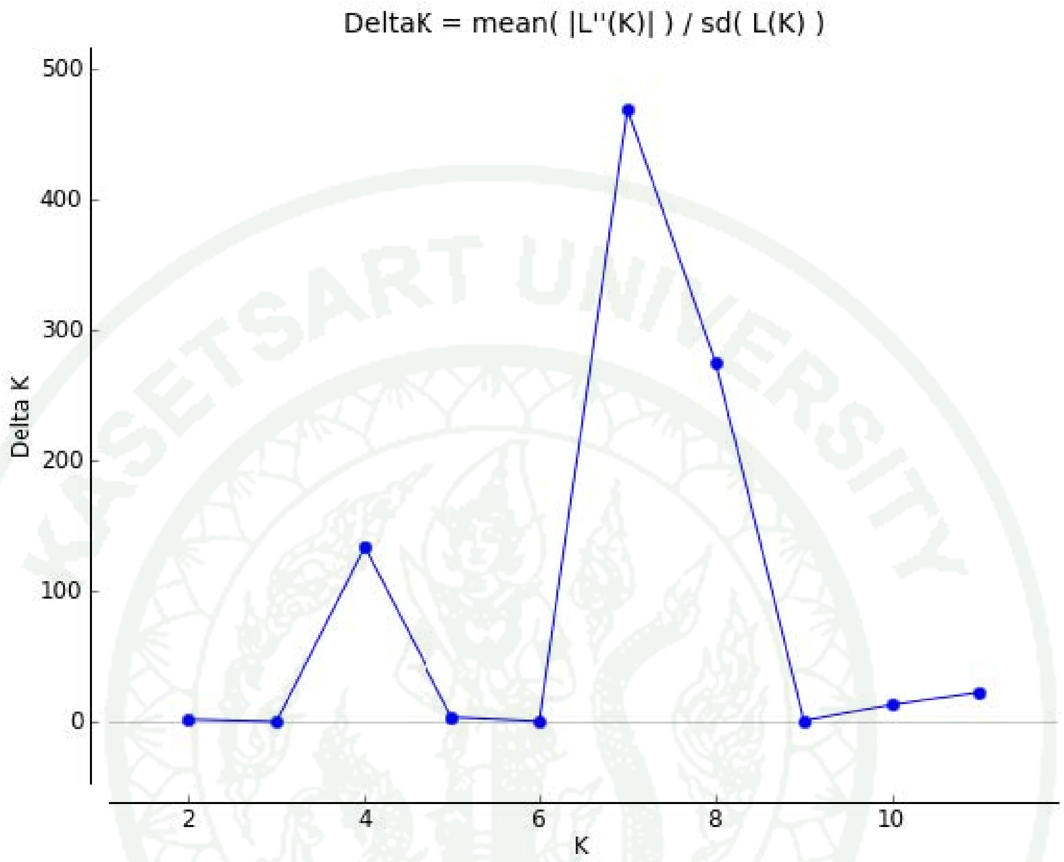

Figure 6 DeltaK (Δ*K*) value calculated for assuming *K* = 1 - 12 clusters of three feral populations of *O. niloticus* in Thailand; four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*

Table 16 Proportion of membership of each pre-defined population in each of the seven clusters (*K*=7). (STRUCTURE: 100,000 Burn-in period; 100,000 Reps)

Given	**Inferred Clusters**							**Number of**
Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Individuals**
ON-BP	0.660	0.198	0.102	0.007	0.024	0.005	0.004	37
ON-BB	0.893	0.073	0.014	0.011	0.003	0.003	0.003	24
ON-SY	0.178	0.007	**0.787**	0.019	0.004	0.002	0.002	44
ON-CD	**0.979**	0.004	0.004	0.004	0.006	0.001	0.003	80
ON-U	0.005	0.008	0.002	**0.978**	0.003	0.002	0.001	20
ON-I	0.011	0.005	0.002	0.004	**0.970**	0.006	0.001	20
ON-GIFT	0.010	**0.970**	0.004	0.004	0.005	0.006	0.002	28
OM-S	0.001	0.002	0.002	0.001	0.002	**0.991**	0.001	40
OA-E	0.004	0.003	0.002	0.011	0.002	0.002	**0.977**	40

An individual bar plot (Figure 8) showed that ON-BP mainly comprised of pure ON-CD (23 individuals; q_1 = 0.888 - 0.996). Seven pure GIFT (q_2 = 0.964 - 0.993) and seven admixed individuals between ON-CD and ON-GIFT (q_1 = 0.172 - 0.880; q_2 = 0.116 - 0.826) were observed in ON-BP. Less degree of admixture was reported for ON-BB which comprised of 21 pure ON-CD (q_1 = 0.961 - 0.997); none of pure GIFT; three admixed individual between ON-CD and ON-GIFT (q_1 = 0.118 - 0.620; q_2 = 0.379 - 0.881). ON-SY comprised of 26 pure ON-CD (q_1 = 0.898 - 0.995); 18 admixed individuals between ON-CD and OM-S (q_1 = 0.515 - 0.896; q_3 = 0.102 - 0.483).

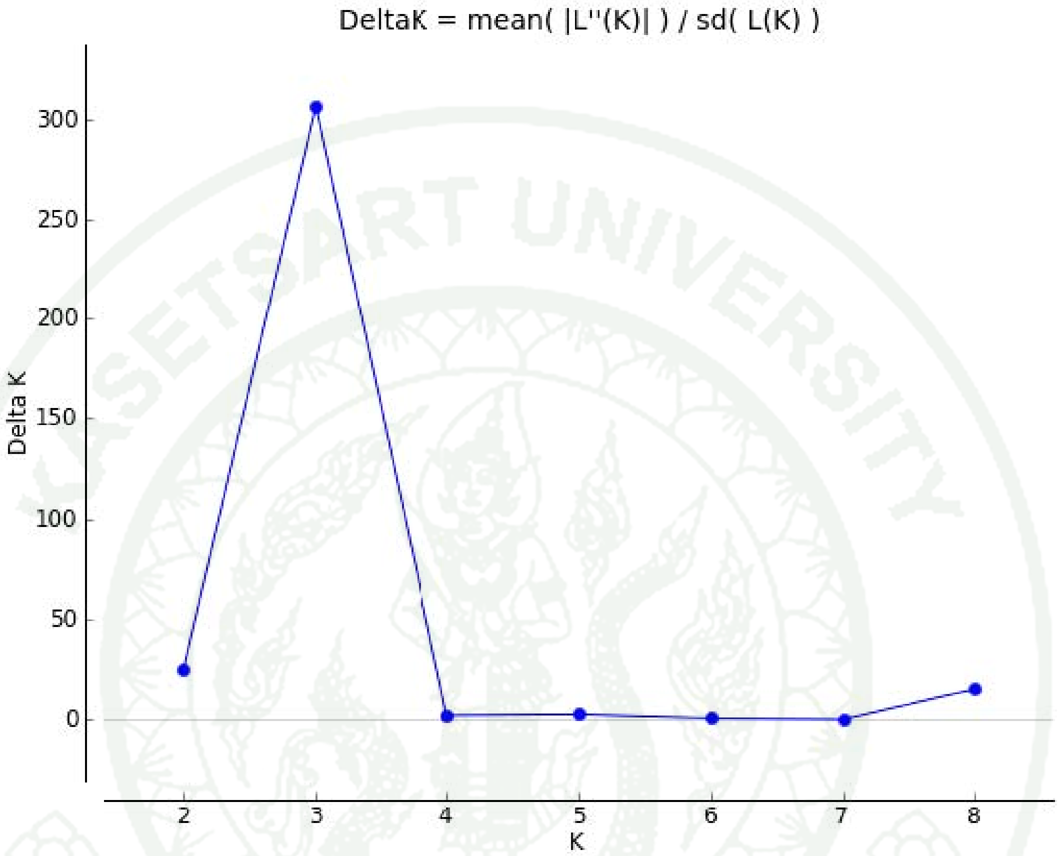

Figure 7 DeltaK (Δ*K*) value calculated for assuming K = 1 - 9 clusters of three feral populations of *O. niloticus* in Thailand; four reference populations of *O. niloticus* and one population each of *O. mossambicus* and *O. aureus*

Table 17 Proportion of membership of each pre-defined population in each of the three clusters (*K*=3). (STRUCTURE: 100,000 Burn-in period; 100,000 Reps)

Given Pop.	Inferred Clusters			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

Genetic variation within populations of the feral populations

One of the feral population (ON-BP) had significantly higher genetic variation than the presumed population of origin (ON-CD) (A_r and H_e of ON-BP population = 7.57±1.68 and 0.77±0.08, respectively; those of ON-CD population = 4.93±1.59 and 0.65±0.15, respectively, Table 18) but equal to those of the newly introduced strain (ON-GIFT) (A_r and H_e = 7.14±2.09 and 0.74±0.13, respectively). The other feral populations (ON-BB and ON-SY) had comparable genetic variation to those of ON-CD and ON-GIFT (Table 18).

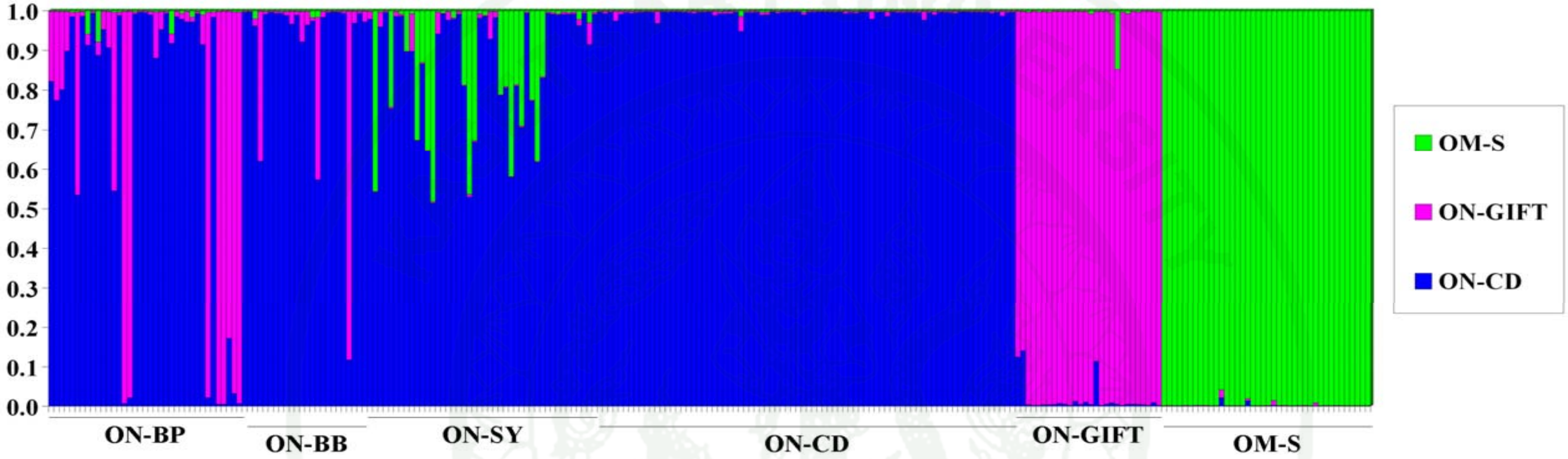

Figure 8 The probability of assignment of individuals to each of the three genetic groups (K = 3) defined in the present study. Each vertical bar represents an individual and each color represents a probability that the individual is assigned to each genepool (admixture proportion, q_i). The values of q_i were provided in the output sheet generated by the program.

