Genetic Diversity of Genetically Improved Farmed Tilapia (GIFT) Broodstocks in Sri Lanka

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Abstract

Microsatellite DNA markers were used to study molecular genetic diversity in three GIFT-derived broodstock Lines in two hatcheries in Sri Lanka. Two Lines set up as replicates from the most recent importation of the GIFT strain from World Fish Center showed no significant differences in allelic diversity, despite having been exposed to very different management practices; one Line exposed to six generations of selection for harvest weight with rotational mating practiced among eight selected groups, while the second Line kept as the original broodstock with no such management. The third Line held at a second hatchery was derived from an older GIFT importation and differed from the first and the second Lines mostly due to presence of a number of private alleles. The most likely explanation for these additional private alleles appears to be admixture with other non-GIFT tilapia stocks held in or near this hatchery.

Keywords: tilapia; hatchery management; microsatellite markers; genetic diversity; private alleles; selective breeding

Introduction

The inland fresh water fishery of Sri Lanka is essentially based on a multitude of reservoirs across the island (Amarasinghe 1998). In recent times, various attempts have been made to develop the inland fishery via fisheries enhancement strategies and land-based aquaculture ventures (Amarasinghe and Weerakoon 2009). The inland fishery in the country was developed following the introduction of the Mozambique tilapia (Oreochromis mossambicus) in 1952, that was later followed by an introduction of Nile tilapia (O. niloticus) in 1975 (De Silva 1988; Amarasinghe and Weerakoon 2009). Recent introductions of the GIFT strain an improved culture line has contributed to inland fisheries production in the country, especially for small-scale pond aquaculture.

Inland fisheries production in Sri Lanka is reported to have reached 68955 Mt in 2012 (www.naqda.gov.lk) and tilapia contribute more than 50% to this. According to the Ten Year Development Policy Framework of the Fisheries and Aquatic Resources of Sri Lanka 2007-2016 (www.fisheries.gov.lk) it is envisaged that this will increase to 74450 Mt by 2016. As the GIFT strain has been selected for fast growth rate over many generations it is considered to be good candidate for aquaculture development in Sri Lanka to help reach envisaged targets from inland fish production by 2016.

Seeds required for the inland fishery are produced solely by Aquaculture Development Centers (AQDC), managed under the National Aquaculture Development Agency (NAQDA) of the Ministry of Fisheries. According to NAQDA (www.naqda.gov.lk), Aquaculture Development Centers are the major tilapia fish breeding stations and produced approximately 24.74 million tilapia fry in 2012. Common broodstock management practices are maintained at all tilapia breeding stations and that face a high demand for supply of fry. To date, the genetic quality of broodstocks and fry used to supply the culture industry in Sri Lanka has not been assessed.

Appropriate management practices to conserve genetic diversity are very important for any captive population, since declines in genetic diversity levels can affect survival and growth of fry in aquaculture systems. Understanding the levels and patterns of genetic variation in broodstock used to produce fry therefore becomes an important requirement to guarantee fingerling quality. A decrease
in genetic diversity levels and/or a change in their genetic composition can decrease fitness in hatchery populations and this has been reported to be a common phenomena in captive populations of fish species; rainbow trout (Miller et al. 2004), Atlantic Salmon (Skaala et al. 2004), Japanese flounder (Sekino et al. 2002), turbot (Coughlan et al. 1998), common carp (Kohlmann et al. 2005) as well as tilapia culture lines (Macaranas et al. 1986; Romana-Eguia et al. 2004; McKinna et al. 2010). Small numbers of broodstock, inbreeding or outbreeding, genetic drift effects and inadvertent selection are all known to be common factors that can contribute to declines in stock quality over time.

To enhance culture performance, selective breeding of broodstocks has been practiced to improve growth rates. When sound base populations have been established and appropriate selection methods are applied, considerable improvements in culture performance are possible for most species. Appropriately designed selective breeding programs can produce genetic gains each generation for various traits in most species (Eknath and Acosta 1998; Ponzoni et al. 2011).

Microsatellite DNA markers have been used widely to characterize and assess levels of genetic diversity in many fish stocks, for broodstock selection (e.g. parental assignment), for mapping economically important quantitative trait loci and in marker assisted breeding programs (Chistiakov et al. 2006). Application of microsatellite markers for genetic diversity studies in tilapia culture lines has been described by Romana-Eguia et al. (2004); Rutten et al. (2004); Hassanien and Gilbey (2005); Ha Hung et al. (2009) and Sukmanomon et al. (2012).