Table 18 Genetic variation within three feral *O. niloticus* 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.

Species	**Population**	**N**	**Genetic variation**					
			P	A	A_e	A_r	H_o	H_e
O. niloticus								
	Bang Phra (ON-BP)	37	14	9.50^{a}	4.68^{a}	7.57^{a}	0.74^{a}	0.77^{a}
				(2.38)	(1.59)	(1.68)	(0.08)	(0.08)
	Bueng Boraphet (ON-BB)	24	14	5.79^{b}	2.98^{bc}	5.42^{bc}	0.61^{ab}	0.62^{ab}
				(2.12)	(1.17)	(1.85)	(0.18)	(0.18)
	Sam Roi Yot (ON-SY)	44	14	6.57^{b}	3.74^{ab}	5.61^{bc}	0.71^{a}	0.72^{a}
				(1.99)	(1.00)	(1.41)	(0.07)	(0.09)
	Chitralada Palace (ON-CD)	80	14	6.07^{b}	3.30^{ab}	4.93^{cd}	0.63^{ab}	0.65^{a}
				(1.90)	(1.29)	(1.59)	(0.14)	(0.15)
	Uganda (ON-U)	20	14	7.50^{ab}	3.99^{ab}	7.50^{a}	0.67^{a}	0.69^{a}
				(3.84)	(2.66)	(3.84)	(0.21)	(0.17)
	Ivory Coast (ON-I)	20	13	3.43^{c}	2.05^{c}	3.43^{de}	0.38^{c}	0.36^{c}
				(2.31)	(1.44)	(2.31)	(0.33)	(0.28)
	GIFT strain (ON-GIFT)	28	14	7.64^{ab}	4.37^{a}	7.14^{ab}	0.74^{a}	0.74^{a}
				(2.24)	(1.87)	(2.09)	(0.10)	(0.13)
O. mossambicus								
	South Africa (OM-S)	40	12	3.14^{c}	2.05^{c}	2.93^{e}	0.51^{bc}	0.43^{c}
				(1.23)	(0.81)	(1.08)	(0.33)	(0.25)
O. aureus								
	Egypt (OA-E)	40	14	5.86^{b}	2.91^{bc}	5.04^{cd}	0.46^{bc}	0.48^{bc}
				(4.47)	(2.48)	(3.65)	(0.24)	(0.26)

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

DISCUSSION

Genetic change of the GIFT-derived hatchery populations comparing to the 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 following factors: genetic drift due to using small number of broodstock during founding event and in later generations, selection (natural and artificial; intentional and unintentional), introduction of genetically different populations, and non-random mating (Norris *et al*., 1999; Fiumera *et al*., 2000; Taniguchi, 2003; Aho *et al*., 2006; Qin *et al*., 2007). Due to the relative high genetic variation especially allelic richness of the GIFT-derived populations, it is assumed that genetic drift which has pronounced effect on allele loss (Allendorf and Phelps, 1980) may only partly responsible for the genetic differentiation observed in this study. This is supported by the large N_e assumed from the following information: 1) breeding of Nile tilapia required 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, personal communication), 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-Equia *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.

Interspecific introgression

The present study found interspecific introgression, as indicated 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 of 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* haplotypes in the sixth generation of GIFT from World Fish 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) suggests that the introgression recently occurred (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 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 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.

The 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). It is possible that the record on the introgression was covered by the owner of ON-CP while the reason of the introgression was unknown.

Possible impacts of interspecific introgression

Introgression in fishes is believed to be detrimental. For examples, 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 Galápapagos finch, Grant and Grant, 1998; Grant and Grant, 2002). 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 *et al*., 2010), while performance of hybrid and introgressed *Mytilus* relative to parental species varied considerably according to 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). On the contrary, the same introgression occurred in GIFT strain did not result in unwanted traits (e.g. DOF, 2006) which may be a consequence of selection which targeted on improving growth and appearances (Bentsen *et al*., 1998; Eknath and Acosta, 1998).

Intraspecific 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. In general, intraspecific introgression occurring between genetically distinct populations or between domesticated and wild populations had adverse impacts due to outbreeding depression (e.g., increased infectious disease susceptibility of introgressed individuals between geographic populations of large mouth 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*; Sušnik *et al*., 2004). The underlying reason for cross breeding occurring in Chitralada-derived, ON-AY, was to restore genetic variation with concern on maintaining Chitralada integrity. Nevertheless, due to the confused naming of the advanced generations of GIFT as Chitralada III, the interstrain crossing occurred (Prayad Soda, personal communication). 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 varation 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 Chitralada (Dey *et al*., 2000; Dan and Little, 2000; Hussain *et al*., 2000; Rutten *et al*., 2004b). 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, GIFT strain has smaller proportion of head and broader trunk than the Chitralada (DOF, 2006). To date, the 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 based population of GIFT rather than recent introgression (model-based clustering). On the contrary, the presence of GIFT alleles in the Chitralada-derived ON-AIT 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 appearances of a strain, and thus possibly causes confusion when the strain is used as founder stock or for genetic improvement programs.

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

High levels of genetic variation in 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 in the production of high quality seed for aquaculture (Taniguchi, 2003). Genetic variation, especially effective number of allele per locus, of the hatchery populations included in this study (A_e = 4.16 - 5.42) 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 was 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, Whitler *et al*., 2005; *Litopenaeus vannamei*, Perez-Enriquez *et al*., 2009). Small $N_{e,}$ which theoretically reduced 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 of neutrality of genetic markers used for the studies.

The genetic variation of GIFT strain in the present study (A_r = 7.14±2.09, H_e = 0.74±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 small number of loci in the earlier study. While the study by Rutten *et al*. (2004a) which was based on 14 loci supported our result (A = 7.5, H_e = 0.70) as did the study of McKinna *et al*. (2010). 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*., 2004a) compared to the average for freshwater fishes (A = 7.5, DeWoody and Avise, 2000) while heterozygosity which was relatively high relative to freshwater fishes (0.46, DeWoody and Avise, 2000), varied considerably (H_e = 0.62 - 0.82, Ambali *et al*., 2000; Romana-Eguia *et al*., 2004; Rutten *et al*., 2004a).

Among the two major origins of Nile tilapia hatchery populations in Thailand, ON-GIFT has slightly higher molecular genetic variation than that of 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 relatively high number of brooders (an average of 92 sires and 163 dams/generation during the first five generations) (Eknath *et al*., 1993; Bentsen *et al*., 1998; Eknath and Acosta, 1998) and N_e of 88 individuals from generation 6-13 (Ponzoni *et al*., 2010). In addition, despite the selection pressure exerted during ten years of the selection program, good broodstock management, e.g. using large number of brooders, relatively equal family contribution, 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 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 alleleic 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 (*Salmo salar*) correlated with body weight, length and fluctuation asymmetry, while microsatellite based genetic variation did not show such relationship (Borrell *et al*., 2004). Despite a complicated relationship between genetic variation and performance, relatively large genetic variation of 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.

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 managed with the brooders for seed production.

According to broodstock management regimes, the hatchery populations used in this study may be divided into four categories; 1) closed population 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 = 8.23±2.74 to 8.54±2.86) comparing to the "closed" populations (6.64±2.36 to 7.73±2.74). However, the relatively low A_e (effective number of alleles per locus) of ON-AY (A_e = 4.88±2.04) 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 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, sign 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.

Contribution of GIFT tilapia in feral populations in Thailand

The present study found evidence that ON-CD (Chitralada strain) was a major contributor to genepools of the three feral populations of *O. niloticus* in Thailand. Genetic differentiation was apparent among the feral populations. The feral population from Bang Phra (ON-BP) has relatively high genetic variation while those of Bueng Boraphet (ON-BB) and Sam Roi Yot (ON-SY) were low. Intraspecific genetic introgression (from ON-GIFT to ON-BP and ON-BB) was observed, while the interspecific (from *O. mossambicus* to *O. niloticus*) introgression was observed only in Sam Roi Yot (ON-SY) population.

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

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

Genetic introgression was detected at intra- and inter-specific levels. The introgressions likely play significant role in shaping genetic constitution of the feral populations. The introgression is an important mechanism to increase genetic variation (Grant and Grant, 1998; Grant and Grant, 2002; Almodȯvar *et al*., 2006; Allendorf and Luikart, 2007). Whereas adverse impacts are also concern due to the so-called “out-breeding depression” which refers to breaking of linkage group favoring local adaptation (Allendorf *et al*., 2001; Goldberg *et al*., 2005; Tymchuk *et al*., 2006). The model-based clustering showed remarkable contribution of ON-GIFT to ON-BP (a proportion of membership of 0.268 sharing with ON-GIFT). 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-CD and ON-GIFT) in ON-BP. The LD coefficient suggested that introgression in ON-BP was not recent (Allendorf *et al*., 2001). The impact of introgression on genetic variation within populations will be discussed in the later part of this manuscript.

Introgression from ON-GIFT was also observed in ON-BB, but at a lower magnitude (un-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 from escapees from nearby cage cultures (Srichareondham *et al*., 1993; Makin *et al*., 2004).

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 definitely 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). The impact of interspecific introgression is discussed in the later part of this manuscript.

Genetic differentiation between the feral populations and the GIFT strain

Genetic differentiation was remarkable between the GIFT strain and the feral tilapia populations as shown by significant difference of allele frequency distribution, genetic distance and the phylogenetic dendrogram which placed ON-GIFT far away from the cluster of the feral populations and their ancestor (ON-CD) with a cluster of ON-I and ON-U in between. This was not unexpected, because although ON-CD was among the eight strains used as founders for ON-GIFT (Eknath *et al*., 1993), the later strain has gone through nine generations of selection which could have substantially modified the genetic background of the population (Falconer and Mackay, 1996).

Genetic diversity of feral populations of Nile tilapia in Thailand

Genetic differentiation between populations

Despite their common origin, the three feral populations were genetically divergent. This may be explained by a combination of; introgression from other genepools (e.g. 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 genetic constitution of the founding stock and its effective population size since the founding event and in successive generations. There is a tendency a population under exploitation to reduce its effective population size over time [e.g. New Zealand snapper, *Pagrus auratus* in Tasman Bay during 1950 and 1998 (Hauser *et al*., 2002); plaice, *Pleuronectes platessa* in North Sea and Iceland during 1924-2002 (Hoarau *et al*., 2004)] which resulted in decline of genetic variation. Therefore, it is relatively unexpected that the population from Bang Phra had higher allele diversity than the population of origin (Chitralada) (A = 5.4 - 6.8, and H_e = 0.62 - 0.82; Ambali *et al*., 2000; Romana-Eguia *et al*., 2004; Rutten *et al*., 2004a). From this it may be inferred that introgression from GIFT, as discussed in the above section, has brought in novel alleles to ON-BP.

Generally, genetic introgression can increase genetic variation of the recipient populations (e.g. Sušnik *et al*., 2004). Genetic variation of ON-SY was not increased as compared to its presumed ancestor, ON-CD, despite of introgression from *O. mossambicus*. This implied that the genetic variation of this population might be reduced due to other factors, e.g. habitat destruction, over-exploitation (Boonthongchuay, 2008), which resulted in small effective population size. Despite of high A_r in ON-BP, the low effective number of alleles per locus observed in all of the feral tilapia populations revealed high proportion of alleles with low frequencies. These alleles are easily to be left out in the later generations. Given the problems on global warming which may result in rising of water temperature, intrusion of saline water into rivers, etc. (Schindler, 2001; Ficke *et al*., 2007) ON-BB and ON-SY will face more serious problems than ON-BP.

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 of the inconclusive empirical data on relationship of genetic variation and performance traits (e.g. Shikano and Taniguchi, 2002; Heath *et al*., 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 the annual production of the feral populations was available only for 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 Bueng Boraphet reservoir, a decline in production (46 mt in 1998 reduced to 12 mt 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 the 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.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study provides the genetic evidence as follow:

1. Genetic change has occurred in the GIFT-derived populations and could eventually result in genetically differentiated strains.

2. The GIFT-derived strains retained high genetic variation, probably through using large number of parents and equal contribution of families.

3. Introduction of new genetic materials into a population with low genetic variation (e.g. introduction of ON-GIFT to ON-CD derived populations) increased genetic variation of the recipient while change of performance should be concern.

4. The Chitralada-derived populations tended to increase genetic variation due to introgression from GIFT.

5. Genetic introgression from *O. massambicus* was observed in GIFT broodstock. Nevertheless, the signature of *O. massambicus* disappeared in the GIFT-derived populations while it still existed in the Chitralada-derived populations (ON-AY). In addition, the introgression from *O. aureus* was observed in ON-CP.

6. ON-CD (Chitralada strain) was a major contributor to genepools of the feral populations of *O. niloticus* in Thailand.

7. Genetic differentiation was apparent among the feral tilapia populations in Thailand and between the feral populations and the newly introduced GIFT strain.

8. Intraspecific genetic introgression (from ON-GIFT to ON-BP and ON-BB) was observed, while the interspecific (from *O. mossambicus* to *O. niloticus*) introgression may have occurred in ON-SY.

Recommendations

1. Although the results revealed that the broodstock were maintained with large effective population size. It is of concern that the good management may be performed according to biology of Nile tilapia (e.g. low fecundity, natural mating favors sex ratio of 1 male: 2-3 females) rather than the active intention to preserve the genetic variation. Therefore, better understanding on broodstock management should be enhanced, especially among private hatchery managers.

2. Due to the information that artificial selection was performed in every hatchery included in this study, genetic monitoring is required to ensure sufficient genetic variation which reflects potential response to selection. In addition, frequent genetic monitoring should be performed in parallel with evaluation of performances. This practices would allow for detection of interspecific introgressed allele(s) at an early stage while information on performance would allow for controlling the alteration towards desirable traits.

3. If the genetic alteration in the GIFT-derived populations is continued with good broodstock management this may end up with genetically diverse strains those can be used for further genetic improvement programs. It is essential that performance of these strains should be regularly evaluated so that they can be efficiently exploited in genetic improvement programs.

4. The Chitralada strain which is relatively pure *O. niloticus* should be maintained in the way that its purity is retained. It is an invaluable genetic resource for further genetic improvement program. Alternatively it may be used to rescue genetic deterioration of hatchery stocks.

5. The interspecific introgression in the hatchery stocks should be kept to minimum. As such, the ON-AY and ON-CP should not be incorporated into any broodstock due to the introgression from *O. mossambicus* and *O. aureus* respectively.

6. High genetic variation of the hatchery populations suggested that they have high potential to evolve under a changing environment. These populations, hence become valuable for the genetic improvement program attempting for the adaptive measure for the global climate change.

7. The interspecific introgression observed in ON-SY should be kept to minimal and this population should not be used for seed propagation and a genetic improvement program. Moreover, translocation of Nile tilapia from Sam Roi Yot reservoir to other places should be avoided to prevent potential genetic contamination.

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APPENDIX

Appendix Table 1 Allele frequencies at fourteen microsatellite loci of six Nile tilapia hatchery populations in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*.

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
UNH172	176	0.160	0.060	0.230	0.080	0.050	0.000	0.000	0.000	0.000	0.125	0.000	0.000	
	178	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	ON-AIT
	180	0.000	0.040	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	
	184	0.030	0.170	0.090	0.250	0.000	0.000	0.000	0.350	0.000	0.036	0.000	0.188	
	186	0.160	0.190	0.070	0.010	0.000	0.000	0.013	0.200	0.000	0.089	0.000	0.025	
	188	0.140	0.090	0.080	0.150	0.040	0.190	0.125	0.050	0.000	0.179	0.000	0.000	
	190	0.030	0.010	0.090	0.020	0.260	0.120	0.075	0.100	0.000	0.179	0.000	0.000	
	192	0.000	0.000	0.000	0.000	0.060	0.160	0.000	0.000	0.000	0.000	0.000	0.000	
	194	0.050	0.110	0.020	0.120	0.070	0.110	0.175	0.050	0.075	0.143	0.000	0.000	
	196	0.080	0.000	0.050	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	198	0.050	0.080	0.130	0.000	0.010	0.040	0.125	0.000	0.000	0.000	0.000	0.050	
	200	0.000	0.000	0.000	0.000	0.000	0.000	0.181	0.075	0.225	0.000	0.000	0.025	
	202	0.020	0.140	0.070	0.060	0.010	0.000	0.000	0.000	0.125	0.071	0.000	0.000	
	204	0.000	0.000	0.000	0.140	0.000	0.030	0.038	0.025	0.325	0.000	0.000	0.013	
	206	0.110	0.080	0.060	0.140	0.000	0.000	0.006	0.125	0.250	0.071	0.000	0.050	
	208	0.170	0.030	0.110	0.010	0.370	0.250	0.263	0.000	0.000	0.107	0.000	0.000	
	210	0.000	0.000	0.000	0.000	0.050	0.040	0.000	0.000	0.000	0.000	0.000	0.000	
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.025	
	214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	216	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-AY
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	OA-E