As such, a selective breeding program was designed for tilapia culture stocks by the World Fish Center (Ponzoni et al. 2010; Nguyen et al. 2011) and implemented by the National Aquaculture Development Authority in Sri Lanka (NAQDA) to improve the performance of the 9th generation GIFT broodstock brought to Dambulla AQDC. Following the introduction, six generations of selection were carried out between 2007 and 2011 to improve growth rate.

The objectives of the present study were to compare the levels of genetic diversity in selected and control lines of GIFT broodstock at Dambulla AQDC and to compare these to the stock at Udawalawa that originated from an earlier introduction of the 6th generation of the GIFT strain, maintained at Udawalawa where no specific genetic management program had been applied. Nine microsatellite markers (Lee et al. 2005) were used to assess the impacts of different management practices on tilapia broodstocks in Sri Lanka.

**Methods and Materials**

**GIFT broodstocks at Dambulla and Udawalawa Hatcheries**

Sri Lanka has three main tilapia hatcheries that produce fingerlings for stocking in reservoirs and seasonal tanks. The three broodstock populations sampled for the present study came from two of these hatcheries. Two populations came from Dambulla AQDC in Matale District, Central Province and the other population (UDA) came from Udawalawa AQDC at Ratnapura District, Sabaragamuwa Province in Sri Lanka. The population GDAM is from 50 families of GIFT (around 25 fingerlings per family, more than one family per bag at import) from the 9th Generation of GIFT (Ponzoni et al. 2010 and Statistics Unit, NAQDA, Sri Lanka) and was brought to Sri Lanka in 2007. According to NAQDA (*Personal communications*) the selective breeding program carried out at Dambulla AQDC is as follows. Fingerlings from each bag were divided into two and each part cohort was placed into a single tank. They constitute the GDAM broodstock at Dambulla station since their original introduction. The other part cohorts from each bag were grouped to form eight different cohorts that were maintained in eight different tanks, separately: they formed the SDAM population.

All tanks including GDAM were maintained separately and basic precautionary measures were taken to keep the stock uncontaminated from external genetic (tilapia) sources. A systematic selective breeding and rotational mating scheme has been practiced among the eight SDAM cohorts since 2007. The best performing fish in terms of body weight and length from each tank (200 males and 200 females making a total of 400) in each generation were selected to become parents for the
next generation and they were bred using rotational mating (males mated to females of another cohort). The current SDAM fish are the sixth generation obtained using this stock management procedure. The UDA population was founded from an earlier generation of GIFT stock (GIFT 6th generation): 1000 fry were brought to Sri Lanka in 2004 from Thailand (further details are unknown) and have been maintained since then as a single stock.

**Sampling**

Sixty four and 54 fish (1:1 male and female) were sampled randomly from the GDAM and UDA stocks, respectively. The SDAM samples came from 64 individuals, representing the eight cohorts, and each cohort was represented by eight individuals (4 males and 4 females) randomly sampled within sexes.

**Microsatellite analysis**

A sample of caudal fin (approximately 5x1 mm) was collected from each individual fish and preserved in 100% ethanol. DNA extraction was performed using the REAL pure DNA extraction kit (Durviz, Spain) following the manufacturer’s instructions. Quality and quantity of extracted DNA were determined using agarose gel electrophoresis and a Nanodrop (ND-1000) spectrophotometer (Labtech International Ltd, UK).

Nine microsatellite markers, each chosen from different linkage groups (Lee et al. 2005) were selected for the study (Table 1). Single locus PCRs were performed using a fluorescent labeled tailed primer method (Boutin-Ganache et al. 2001). Three types of tails - Godde (catgcgtgatctgacat), CAG (cagtcgggcgtcatca) and M13R (ggataaacttcacacagg) - were used and the tail was added to the 5’ end of either the reverse or forward primer. To label the PCR product, another primer containing the complimentary sequence to the tail, labeled with a fluorescent dye, was also included in the PCR reaction. Each of the tailed primers had a different fluorescent dye to allow identification of the PCR products.