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	222	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	OA-E
	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	OA-E
	230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.188	OA-E
	238	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-CP
	246	0.000	0.000	0.000	0.000	0.030	0.020	0.000	0.000	0.000	0.000	1.000	0.000	
UNH211	106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	112	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.025	0.000	0.000	0.000	0.000	
	114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.013	
	118	0.000	0.000	0.000	0.090	0.090	0.190	0.456	0.075	0.075	0.000	0.000	0.000	
	120	0.190	0.170	0.540	0.240	0.240	0.170	0.144	0.000	0.050	0.036	0.000	0.000	
	122	0.190	0.370	0.070	0.080	0.020	0.100	0.025	0.025	0.025	0.089	0.000	0.000	
	124	0.000	0.070	0.000	0.030	0.060	0.000	0.000	0.000	0.000	0.196	0.000	0.000	
	126	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.071	0.000	0.000	
	128	0.160	0.040	0.030	0.020	0.030	0.020	0.000	0.100	0.000	0.018	0.000	0.013	
	130	0.050	0.050	0.010	0.110	0.060	0.030	0.019	0.050	0.000	0.125	0.000	0.025	
	132	0.070	0.040	0.030	0.070	0.070	0.110	0.088	0.200	0.075	0.071	0.000	0.000	
	134	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.075	0.075	0.000	0.000	0.000	
	136	0.000	0.000	0.000	0.030	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	140	0.000	0.000	0.000	0.020	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	142	0.010	0.010	0.020	0.000	0.000	0.020	0.000	0.000	0.000	0.054	0.938	0.000	
	144	0.140	0.070	0.070	0.100	0.040	0.000	0.006	0.000	0.000	0.036	0.000	0.138	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	146	0.070	0.100	0.120	0.030	0.060	0.010	0.013	0.075	0.025	0.179	0.013	0.000	
	150	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.125	0.000	0.000	0.000	0.113	
	152	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	
	154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.175	0.000	0.000	0.000	
	156	0.000	0.000	0.000	0.060	0.070	0.110	0.069	0.025	0.075	0.000	0.050	0.000	
	162	0.040	0.020	0.000	0.010	0.070	0.080	0.119	0.000	0.425	0.018	0.000	0.000	
	168	0.000	0.000	0.000	0.030	0.070	0.130	0.063	0.000	0.000	0.000	0.000	0.013	
	170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.288	OA-E
	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	OA-E
	184	0.080	0.040	0.110	0.010	0.070	0.020	0.000	0.000	0.000	0.107	0.000	0.000	
	190	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	OA-E
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088	OA-E
UNH216	120	0.010	0.010	0.000	0.020	0.000	0.000	0.025	0.000	0.000	0.054	0.000	0.775	
	122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	124	0.560	0.420	0.570	0.420	0.210	0.040	0.163	0.050	0.050	0.518	0.000	0.138	
	126	0.310	0.410	0.280	0.430	0.490	0.750	0.706	0.700	0.950	0.214	0.000	0.038	
	128	0.040	0.060	0.010	0.090	0.070	0.050	0.088	0.250	0.000	0.089	0.000	0.025	
	138	0.070	0.050	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.288	0.000	
	144	0.000	0.000	0.000	0.010	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	156	0.000	0.000	0.000	0.030	0.090	0.090	0.019	0.000	0.000	0.000	0.000	0.000	
	158	0.010	0.050	0.080	0.000	0.100	0.070	0.000	0.000	0.000	0.107	0.000	0.000	
	172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.313	0.000	OM-S
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.375	0.000	OM-S
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S
UNH222	160	0.000	0.000	0.000	0.010	0.010	0.030	0.000	0.000	0.000	0.000	0.000	0.000	
	162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.525	0.563	
	164	0.000	0.000	0.000	0.000	0.000	0.000	0.138	0.000	0.000	0.054	0.000	0.413	
	166	0.020	0.010	0.170	0.060	0.030	0.160	0.000	0.000	0.000	0.000	0.063	0.000	
	168	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	ON-CD
	174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.413	0.000	OM-S
	178	0.030	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	180	0.030	0.030	0.100	0.060	0.030	0.070	0.000	0.000	0.000	0.000	0.000	0.000	
	182	0.140	0.040	0.110	0.040	0.060	0.020	0.000	0.075	0.000	0.089	0.000	0.000	
	184	0.000	0.000	0.000	0.000	0.000	0.010	0.081	0.525	1.000	0.000	0.000	0.000	
	186	0.610	0.670	0.510	0.540	0.700	0.000	0.094	0.100	0.000	0.625	0.000	0.000	
	188	0.120	0.080	0.030	0.250	0.160	0.530	0.394	0.150	0.000	0.143	0.000	0.025	
	190	0.000	0.000	0.000	0.000	0.000	0.160	0.288	0.075	0.000	0.000	0.000	0.000	
	192	0.010	0.040	0.040	0.000	0.000	0.010	0.000	0.025	0.000	0.000	0.000	0.000	
	196	0.040	0.110	0.040	0.040	0.010	0.010	0.000	0.050	0.000	0.089	0.000	0.000	
UNH212	178	0.020	0.000	0.000	0.040	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.900	
	180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	182	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	
	186	0.260	0.290	0.330	0.180	0.140	0.040	0.175	0.000	0.925	0.214	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	188	0.080	0.030	0.010	0.180	0.250	0.320	0.363	0.000	0.000	0.089	0.000	0.000	
	190	0.130	0.140	0.180	0.220	0.310	0.200	0.238	0.525	0.000	0.071	0.000	0.000	
	192	0.350	0.340	0.350	0.270	0.160	0.050	0.056	0.100	0.075	0.464	0.000	0.013	
	194	0.050	0.060	0.030	0.010	0.010	0.000	0.000	0.025	0.000	0.018	0.000	0.000	
	200	0.060	0.130	0.100	0.080	0.030	0.150	0.013	0.000	0.000	0.143	0.000	0.000	
	202	0.040	0.000	0.000	0.020	0.080	0.110	0.156	0.325	0.000	0.000	0.488	0.000	
	204	0.010	0.000	0.000	0.000	0.000	0.050	0.000	0.025	0.000	0.000	0.000	0.000	
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	OM-S
	216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S
	218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000	OM-S
	226	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.000	ON-AIT
	228	0.000	0.000	0.000	0.000	0.010	0.020	0.000	0.000	0.000	0.000	0.363	0.000	
UNH160	132	0.090	0.130	0.090	0.020	0.030	0.030	0.000	0.000	0.000	0.089	0.000	0.000	
	168	0.080	0.060	0.000	0.040	0.040	0.030	0.000	0.000	0.000	0.000	0.000	0.650	
	172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	ON-GIFT
	180	0.160	0.130	0.080	0.280	0.080	0.060	0.094	0.000	0.000	0.232	0.000	0.000	
	182	0.010	0.010	0.020	0.100	0.030	0.040	0.188	0.150	0.000	0.089	0.000	0.350	
	184	0.250	0.300	0.270	0.140	0.160	0.060	0.025	0.050	0.000	0.357	0.550	0.000	
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.425	0.000	0.000	0.350	0.000	
	190	0.020	0.000	0.180	0.020	0.020	0.000	0.188	0.050	0.050	0.018	0.000	0.000	
	192	0.000	0.000	0.000	0.030	0.020	0.000	0.006	0.025	0.000	0.000	0.000	0.000	
	194	0.010	0.010	0.060	0.010	0.090	0.070	0.006	0.000	0.000	0.000	0.100	0.000	
	196	0.060	0.060	0.090	0.050	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	200	0.130	0.120	0.020	0.020	0.030	0.000	0.000	0.100	0.000	0.071	0.000	0.000	
	202	0.070	0.010	0.010	0.080	0.210	0.420	0.263	0.000	0.950	0.089	0.000	0.000	
	204	0.060	0.020	0.000	0.010	0.000	0.000	0.000	0.025	0.000	0.018	0.000	0.000	
	206	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.025	0.000	0.000	0.000	0.000	
	208	0.010	0.000	0.000	0.120	0.190	0.270	0.231	0.050	0.000	0.000	0.000	0.000	
	210	0.050	0.150	0.180	0.080	0.040	0.010	0.000	0.050	0.000	0.018	0.000	0.000	
UNH213	182	0.010	0.040	0.000	0.050	0.160	0.130	0.006	0.000	0.000	0.000	0.000	0.900	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S
	190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.625	0.013	
	192	0.000	0.000	0.000	0.030	0.170	0.120	0.075	0.000	0.375	0.000	0.325	0.000	
	194	0.000	0.000	0.000	0.000	0.000	0.120	0.000	0.000	0.000	0.000	0.000	0.000	ON-AIT
	196	0.100	0.120	0.140	0.060	0.050	0.080	0.000	0.125	0.000	0.107	0.000	0.000	
	198	0.020	0.040	0.000	0.030	0.020	0.000	0.000	0.025	0.000	0.071	0.013	0.050	
	200	0.090	0.030	0.000	0.050	0.050	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
	204	0.390	0.470	0.640	0.300	0.120	0.050	0.019	0.350	0.575	0.446	0.000	0.000	
	206	0.170	0.160	0.110	0.100	0.070	0.050	0.375	0.000	0.000	0.161	0.000	0.000	
	208	0.080	0.070	0.100	0.330	0.310	0.450	0.519	0.225	0.000	0.071	0.013	0.000	
	210	0.000	0.000	0.000	0.010	0.010	0.000	0.006	0.150	0.025	0.000	0.000	0.000	
	212	0.030	0.060	0.010	0.020	0.030	0.000	0.000	0.075	0.000	0.107	0.000	0.000	
	214	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.050	0.000	0.000	0.000	0.000	
	218	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
	238	0.070	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	**ON-UT**	**ON-PB**	**ON-CP**	**ON-AY**	**ON-AIT**	**ON-CD**	**ON-U**	**ON-I**	**ON-GIFT**	**OM-S**	**OA-E**	
UNH153	197	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.050	0.000	0.850	0.000	
	205	0.000	0.000	0.000	0.050	0.090	0.170	0.006	0.000	0.000	0.000	0.000	0.000	
	209	0.000	0.000	0.000	0.050	0.010	0.040	0.000	0.000	0.000	0.000	0.125	0.000	
	211	0.700	0.620	0.430	0.520	0.660	0.640	0.825	0.800	0.950	0.750	0.025	0.025	
	213	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	ON-U
	215	0.050	0.040	0.150	0.180	0.080	0.060	0.169	0.000	0.000	0.018	0.000	0.450	
	217	0.020	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	
	231	0.160	0.320	0.340	0.170	0.160	0.080	0.000	0.000	0.000	0.179	0.000	0.525	
	233	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	ON-GIFT
	243	0.070	0.020	0.080	0.010	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
UNH132	125	0.000	0.000	0.000	0.160	0.230	0.300	0.488	0.025	0.025	0.000	0.000	0.000	
	127	0.000	0.000	0.020	0.030	0.150	0.100	0.119	0.325	0.400	0.000	0.000	0.375	
	131	0.440	0.420	0.530	0.280	0.200	0.000	0.188	0.475	0.125	0.464	0.000	0.000	
	133	0.330	0.270	0.230	0.190	0.040	0.160	0.006	0.025	0.000	0.179	0.825	0.000	
	135	0.000	0.000	0.000	0.040	0.080	0.070	0.000	0.025	0.425	0.018	0.025	0.000	
	137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	139	0.230	0.210	0.220	0.300	0.280	0.300	0.194	0.075	0.025	0.250	0.000	0.000	
	141	0.000	0.100	0.000	0.000	0.020	0.070	0.000	0.025	0.000	0.089	0.150	0.000	
	143	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.600	
	149	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
UNH138	164	0.000	0.000	0.000	0.060	0.070	0.070	0.013	0.000	0.000	0.143	1.000	0.000	
	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.200	0.000	0.000	0.000	
	180	0.060	0.170	0.070	0.080	0.040	0.010	0.000	0.075	0.000	0.196	0.000	0.000	
	182	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-UT

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	186	0.060	0.150	0.100	0.170	0.470	0.320	0.438	0.300	0.000	0.054	0.000	0.050	
	188	0.350	0.210	0.200	0.100	0.110	0.070	0.100	0.025	0.800	0.304	0.000	0.263	
	190	0.000	0.010	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	192	0.220	0.080	0.030	0.290	0.160	0.330	0.288	0.050	0.000	0.179	0.000	0.438	
	194	0.020	0.000	0.160	0.060	0.010	0.020	0.163	0.000	0.000	0.000	0.000	0.113	
	196	0.020	0.010	0.020	0.000	0.010	0.010	0.000	0.175	0.000	0.036	0.000	0.000	
	200	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	
	202	0.010	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	204	0.150	0.090	0.140	0.070	0.010	0.000	0.000	0.025	0.000	0.018	0.000	0.113	
	208	0.000	0.070	0.040	0.020	0.010	0.160	0.000	0.075	0.000	0.000	0.000	0.013	
	210	0.000	0.010	0.000	0.000	0.010	0.000	0.000	0.050	0.000	0.018	0.000	0.000	
	218	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	ON-AIT
	228	0.110	0.160	0.210	0.130	0.100	0.000	0.000	0.000	0.000	0.054	0.000	0.000	
UNH192	144	0.010	0.010	0.000	0.030	0.030	0.000	0.000	0.000	0.000	0.018	0.050	0.000	
	146	0.260	0.160	0.230	0.120	0.100	0.020	0.006	0.175	0.000	0.250	0.000	0.038	
	148	0.150	0.160	0.160	0.030	0.000	0.010	0.000	0.000	0.000	0.034	0.363	0.900	
	150	0.210	0.340	0.240	0.440	0.370	0.560	0.531	0.250	0.050	0.304	0.000	0.038	
	152	0.310	0.290	0.340	0.330	0.350	0.250	0.444	0.575	0.875	0.357	0.000	0.025	
	154	0.050	0.030	0.010	0.040	0.010	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
	156	0.000	0.000	0.000	0.000	0.000	0.010	0.019	0.000	0.000	0.000	0.000	0.000	
	164	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.018	0.000	0.000	
	168	0.000	0.000	0.020	0.010	0.140	0.150	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	OM-S
	190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.388	0.000	OM-S
UNH1004	182	0.000	0.000	0.000	0.010	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.763	
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	OA-E
	194	0.150	0.250	0.080	0.130	0.090	0.050	0.000	0.150	0.000	0.357	0.000	0.000	
	196	0.040	0.000	0.050	0.010	0.000	0.000	0.000	0.050	0.000	0.018	0.000	0.125	
	198	0.060	0.190	0.110	0.170	0.370	0.330	0.288	0.275	0.000	0.089	0.000	0.000	
	200	0.040	0.060	0.050	0.050	0.060	0.030	0.000	0.000	0.000	0.125	0.000	0.000	
	202	0.080	0.060	0.340	0.270	0.240	0.260	0.300	0.025	0.000	0.089	0.000	0.000	
	206	0.000	0.010	0.140	0.000	0.020	0.020	0.000	0.075	0.000	0.000	0.000	0.000	
	208	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-AY
	214	0.040	0.030	0.000	0.010	0.010	0.000	0.000	0.300	0.000	0.000	0.000	0.000	
	218	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	
	220	0.080	0.020	0.000	0.020	0.010	0.060	0.000	0.000	0.000	0.143	0.000	0.000	
	222	0.130	0.090	0.060	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.013	
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	228	0.160	0.060	0.070	0.000	0.020	0.010	0.000	0.000	0.000	0.036	0.000	0.000	
	230	0.040	0.090	0.000	0.070	0.000	0.000	0.056	0.000	0.525	0.036	0.000	0.000	
	232	0.020	0.010	0.020	0.100	0.040	0.080	0.275	0.000	0.075	0.071	0.000	0.000	
	234	0.080	0.060	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	
	238	0.000	0.010	0.030	0.010	0.010	0.040	0.006	0.000	0.000	0.000	0.000	0.000	
	246	0.010	0.000	0.000	0.090	0.080	0.120	0.000	0.000	0.000	0.000	0.000	0.000	
	248	0.070	0.060	0.050	0.020	0.020	0.000	0.000	0.000	0.400	0.000	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.713	0.000	OM-S
	256	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.288	0.000	
UNH982	120	0.020	0.100	0.080	0.050	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	ON-U
	124	0.030	0.000	0.000	0.010	0.010	0.010	0.000	0.025	0.000	0.000	0.400	0.000	
	126	0.290	0.300	0.400	0.220	0.350	0.480	0.056	0.400	0.000	0.304	0.000	0.000	
	128	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.275	0.000	0.000	0.000	0.000	ON-U
	132	0.050	0.060	0.060	0.080	0.050	0.010	0.000	0.075	0.000	0.018	0.000	0.000	
	134	0.050	0.100	0.190	0.190	0.270	0.360	0.663	0.000	0.000	0.125	0.000	0.000	
	136	0.120	0.120	0.180	0.160	0.030	0.000	0.000	0.050	0.000	0.214	0.500	0.388	
	138	0.030	0.010	0.000	0.060	0.010	0.010	0.000	0.025	0.000	0.000	0.000	0.000	
	140	0.030	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	
	142	0.000	0.000	0.010	0.010	0.000	0.010	0.006	0.025	0.000	0.000	0.000	0.088	
	144	0.100	0.060	0.020	0.060	0.050	0.000	0.000	0.000	0.000	0.143	0.100	0.175	
	146	0.000	0.000	0.000	0.020	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.025	
	148	0.040	0.010	0.020	0.010	0.020	0.010	0.000	0.000	0.000	0.018	0.000	0.075	
	150	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.050	
	152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.025	
	156	0.170	0.120	0.040	0.010	0.030	0.050	0.000	0.000	0.075	0.071	0.000	0.125	
	160	0.070	0.100	0.000	0.030	0.050	0.020	0.219	0.025	0.775	0.071	0.000	0.000	
	168	0.000	0.010	0.000	0.090	0.040	0.010	0.056	0.025	0.000	0.036	0.000	0.000	

Appendix Table 1 (Continued)

Locus	Allele Size	Population												Private Allele
		ON-PT	ON-UT	ON-PB	ON-CP	ON-AY	ON-AIT	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
UNH173	123	0.010	0.010	0.020	0.170	0.230	0.310	0.463	0.150	0.000	0.125	0.000	0.000	
	127	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	ON-U
	133	0.000	0.010	0.030	0.040	0.180	0.140	0.019	0.700	0.000	0.000	0.300	0.000	
	135	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.100	0.000	
	175	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	
	187	0.420	0.380	0.470	0.360	0.210	0.130	0.031	0.000	0.900	0.375	0.600	0.938	
	189	0.030	0.000	0.080	0.120	0.120	0.100	0.200	0.000	0.000	0.000	0.000	0.000	
	193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	195	0.020	0.030	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	
	201	0.180	0.200	0.050	0.100	0.020	0.040	0.000	0.000	0.000	0.036	0.000	0.000	
	205	0.270	0.280	0.330	0.180	0.220	0.270	0.288	0.000	0.075	0.304	0.000	0.050	
	207	0.070	0.090	0.010	0.010	0.020	0.010	0.000	0.000	0.000	0.036	0.000	0.000	

Appendix Table 2 Results of linkage disequilibrium tests based on the Chi square test of six Nile tilapia hatchery populations in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. R = correlation coefficient, *D* = disequilibrium coefficient

Population	Loci pairs	R	R Probability	*D*
ON-PT	*UNH216 - UNH212*	0.2232	0.0004	0.0100
	UNH222 - UNH982	0.1242	0.0001	0.0246
	UNH212 - UNH1004	0.1335	0.0001	0.0157
	UNH160 - UNH153	0.1467	0.0007	0.0098
	UNH160 - UNH173	0.1820	0.0001	0.0182
	UNH153 - UNH138	0.1554	0.0001	0.0252
	UNH138 - UNH173	0.1414	0.0004	0.0227
	UNH1004 - UNH982	0.1422	0.0001	0.0327
	UNH1004 - UNH173	0.1305	0.0003	0.0155
Mean±SE				0.0194±0.0025
ON-UT	*UNH222 - UNH212*	0.1307	0.0001	0.0145
	UNH160 - UNH173	0.1942	0.0001	0.0098
	UNH213 - UNH192	0.2033	0.0003	0.0100
	UNH982 - UNH173	0.1853	0.0001	0.0143
Mean±SE				0.0122±0.0013
ON-PB	*UNH172 - UNH192*	0.1256	0.0008	0.0192
	UNH160 - UNH153	0.1578	0.0013	0.0188
Mean±SE				0.0190±0.0002
ON-CP	*UNH172 - UNH212*	0.1253	0.0001	0.0261
	UNH172 - UNH213	0.1652	0.0001	0.0099
	UNH172 - UNH982	0.1242	0.0001	0.0098
	UNH172 - UNH173	0.1817	0.0001	0.0098
	UNH211 - UNH982	0.1153	0.0001	0.0268
	UNH212 - UNH160	0.1459	0.0001	0.0227
	UNH212 - UNH192	0.2185	0.0001	0.0100
	UNH212 - UNH982	0.1336	0.0001	0.0348

Appendix Table 2 (Continued)

Population	**Loci pairs**	**R**	**R Probability**	***D***
	UNH160 - UNH982	0.1247	0.0001	0.0220
	UNH213 - UNH982	0.1416	0.0001	0.0213
	UNH213 - UNH173	0.1759	0.0001	0.0098
	UNH153 - UNH1004	0.1946	0.0001	0.0099
	UNH982 - UNH173	0.1244	0.0001	0.0098
Mean±SE				0.0171±0.0024
ON-AY	*UNH172 - UNH211*	0.1497	0.0001	0.0129
	UNH172 - UNH212	0.1727	0.0001	0.0098
	UNH172 - UNH192	0.1373	0.0002	0.0125
	UNH211 - UNH216	0.1426	0.0001	0.0196
	UNH211 - UNH212	0.1692	0.0001	0.0238
	UNH211 - UNH160	0.1358	0.0001	0.0098
	UNH211 - UNH213	0.1614	0.0001	0.0210
	UNH211 - UNH138	0.1457	0.0002	0.0098
	UNH211 - UNH1004	0.1386	0.0001	0.0118
	UNH216 - UNH212	0.1556	0.0001	0.0282
	UNH216 - UNH160	0.1489	0.0001	0.0516
	UNH216 - UNH982	0.1327	0.0001	0.0265
	UNH212 - UNH160	0.1446	0.0001	0.0401
	UNH212 - UNH192	0.1577	0.0001	0.0459
	UNH212 - UNH982	0.1245	0.0001	0.0096
	UNH160 - UNH1004	0.1223	0.0001	0.0196
	UNH160 - UNH982	0.1200	0.0001	0.0187
	UNH213 - UNH132	0.1576	0.0001	0.0192
	UNH213 - UNH138	0.1979	0.0001	0.0098
	UNH213 - UNH1004	0.1474	0.0001	0.0230
	UNH138 - UNH192	0.1998	0.0001	0.0142
	UNH138 - UNH1004	0.2062	0.0001	0.0114

Appendix Table 2 (Continued)

Population	**Loci pairs**	**R**	**R Probability**	***D***
	UNH138 - *UNH173*	0.1519	0.0001	0.0247
	UNH192 - *UNH173*	0.1615	0.0001	0.0098
	UNH982 - *UNH173*	0.1359	0.0001	0.0098
Mean±SE				0.0197±0.0023
ON-AIT	*UNH172* - *UNH160*	0.1211	0.0001	0.0216
	UNH172 - *UNH982*	0.1250	0.0001	0.0120
	UNH211 - *UNH216*	0.1283	0.0001	0.0192
	UNH211 - *UNH222*	0.1619	0.0001	0.0140
	UNH211 - *UNH160*	0.1690	0.0001	0.0126
	UNH211 - *UNH982*	0.1388	0.0001	0.0120
	UNH216 - *UNH222*	0.1721	0.0001	0.0196
	UNH216 - *UNH160*	0.1353	0.0001	0.0196
	UNH216 - *UNH982*	0.1541	0.0001	0.0192
	UNH222 - *UNH160*	0.1791	0.0001	0.0098
	UNH222 - *UNH982*	0.2073	0.0001	0.0099
	UNH160 - *UNH982*	0.1904	0.0001	0.0099
	UNH153 - *UNH982*	0.1910	0.0001	0.0097
	UNH138 - *UNH982*	0.2002	0.0001	0.0099
Mean±SE				0.0142±0.0012
ON-CD	*UNH216* - *UNH222*	0.1180	0.0001	0.0180
	UNH216 - *UNH213*	0.1209	0.0004	0.0108
	UNH213 - *UNH132*	0.2385	0.0001	0.0063
	UNH982 - *UNH173*	0.1256	0.0005	0.0061
Mean±SE				0.0103±0.0028
ON-U	*UNH216* - *UNH153*	0.5589	0.0002	0.0000
	UNH160 - *UNH982*	0.3688	0.0001	0.0250
Mean±SE				0.0125±0.0125

Appendix Table 2 (Continued)