**Table 1** Details of the microsatellites studied and the primer sequences

<table>
<thead>
<tr>
<th>Locus ID</th>
<th>*LG</th>
<th>Repeat Type</th>
<th>Size range (bp)</th>
<th>Primer sequence (5’-3’)</th>
</tr>
</thead>
</table>
| UNH995   | 1   | (CA)14      | 184-265         | F: CCAGCCCTCTGCAAAAAAGAC  
R: GCAGCAACAACCACAGTGCTA |
| UNH982   | 3   | (GT)21GC(GT)9 | 120-168         | TCAATACGTGGTCCCCCTCTTT  
TTCAGAGCGCATATCTTCTTG |
| UNH172   | 4   | (CA)17      | 176-246         | AATGCTTATAATGCTTTCA  
CTTTTATAGTCGCCCTTTGTTA |
| UNH132   | 9   | (GA)6GC(GA)7 | 125-149         | ATATAAAGAATGAGTCGCTGAG  
TGGAATAGAGGCTGGTGAG |
| UNH192   | 11  | (CA)10      | 144-190         | GGAATCCATAAGATCAGTTA  
CTTTTCAGGATTTACTGCTAAG |
| UNH173   | 13  | (CA)8       | 123-207         | CGTGAGAAAAACATGTT  
TATTGATTATAGCTGCTG |
| UNH138   | 16  | (CA)26      | 164-228         | TTCAGCTCTCATCTCTGT  
CCATTTAACCTCTCCATCT |
| UNH153   | 18  | (CA)9       | 197-243         | TCTGCTTTGCTTTTCTCATTTCT  
TACGGCACACTCCCTCCCAT |
| UNH216   | 23  | (CA)11      | 120-186         | GGGAACTAAAGCTGAAATA  
TGCAAGGAATATCAGCA |

*Linkage groups
PCR was performed in a Temperature Gradient thermal cycler (Biometra, Gottingen, Germany) using the Klear Taq kit (K Bioscience, UK). Fifteen micro liter reactions were set up containing a final concentration of 1X KlearTaq buffer, 1.5mM MgCl$_2$, 200nm (each) dNTPs, 300nM labeled primer, 300nM forward/reverse primer (depending on the labeled direction of the primer), 20nM tailed primer, 0.75U KlearTaq enzyme and 20ng DNA. The PCR conditions were as follows: 95°C for 15min (initial denaturation and enzyme activation) followed by 40 cycles of 95°C for 30s, 56°C for 30s and 72°C for 1min and a final extension time of 20min at 72°C. PCR products were checked by electrophoresis in 1.0% agarose gels made using 0.5X TAE buffer containing ethidium bromide (10µg/ml).

Two or three different PCR products with different fluorescent labels were multiplexed and the sizing of the products was performed using a CEQ 8800 Genetic Analysis System automated capillary sequencer (Beckman Coulter, USA). Allele sizes were analysed using CEQ 8800 software.

Data Analysis

Population genetic variability parameters, including number of alleles (A), allele frequencies per locus, allelic richness (Ar, calculated from the smallest sample size) observed and expected heterozygosities (Ho and He respectively) of each population, and Fis were estimated using FSTAT software for windows version 2.9.3 (Goudet 2002). The effective number of alleles per locus (Ae) was calculated according to Ferguson (1980). Differences between populations for each parameter (A, Ae, Ar) were tested for significance using One Way Analysis of Variance in SPSS statistical package, version 17.0 (SPSS Inc., Chicago). The total number of alleles and the total number of private alleles were estimated for each locus in each tilapia strain.

GENEPOP version 4.0 (Raymond and Rousset 1995a; Rousset 2008) was utilized to determine if each locus and population conformed to Hardy-Weinberg equilibrium (HWE) by testing observed and expected heterozygosities and fixation indices (Fis) (Weir and Cockerham 1984) using a Markov chain exact method (dememorisation:1000; batches:100; iterations per batch:1000) (Guo and Thompson 1992). The same program was used to test the exact probability of significant deviations from HWE (Fisher’s method) for all loci and for all populations to determine genetic differentiation between each population pair (exact G test). Most loci did not conform to HWE, the program Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004), was used to detect for presence of null alleles.

As the UDA population contained a comparatively higher number of private alleles than the other two populations, hierarchical cluster analysis was performed using the between groups method, Euclidean distances, standardized with Z scores (SPSS version 17) on UDA population to identify whether the individuals possessing private alleles could be separated. Only 46 individuals (from 54) were used for the analysis as the remaining ones did not have a complete allele set across all nine loci.

Results

The number of alleles per locus varied from a minimum of 3 (locus UNH 153) to a maximum of 12 (locus UNH 995) across the 3 Lines. The mean number of alleles per locus (A) was lowest in GDAM (5.8) and highest in UDA (7.7) (Table 2). Allelic richness (Ar) was also lowest in GDAM and highest in UDA. SDAM had the lowest effective number of alleles (Ae) while the other two populations had very similar values. No significant differences were evident in A, Ae or Ar among the three Lines. Private alleles were present in all three populations and UDA showed the highest number of private alleles (Table 2).