Population	Loci pairs	R	R Probability	*D*
ON-I	*UNH211 - UNH213*	0.5094	0.0001	-0.0031
	UNH211 - UNH153	0.6848	0.0001	0.0000
	UNH211 - UNH132	0.4432	0.0001	0.0250
	UNH211 - UNH192	0.2352	0.0001	0.0974
	UNH216 - UNH212	0.8353	0.0005	0.0000
	UNH160 - UNH982	0.5485	0.0020	0.0000
	UNH160 - UNH173	0.6162	0.0023	0.0000
	UNH213 - UNH153	0.9974	0.0001	0.0000
	UNH213 - UNH132	0.9216	0.0001	0.0358
	UNH153 - UNH132	0.9901	0.0001	0.0000
	UNH138 - UNH192	0.5915	0.0001	0.0000
	UNH138 - UNH173	0.4436	0.0062	0.0000
Mean±SE				0.0129±0.0084
ON-GIFT	*UNH172 - UNH211*	0.1790	0.0001	0.0344
	UNH211 - UNH138	0.3209	0.0001	0.0428
	UNH153 - UNH192	0.3614	0.0002	0.0599
Mean±SE				0.0457±0.0075
OM-S	*UNH211 - UNH222*	0.3552	0.0001	-0.0056
	UNH211 - UNH212	0.2465	0.0011	-0.0076
Mean±SE				-0.0066±0.0010
OA-E	*UNH172 - UNH222*	0.2583	0.0001	0.0244
	UNH172 - UNH153	0.2561	0.0001	0.0244
	UNH172 - UNH192	0.2363	0.0001	0.0181
	UNH216 - UNH212	0.2144	0.0001	0.0006
	UNH222 - UNH212	0.2201	0.0002	0.0122
	UNH222 - UNH153	0.6406	0.0001	0.0244
	UNH222 - UNH138	0.1715	0.0003	0.0122
	UNH222 - UNH192	0.5917	0.0001	0.0244

Appendix Table 2 (Continued)

Population	**Loci pairs**	**R**	**R Probability**	***D***
	UNH222 - *UNH1004*	0.2705	0.0020	-0.0032
	UNH222 - *UNH173*	0.2753	0.0003	0.0003
	UNH212 - *UNH153*	0.2596	0.0001	0.0122
	UNH212 - *UNH138*	0.3690	0.0001	-0.0053
	UNH212 - *UNH192*	0.2218	0.0006	0.0122
	UNH153 - *UNH138*	0.1903	0.0002	0.0122
	UNH153 - *UNH192*	0.5746	0.0001	0.0244
	UNH153 - *UNH173*	0.2611	0.0005	0.0003
	UNH138 - *UNH192*	0.1932	0.0008	0.0122
Mean±SE				0.0121±0.0025

Appendix Table 3 Genic differentiation for each population pair across all loci of six Nile tilapia hatchery populations in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. (Fisher's method; $P < 0.0008$ after Bonferroni correction for multiple tests).

Population pair	χ^2	**df**	**P-value**
ON-PT & ON-UT	Infinity	28	0.0000
ON-PT & ON-PC	Infinity	28	0.0000
ON-PT & ON-CP	Infinity	28	0.0000
ON-PT & ON-AY	Infinity	28	0.0000
ON-PT & ON-AIT	Infinity	28	0.0000
ON-PT & ON-CD	Infinity	28	0.0000
ON-PT & ON-U	Infinity	28	0.0000
ON-PT & ON-I	Infinity	28	0.0000
ON-PT & ON-GIFT	Infinity	28	0.0000
ON-PT & OM-S	Infinity	28	0.0000
ON-PT & OA-E	Infinity	28	0.0000
ON-UT & ON-PC	Infinity	28	0.0000
ON-UT & ON-CP	Infinity	28	0.0000
ON-UT & ON-AY	Infinity	28	0.0000
ON-UT & ON-AIT	Infinity	28	0.0000
ON-UT & ON-CD	Infinity	28	0.0000
ON-UT & ON-U	Infinity	28	0.0000
ON-UT & ON-I	Infinity	28	0.0000
ON-UT & ON-GIFT	140.50	28	0.0000
ON-UT & OM-S	Infinity	28	0.0000
ON-UT & OA-E	Infinity	28	0.0000
ON-PC & ON-CP	Infinity	28	0.0000
ON-PC & ON-AY	Infinity	28	0.0000
ON-PC & ON-AIT	Infinity	28	0.0000

Appendix Table 3 (Continued)

Population pair	χ^2	df	P-value
ON-PC & ON-CD	Infinity	28	0.0000
ON-PC & ON-U	Infinity	28	0.0000
ON-PC & ON-I	Infinity	28	0.0000
ON-PC & ON-GIFT	Infinity	28	0.0000
ON-PC & OM-S	Infinity	28	0.0000
ON-PC & OA-E	Infinity	28	0.0000
ON-CP & ON-AY	Infinity	28	0.0000
ON-CP & ON-AIT	Infinity	28	0.0000
ON-CP & ON-CD	Infinity	28	0.0000
ON-CP & ON-U	Infinity	28	0.0000
ON-CP & ON-I	Infinity	28	0.0000
ON-CP & ON-GIFT	Infinity	28	0.0000
ON-CP & OM-S	Infinity	28	0.0000
ON-CP & OA-E	Infinity	28	0.0000
ON-AY & ON-AIT	Infinity	28	0.0000
ON-AY & ON-CD	Infinity	28	0.0000
ON-AY & ON-U	Infinity	28	0.0000
ON-AY & ON-I	Infinity	28	0.0000
ON-AY & ON-GIFT	Infinity	28	0.0000
ON-AY & OM-S	Infinity	28	0.0000
ON-AY & OA-E	Infinity	28	0.0000
ON-AIT & ON-CD	Infinity	28	0.0000
ON-AIT & ON-U	Infinity	28	0.0000
ON-AIT & ON-I	Infinity	28	0.0000
ON-AIT & ON-GIFT	Infinity	28	0.0000
ON-AIT & OM-S	Infinity	28	0.0000
ON-AIT & OA-E	Infinity	28	0.0000
ON-CD & ON-U	Infinity	28	0.0000

Appendix Table 3 (Continued)

Population pair	χ^2	**df**	**P-value**
ON-CD & ON-I	Infinity	28	0.0000
ON-CD & ON-GIFT	Infinity	28	0.0000
ON-CD & OM-S	Infinity	28	0.0000
ON-CD & OA-E	Infinity	28	0.0000
ON-U & ON-I	Infinity	28	0.0000
ON-U & ON-GIFT	Infinity	28	0.0000
ON-U & OM-S	Infinity	28	0.0000
ON-U & OA-E	Infinity	28	0.0000
ON-I & ON-GIFT	Infinity	28	0.0000
ON-I & OM-S	Infinity	28	0.0000
ON-I & OA-E	Infinity	28	0.0000
ON-GIFT & OM-S	Infinity	28	0.0000
ON-GIFT & OA-E	Infinity	28	0.0000
OM-S & OA-E	Infinity	28	0.0000

Appendix Table 4 Allele frequencies at fourteen microsatellite loci of three feral populations of *O. niloticus* in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*.

Locus	Allele Size	Population									Private allele
		ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
UNH172	176	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	ON-GIFT
	184	0.054	0.021	0.000	0.000	0.350	0.000	0.036	0.000	0.188	
	186	0.027	0.146	0.034	0.013	0.200	0.000	0.089	0.000	0.025	
	188	0.203	0.063	0.057	0.125	0.050	0.000	0.179	0.000	0.000	
	190	0.122	0.292	0.102	0.075	0.100	0.000	0.179	0.000	0.000	
	194	0.243	0.063	0.102	0.175	0.050	0.075	0.143	0.000	0.000	
	198	0.054	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.050	
	200	0.000	0.063	0.080	0.181	0.075	0.225	0.000	0.000	0.025	
	202	0.000	0.000	0.000	0.000	0.000	0.125	0.071	0.000	0.000	
	204	0.054	0.000	0.011	0.038	0.025	0.325	0.000	0.000	0.013	
	206	0.000	0.000	0.011	0.006	0.125	0.250	0.071	0.000	0.050	
	208	0.176	0.354	0.125	0.263	0.000	0.000	0.107	0.000	0.000	
	210	0.041	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	212	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.025	
	214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	OA-E
	222	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	OA-E
	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	OA-E
	230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E

Appendix Table 4 (Continued)

Locus	Allele Size	Population									Private allele
		ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.188	OA-E
	246	0.027	0.000	0.477	0.000	0.000	0.000	0.000	1.000	0.000	
UNH211	106	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	112	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	114	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.013	
	118	0.216	0.583	0.136	0.456	0.075	0.075	0.000	0.000	0.000	
	120	0.189	0.104	0.023	0.144	0.000	0.050	0.036	0.000	0.000	
	122	0.108	0.021	0.000	0.025	0.025	0.025	0.089	0.000	0.000	
	124	0.014	0.000	0.000	0.000	0.000	0.000	0.196	0.000	0.000	
	126	0.000	0.000	0.000	0.000	0.025	0.000	0.071	0.000	0.000	
	128	0.000	0.000	0.000	0.000	0.100	0.000	0.018	0.000	0.013	
	130	0.000	0.000	0.000	0.019	0.050	0.000	0.125	0.000	0.025	
	132	0.054	0.042	0.068	0.088	0.200	0.075	0.071	0.000	0.000	
	134	0.000	0.021	0.034	0.000	0.075	0.075	0.000	0.000	0.000	
	142	0.000	0.021	0.000	0.000	0.000	0.000	0.054	0.938	0.000	
	144	0.014	0.000	0.000	0.006	0.000	0.000	0.036	0.000	0.138	
	146	0.068	0.083	0.171	0.013	0.075	0.025	0.179	0.013	0.000	
	150	0.000	0.021	0.068	0.000	0.125	0.000	0.000	0.000	0.113	
	152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	154	0.014	0.000	0.000	0.000	0.100	0.175	0.000	0.000	0.000	
	156	0.068	0.021	0.057	0.069	0.025	0.075	0.000	0.050	0.000	
	162	0.162	0.021	0.398	0.119	0.000	0.425	0.018	0.000	0.000	
	168	0.081	0.063	0.046	0.063	0.000	0.000	0.000	0.000	0.013	
	170	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U

Appendix Table 4 (Continued)

Locus	Allele Size	Population ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	Private allele
	174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.288	OA-E
	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	182	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.000	0.000	ON-GIFT
	190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	OA-E
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	OA-E
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088	OA-E
UNH216	120	0.068	0.021	0.057	0.025	0.000	0.000	0.054	0.000	0.775	
	122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	124	0.122	0.000	0.000	0.163	0.050	0.050	0.518	0.000	0.138	
	126	0.568	0.875	0.421	0.706	0.700	0.950	0.214	0.000	0.038	
	128	0.176	0.104	0.091	0.088	0.250	0.000	0.089	0.000	0.025	
	138	0.014	0.000	0.000	0.000	0.000	0.000	0.018	0.288	0.000	
	156	0.041	0.000	0.330	0.019	0.000	0.000	0.000	0.000	0.000	
	158	0.014	0.000	0.102	0.000	0.000	0.000	0.107	0.000	0.000	
	172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.313	0.000	OM-S
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.375	0.000	OM-S
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S
UNH222	162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.525	0.563	
	164	0.041	0.021	0.102	0.138	0.000	0.000	0.054	0.000	0.413	
	166	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	OM-S
	168	0.041	0.000	0.466	0.006	0.000	0.000	0.000	0.000	0.000	

Appendix Table 4 (Continued)

Locus	Allele Size	Population									Private allele
		ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	174	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.413	0.000	
	182	0.000	0.063	0.011	0.000	0.075	0.000	0.089	0.000	0.000	
	184	0.176	0.000	0.011	0.081	0.525	1.000	0.000	0.000	0.000	
	186	0.230	0.250	0.068	0.094	0.100	0.000	0.625	0.000	0.000	
	188	0.378	0.375	0.296	0.394	0.150	0.000	0.143	0.000	0.025	
	190	0.108	0.271	0.034	0.288	0.075	0.000	0.000	0.000	0.000	
	192	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	194	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	196	0.000	0.021	0.011	0.000	0.050	0.000	0.089	0.000	0.000	
UNH212	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.900	OA-E
	180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	186	0.081	0.000	0.000	0.175	0.000	0.925	0.214	0.000	0.000	
	188	0.162	0.250	0.159	0.363	0.000	0.000	0.089	0.000	0.000	
	190	0.351	0.292	0.148	0.238	0.525	0.000	0.071	0.000	0.000	
	192	0.230	0.229	0.261	0.056	0.100	0.075	0.464	0.000	0.013	
	194	0.014	0.021	0.000	0.000	0.025	0.000	0.018	0.000	0.000	
	200	0.068	0.021	0.000	0.013	0.000	0.000	0.143	0.000	0.000	
	202	0.081	0.188	0.057	0.156	0.325	0.000	0.000	0.488	0.000	
	204	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	OM-S
	216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S
	218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000	OM-S

Appendix Table 4 (Continued)

Locus	Allele Size	Population									Private allele
		ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	226	0.014	0.000	0.227	0.000	0.000	0.000	0.000	0.000	0.000	
	228	0.000	0.000	0.148	0.000	0.000	0.000	0.000	0.363	0.000	
UNH160	132	0.027	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	
	168	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.650	
	172	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	ON-GIFT
	180	0.095	0.396	0.466	0.094	0.000	0.000	0.232	0.000	0.000	
	182	0.068	0.000	0.011	0.188	0.150	0.000	0.089	0.000	0.350	
	184	0.068	0.063	0.171	0.025	0.050	0.000	0.357	0.550	0.000	
	186	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	188	0.000	0.000	0.000	0.000	0.425	0.000	0.000	0.350	0.000	
	190	0.027	0.000	0.000	0.188	0.050	0.050	0.018	0.000	0.000	
	192	0.000	0.021	0.000	0.006	0.025	0.000	0.000	0.000	0.000	
	194	0.162	0.042	0.034	0.006	0.000	0.000	0.000	0.100	0.000	
	196	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	198	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	200	0.014	0.000	0.023	0.000	0.100	0.000	0.071	0.000	0.000	
	202	0.189	0.188	0.261	0.263	0.000	0.950	0.089	0.000	0.000	
	204	0.000	0.000	0.000	0.000	0.025	0.000	0.018	0.000	0.000	
	206	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	208	0.324	0.271	0.034	0.231	0.050	0.000	0.000	0.000	0.000	
	210	0.000	0.021	0.000	0.000	0.050	0.000	0.018	0.000	0.000	
UNH213	182	0.054	0.104	0.068	0.006	0.000	0.000	0.000	0.000	0.900	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	OA-E
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	OM-S

Appendix Table 4 (Continued)

Locus	Allele Size	Population									Private allele
		ON-BP	**ON-BB**	**ON-SY**	**ON-CD**	**ON-U**	**ON-I**	**ON-GIFT**	**OM-S**	**OA-E**	
	190	0.014	0.000	0.000	0.000	0.000	0.025	0.000	0.625	0.013	
	192	0.284	0.042	0.557	0.075	0.000	0.375	0.000	0.325	0.000	
	196	0.027	0.000	0.000	0.000	0.125	0.000	0.107	0.000	0.000	
	198	0.014	0.000	0.000	0.000	0.025	0.000	0.071	0.013	0.050	
	200	0.014	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
	204	0.108	0.042	0.000	0.019	0.350	0.575	0.446	0.000	0.000	
	206	0.041	0.042	0.000	0.375	0.000	0.000	0.161	0.000	0.000	
	208	0.419	0.771	0.375	0.519	0.225	0.000	0.071	0.013	0.000	
	210	0.000	0.000	0.000	0.006	0.150	0.025	0.000	0.000	0.000	
	212	0.014	0.000	0.000	0.000	0.075	0.000	0.107	0.000	0.000	
	214	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	ON-U
	218	0.014	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
UNH153	197	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.850	0.000	
	205	0.203	0.083	0.500	0.006	0.000	0.000	0.000	0.000	0.000	
	209	0.014	0.000	0.046	0.000	0.000	0.000	0.000	0.125	0.000	
	211	0.716	0.771	0.432	0.825	0.800	0.950	0.750	0.025	0.025	
	213	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	ON-U
	215	0.027	0.125	0.023	0.169	0.000	0.000	0.018	0.000	0.450	
	217	0.014	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	231	0.027	0.000	0.000	0.000	0.000	0.000	0.179	0.000	0.525	
	233	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	ON-GIFT
	243	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	ON-GIFT
UNH132	125	0.203	0.333	0.102	0.488	0.025	0.025	0.000	0.000	0.000	
	127	0.378	0.292	0.443	0.119	0.325	0.400	0.000	0.000	0.375	

Appendix Table 4 (Continued)

		Population									
Locus	**Allele Size**	**ON-BP**	**ON-BB**	**ON-SY**	**ON-CD**	**ON-U**	**ON-I**	**ON-GIFT**	**OM-S**	**OA-E**	**Private allele**
UNH138	131	0.068	0.042	0.000	0.188	0.475	0.125	0.464	0.000	0.000	
	133	0.068	0.000	0.000	0.006	0.025	0.000	0.179	0.825	0.000	
	135	0.027	0.000	0.148	0.000	0.025	0.425	0.018	0.025	0.000	
	137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E
	139	0.257	0.333	0.296	0.194	0.075	0.025	0.250	0.000	0.000	
	141	0.000	0.000	0.000	0.000	0.025	0.000	0.089	0.150	0.000	
	143	0.000	0.000	0.011	0.006	0.000	0.000	0.000	0.000	0.600	
	149	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	164	0.054	0.021	0.421	0.013	0.000	0.000	0.143	1.000	0.000	
	178	0.000	0.000	0.000	0.000	0.150	0.200	0.000	0.000	0.000	
	180	0.027	0.000	0.000	0.000	0.075	0.000	0.196	0.000	0.000	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	186	0.473	0.354	0.364	0.438	0.300	0.000	0.054	0.000	0.050	
	188	0.095	0.042	0.034	0.100	0.025	0.800	0.304	0.000	0.263	
	190	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	192	0.270	0.500	0.148	0.288	0.050	0.000	0.179	0.000	0.438	
	194	0.027	0.000	0.023	0.163	0.000	0.000	0.000	0.000	0.113	
	196	0.014	0.000	0.011	0.000	0.175	0.000	0.036	0.000	0.000	
	200	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	ON-U
	204	0.000	0.063	0.000	0.000	0.025	0.000	0.018	0.000	0.113	
	208	0.000	0.021	0.000	0.000	0.075	0.000	0.000	0.000	0.013	
	210	0.014	0.000	0.000	0.000	0.050	0.000	0.018	0.000	0.000	
	228	0.014	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	

Appendix Table 4 (Continued)

Locus	Allele	Population									Private
	Size	ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	allele
UNH192	144	0.000	0.000	0.102	0.000	0.000	0.000	0.018	0.050	0.000	
	146	0.014	0.000	0.023	0.006	0.175	0.000	0.250	0.000	0.038	
	148	0.041	0.000	0.000	0.000	0.000	0.000	0.036	0.363	0.900	
	150	0.351	0.417	0.307	0.531	0.250	0.050	0.304	0.000	0.038	
	152	0.392	0.563	0.239	0.444	0.575	0.875	0.357	0.000	0.025	
	154	0.041	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	
	156	0.041	0.021	0.046	0.019	0.000	0.000	0.000	0.000	0.000	
	164	0.000	0.000	0.000	0.000	0.000	0.075	0.018	0.000	0.000	
	168	0.122	0.000	0.284	0.000	0.000	0.000	0.000	0.000	0.000	
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	OM-S
	190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.388	0.000	OM-S
UNH1004	180	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	182	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.763	
	186	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	
	194	0.000	0.021	0.000	0.000	0.150	0.000	0.357	0.000	0.000	
	196	0.000	0.000	0.000	0.000	0.050	0.000	0.018	0.000	0.125	
	198	0.311	0.250	0.102	0.288	0.275	0.000	0.089	0.000	0.000	
	200	0.081	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	
	202	0.189	0.479	0.182	0.300	0.025	0.000	0.089	0.000	0.000	
	206	0.027	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	
	214	0.000	0.000	0.000	0.000	0.300	0.000	0.000	0.000	0.000	ON-U
	218	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	ON-U
	220	0.014	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000	
	222	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	

Appendix Table 4 (Continued)

Locus	Allele Size	Population									Private allele
		ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	
	224	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	228	0.000	0.021	0.000	0.000	0.000	0.000	0.036	0.000	0.000	
	230	0.027	0.021	0.000	0.056	0.000	0.525	0.036	0.000	0.000	
	232	0.108	0.167	0.091	0.275	0.000	0.075	0.071	0.000	0.000	
	234	0.014	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	
	238	0.176	0.042	0.352	0.006	0.000	0.000	0.000	0.000	0.000	
	246	0.000	0.000	0.193	0.000	0.000	0.000	0.000	0.000	0.000	ON-SY
	248	0.000	0.000	0.000	0.000	0.000	0.400	0.000	0.000	0.000	ON-I
	250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.713	0.000	OM-S
	256	0.014	0.000	0.080	0.000	0.000	0.000	0.000	0.288	0.000	
UNH982	120	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ON-BP
	122	0.000	0.000	0.080	0.000	0.050	0.000	0.000	0.000	0.000	
	124	0.014	0.000	0.205	0.000	0.025	0.000	0.000	0.400	0.000	
	126	0.203	0.542	0.284	0.056	0.400	0.000	0.304	0.000	0.000	
	128	0.041	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	
	130	0.000	0.000	0.000	0.000	0.275	0.000	0.000	0.000	0.000	ON-U
	132	0.014	0.000	0.000	0.000	0.075	0.000	0.018	0.000	0.000	
	134	0.405	0.375	0.216	0.663	0.000	0.000	0.125	0.000	0.000	
	136	0.027	0.021	0.000	0.000	0.050	0.000	0.214	0.500	0.388	
	138	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	ON-U
	140	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	
	142	0.000	0.000	0.000	0.006	0.025	0.000	0.000	0.000	0.088	
	144	0.000	0.000	0.000	0.000	0.000	0.000	0.143	0.100	0.175	
	146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	OA-E