UDA and SDAM deviated significantly from HWE overall, while GDAM did not depart significantly from HWE (Table 3). GDAM only had a single locus that departed from HWE while SDAM had six (3 loci with Ho>He, and 3 loci with He>Ho), and UDA had six (all with He>Ho). High Fis values for most loci in the UDA Line indicated an excess of homozygotes, Testing for null
alleles resulted two (UNH138, UNH172) and three (UNH132, UNH172, UNH995) loci in SDAM and UDA respectively, while GDAM showed no indication of null alleles being present (Table 2).

Table 2 Summary statistics on genetic variation in three hatchery populations derived from GIFT tilapia

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>No. of Loci</th>
<th>A</th>
<th>Ae</th>
<th>Ar</th>
<th>Null alleles</th>
<th>Private alleles**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDAM</td>
<td>65</td>
<td>9</td>
<td>5.8</td>
<td>3.6</td>
<td>5.7</td>
<td>ND</td>
<td>UNH216(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.9)</td>
<td>(1.1)</td>
<td>(1.9)</td>
<td>UNH138</td>
<td>UNH982(1)</td>
</tr>
<tr>
<td>SDAM</td>
<td>64</td>
<td>9</td>
<td>6.1</td>
<td>3.2</td>
<td>6.0</td>
<td>UNH138</td>
<td>UNH132(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.4)</td>
<td>(0.7)</td>
<td>(1.3)</td>
<td>UNH172</td>
<td>UNH192(2)</td>
</tr>
<tr>
<td>UDA</td>
<td>54</td>
<td>9</td>
<td>7.7</td>
<td>3.7</td>
<td>7.6</td>
<td>UNH132</td>
<td>UNH132(5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.8)</td>
<td>(2.0)</td>
<td>(2.8)</td>
<td>UNH172</td>
<td>UNH995(4)</td>
</tr>
</tbody>
</table>

N-sample size, A-mean number of alleles per locus, Ae-effective number of alleles, Ar- allelic richness (standard deviations in parentheses)

**Number of private alleles at a locus are given in parenthesis and superscript denotes the total allele frequencies of these private allele, ND not detected

Table 3 Tests for conformation to Hardy-Weinberg equilibrium showing observed and expected heterozygosities

<table>
<thead>
<tr>
<th>Locus</th>
<th>Ho</th>
<th>He</th>
<th>P*</th>
<th>Fis</th>
<th>Ho</th>
<th>He</th>
<th>P*</th>
<th>Fis</th>
<th>Ho</th>
<th>He</th>
<th>P*</th>
<th>Fis</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNH216</td>
<td>0.646</td>
<td>0.738</td>
<td>0.053</td>
<td>0.125</td>
<td>0.714</td>
<td>0.676</td>
<td>0.004</td>
<td>-0.058</td>
<td>0.685</td>
<td>0.666</td>
<td>0.182</td>
<td>-0.030</td>
</tr>
<tr>
<td>UNH173</td>
<td>0.584</td>
<td>0.590</td>
<td>0.610</td>
<td>0.008</td>
<td>0.750</td>
<td>0.565</td>
<td>0.007</td>
<td>-0.331</td>
<td>0.528</td>
<td>0.599</td>
<td>0.026</td>
<td>0.119</td>
</tr>
<tr>
<td>UNH132</td>
<td>0.723</td>
<td>0.723</td>
<td>0.238</td>
<td>0.000</td>
<td>0.641</td>
<td>0.700</td>
<td>0.024</td>
<td>0.086</td>
<td>0.660</td>
<td>0.809</td>
<td>&lt;0.001</td>
<td>0.185</td>
</tr>
<tr>
<td>UNH995</td>
<td>0.810</td>
<td>0.787</td>
<td>0.053</td>
<td>-0.029</td>
<td>0.714</td>
<td>0.756</td>
<td>0.326</td>
<td>0.055</td>
<td>0.673</td>
<td>0.889</td>
<td>&lt;0.001</td>
<td>0.245</td>
</tr>
<tr>
<td>UNH153</td>
<td>0.159</td>
<td>0.149</td>
<td>1.000</td>
<td>-0.064</td>
<td>0.590</td>
<td>0.617</td>
<td>0.006</td>
<td>0.044</td>
<td>0.615</td>
<td>0.480</td>
<td>0.059</td>
<td>-0.2835</td>
</tr>
<tr>
<td>UNH192</td>
<td>0.646</td>
<td>0.753</td>
<td>0.023</td>
<td>0.143</td>
<td>0.635</td>
<td>0.620</td>
<td>&lt;0.001</td>
<td>-0.026</td>
<td>0.519</td>
<td>0.526</td>
<td>0.007</td>
<td>0.0146</td>
</tr>
<tr>
<td>UNH138</td>
<td>0.873</td>
<td>0.841</td>
<td>0.606</td>
<td>-0.039</td>
<td>0.630</td>
<td>0.753</td>
<td>0.001</td>
<td>0.165</td>
<td>0.750</td>
<td>0.848</td>
<td>0.200</td>
<td>0.1160</td>
</tr>
<tr>
<td>UNH172</td>
<td>0.769</td>
<td>0.767</td>
<td>0.234</td>
<td>-0.003</td>
<td>0.460</td>
<td>0.730</td>
<td>&lt;0.001</td>
<td>0.371</td>
<td>0.685</td>
<td>0.843</td>
<td>&lt;0.001</td>
<td>0.189</td>
</tr>
<tr>
<td>UNH982</td>
<td>0.820</td>
<td>0.769</td>
<td>0.698</td>
<td>-0.066</td>
<td>0.688</td>
<td>0.700</td>
<td>0.207</td>
<td>0.019</td>
<td>0.740</td>
<td>0.795</td>
<td>0.822</td>
<td>0.069</td>
</tr>
<tr>
<td>Mean</td>
<td>0.670</td>
<td>0.680</td>
<td>0.0659</td>
<td>0.647</td>
<td>0.680</td>
<td>HS</td>
<td>0.651</td>
<td>0.717</td>
<td>HS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard error for pseudo exact tests is in parentheses. (---) denotes complete enumeration of exact test for four alleles per locus, HS-highly significant
Results of the hierarchical cluster analysis show that most of the individuals possessing private alleles for nine loci in the UDA population formed a separate cluster (denoted as A) in the dendrogram (Fig 1). Of interest, most of these individuals had more than two private alleles per individual (Table 4).