Appendix Table 4 (Continued)

Locus	Allele	Population									Private
	Size	ON-BP	ON-BB	ON-SY	ON-CD	ON-U	ON-I	ON-GIFT	OM-S	OA-E	allele
	148	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.075	
	150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	OA-E
	152	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.025	
	156	0.014	0.021	0.011	0.000	0.000	0.075	0.071	0.000	0.125	
	160	0.122	0.000	0.182	0.219	0.025	0.775	0.071	0.000	0.000	
	168	0.135	0.042	0.023	0.056	0.025	0.000	0.036	0.000	0.000	
UNH173	123	0.230	0.271	0.296	0.463	0.150	0.000	0.125	0.000	0.000	
	127	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	ON-U
	133	0.054	0.125	0.057	0.019	0.700	0.000	0.000	0.300	0.000	
	135	0.000	0.000	0.011	0.000	0.025	0.000	0.000	0.100	0.000	
	175	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	ON-I
	187	0.270	0.188	0.523	0.031	0.000	0.900	0.375	0.600	0.938	
	189	0.284	0.104	0.057	0.200	0.000	0.000	0.000	0.000	0.000	
	193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	OA-E
	195	0.014	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	
	201	0.041	0.042	0.000	0.000	0.000	0.000	0.036	0.000	0.000	
	205	0.108	0.271	0.046	0.288	0.000	0.075	0.304	0.000	0.050	
	207	0.000	0.000	0.011	0.000	0.000	0.000	0.036	0.000	0.000	

Appendix Table 5 Results of linkage disequilibrium tests based on the Chi square test of three feral populations of *O. niloticus* in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. R = correlation coefficient, *D* = disequilibrium coefficient

Population	**Loci pairs**	**R**	**R Probability**	***D***
ON-BP	*UNH172 - UNH211*	0.1487	0.0001	0.0298
	UNH172 - UNH222	0.1434	0.0001	0.0574
	UNH172 - UNH213	0.1540	0.0001	0.0131
	UNH172 - UNH132	0.1808	0.0001	0.0619
	UNH172 - UNH192	0.1609	0.0001	0.0255
	UNH211 - UNH216	0.2256	0.0001	0.0215
	UNH211 - UNH222	0.1848	0.0001	0.0135
	UNH211 - UNH212	0.1986	0.0001	0.0135
	UNH211 - UNH160	0.1830	0.0001	0.0189
	UNH211 - UNH213	0.2669	0.0001	0.0134
	UNH211 - UNH132	0.1888	0.0001	0.0407
	UNH211 - UNH138	0.2705	0.0001	0.0360
	UNH211 - UNH1004	0.1993	0.0001	0.0315
	UNH211 - UNH982	0.2216	0.0001	0.0208
	UNH211 - UNH173	0.1694	0.0001	0.0300
	UNH216 - UNH138	0.2051	0.0002	0.0312
	UNH216 - UNH192	0.1613	0.0001	0.0267
	UNH222 - UNH153	0.2590	0.0001	0.0133
	UNH222 - UNH138	0.1877	0.0001	0.0270
	UNH212 - UNH160	0.1502	0.0001	0.0195
	UNH212 - UNH213	0.2217	0.0001	0.0443
	UNH212 - UNH153	0.1676	0.0001	0.0379
	UNH212 - UNH138	0.2064	0.0001	0.0195
	UNH212 - UNH192	0.1609	0.0001	0.0255
	UNH212 - UNH1004	0.2101	0.0001	0.0133

Appendix Table 5 (Continued)

Population	Loci pairs	R	R Probability	*D*
	UNH160 - UNH213	0.2083	0.0001	0.0177
	UNH160 - UNH153	0.1506	0.0001	0.0259
	UNH160 - UNH132	0.1790	0.0001	0.0360
	UNH160 - UNH138	0.1877	0.0001	0.0176
	UNH160 - UNH1004	0.1478	0.0001	0.0182
	UNH160 - UNH982	0.1979	0.0001	0.0196
	UNH160 - UNH173	0.2229	0.0001	0.0135
	UNH213 - UNH138	0.3188	0.0001	0.0135
	UNH213 - UNH1004	0.2140	0.0001	0.0203
	UNH213 - UNH982	0.2217	0.0001	0.0133
	UNH213 - UNH173	0.2581	0.0001	0.0170
	UNH153 - UNH138	0.1343	0.0002	0.0197
	UNH153 - UNH1004	0.2077	0.0001	0.0192
	UNH153 - UNH982	0.2986	0.0001	0.0134
	UNH138 - UNH1004	0.2048	0.0001	0.0297
	UNH138 - UNH982	0.1802	0.0001	0.0216
	UNH138 - UNH173	0.2316	0.0001	0.0135
	UNH192 - UNH1004	0.1508	0.0001	0.0199
	UNH1004 - UNH982	0.2072	0.0001	0.0153
	UNH982 - UNH173	0.2302	0.0001	0.0135
Mean±SE				0.0237±0.0017
ON-BB	*UNH172 - UNH153*	0.3831	0.0001	0.0208
	UNH211 - UNH212	0.3410	0.0003	0.0208
	UNH211 - UNH1004	0.2959	0.0003	0.0208
	UNH212 - UNH160	0.4122	0.0001	0.0208
	UNH212 - UNH173	0.3422	0.0001	0.0417
	UNH160 - UNH213	0.3153	0.0001	0.0380
	UNH160 - UNH173	0.3169	0.0001	0.0417
	UNH213 - UNH138	0.2954	0.0001	0.0380

Appendix Table 5 (Continued)

Population	Loci pairs	R	R Probability	*D*
	UNH1004 - UNH982	0.4549	0.0001	0.0208
	UNH1004 - UNH173	0.3125	0.0001	0.0417
Mean±SE				0.0305±0.0033
ON-SY	*UNH172 - UNH211*	0.1412	0.0001	0.1216
	UNH172 - UNH222	0.1621	0.0001	0.0221
	UNH172 - UNH160	0.1525	0.0001	0.1728
	UNH172 - UNH192	0.1701	0.0001	0.0780
	UNH172 - UNH1004	0.1523	0.0001	0.0402
	UNH172 - UNH173	0.1244	0.0001	0.0217
	UNH211 - UNH1004	0.1536	0.0001	0.0671
	UNH222 - UNH160	0.1437	0.0001	0.0110
Mean±SE				0.0668±0.0199
ON-CD	*UNH216 - UNH222*	0.1180	0.0001	0.0061
	UNH213 - UNH132	0.2385	0.0001	0.0063
Mean±SE				0.0062±0.0001
ON-U	*UNH160 - UNH982*	0.3688	0.0001	0.0299
Mean±SE				0.0299±0.0049
ON-I	*UNH211 - UNH213*	0.5094	0.0001	0.0250
	UNH211 - UNH153	0.6848	0.0001	0.0000
	UNH211 - UNH132	0.4432	0.0001	0.0250
	UNH211 - UNH192	0.2352	0.0001	0.0974
	UNH213 - UNH153	0.9974	0.0001	0.0000
	UNH213 - UNH132	0.9216	0.0001	0.0385
	UNH153 - UNH132	0.9901	0.0001	0.0000
Mean±SE				0.0266±0.0132
ON-GIFT	*UNH172 - UNH211*	0.1790	0.0001	0.0344
	UNH211 - UNH138	0.2221	0.0001	0.0428
Mean±SE				0.0386±0.0042

Appendix Table 5 (Continued)

Population	Loci pairs	R	R Probability	*D*
OM-S	*UNH211* - *UNH212*	0.3552	0.0001	-0.0076
Mean±SE				-0.0076±0.0103
OA-E	*UNH172* - *UNH222*	0.2583	0.0001	0.0244
	UNH172 - *UNH153*	0.2561	0.0001	0.0244
	UNH216 - *UNH212*	0.2144	0.0001	-0.0333
	UNH222 - *UNH212*	0.6406	0.0002	0.0122
	UNH222 - *UNH153*	0.2201	0.0001	0.0244
	UNH222 - *UNH192*	0.5917	0.0001	0.0244
	UNH222 - *UNH173*	0.2753	0.0003	0.0003
	UNH212 - *UNH153*	0.2596	0.0001	0.0122
	UNH212 - *UNH138*	0.3690	0.0001	-0.0053
Mean±SE				0.0093±0.0065

Appendix Table 6 Genic differentiation for each population pair across all loci of three feral populations of *O. niloticus* in Thailand, four reference populations of *O. niloticus*, and one population each of *O. mossambicus* and *O. aureus*. (Fisher's method; $P < 0.0014$ after Bonferroni correction for multiple tests).

Population pair	χ^2	**df**	**P-value**
ON-BP & ON-BB	Infinity	28	0.0000
ON-BP & ON-SY	Infinity	28	0.0000
ON-BP & ON-CD	Infinity	28	0.0000
ON-BP & ON-U	Infinity	28	0.0000
ON-BP & ON-I	Infinity	28	0.0000
ON-BP & ON-GIFT	Infinity	28	0.0000
ON-BP & OM-S	Infinity	28	0.0000
ON-BP & OA-E	Infinity	28	0.0000
ON-BB & ON-SY	Infinity	28	0.0000
ON-BB & ON-CD	Infinity	28	0.0000
ON-BB & ON-U	Infinity	28	0.0000
ON-BB & ON-I	Infinity	28	0.0000
ON-BB & ON-GIFT	Infinity	28	0.0000
ON-BB & OM-S	Infinity	28	0.0000
ON-BB & OA-E	Infinity	28	0.0000
ON-SY & ON-CD	Infinity	28	0.0000
ON-SY & ON-U	Infinity	28	0.0000
ON-SY & ON-I	Infinity	28	0.0000
ON-SY & ON-GIFT	Infinity	28	0.0000
ON-SY & OM-S	Infinity	28	0.0000
ON-SY & OA-E	Infinity	28	0.0000
ON-CD & ON-U	Infinity	28	0.0000
ON-CD & ON-I	Infinity	28	0.0000
ON-CD & ON-GIFT	Infinity	28	0.0000

Appendix Table 6 (Continued)

Population pair	χ^2	**df**	**P-value**
ON-CD & OM-S	Infinity	28	0.0000
ON-CD & OA-E	Infinity	28	0.0000
ON-U & ON-I	Infinity	28	0.0000
ON-U & ON-GIFT	Infinity	28	0.0000
ON-U & OM-S	Infinity	28	0.0000
ON-U & OA-E	Infinity	28	0.0000
ON-I & ON-GIFT	Infinity	28	0.0000
ON-I & OM-S	Infinity	28	0.0000
ON-I & OA-E	Infinity	28	0.0000
ON-GIFT & OM-S	Infinity	28	0.0000
ON-GIFT & OA-E	Infinity	28	0.0000
OM-S & OA-E	Infinity	28	0.0000

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