Figure 1 Dendrogram showing the GIFT fish of UDA having private alleles forming a single cluster (A)
Table 4 UDA individuals in the cluster A of the dendrogram and the number of private alleles present in them

<table>
<thead>
<tr>
<th>Locus</th>
<th>UNH173</th>
<th>UNH132</th>
<th>UNH 995</th>
<th>UNH 138</th>
<th>UNH 172</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Number</td>
<td>195</td>
<td>142, 144, 147, 153, 157</td>
<td>230, 234, 240, 253</td>
<td>193</td>
<td>192, 230, 226, 240</td>
<td></td>
</tr>
<tr>
<td>UDA36</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>UDA 50</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>UDA 58</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>UDA 29</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>UDA 54</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>UDA 3</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>UDA 6</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>UDA 38</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>UDA 60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>UDA 20</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>UDA 21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>UDA 32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion and Conclusions
The present study characterized molecular genetic variation in two tilapia culture lines founded from the same stock (GIFT, Generation 9) that had been subjected to very different stock management regimes (“minimal change” vs selection with rotational mating), carried out at Dambulla Aquaculture Development Center. Diversity in the two GIFT lines of Generation 9 was then compared with another hatchery line (Udawalawa) founded from an earlier 6th Generation GIFT stock. The time difference between introductions of the different GIFT generations (6 vs 9) into Sri Lanka and the present study was five and eight years respectively.

There were no significant differences in the number of alleles (A), the effective number of alleles (Ae) or allelic richness (Ar) among the three populations. The UDA Line (that had the highest values for all parameters) also had several private alleles with fairly high combined frequencies (up to 0.33 for UNH172, Table 2). The small number of private alleles at low frequencies in the GDAM and SDAM samples may have resulted from genetic drift and/or sampling error in these populations.

Potential reasons for private alleles being present in the UDA Line
In addition to the GIFT stock, Udawalawa AQDC maintains another non-GIFT O. niloticus broodstock. Water for broodstock ponds is supplied from the Udawalawa reservoir where tilapia stocks of more than a single species (O. mossambicus, O. niloticus and O. rendalli) are present (Chandrasoma 1986; De Silva et al. 2005). Accidental escapes and gene introgression from these sources in the hatchery could have been responsible for the private alleles observed in the UDA GIFT-derived population. The majority of loci in the UDA population showed a deficit of heterozygotes and significant deviation from HWE (Table 3). Most of the private alleles were shared by the same individuals (Table 4). This suggests recent introgression of foreign genes into the UDA Line from other tilapia stocks.

Comparison of GDAM and SDAM
GDAM, SDAM Lines were very similar with respect to He and all diversity measures indicating little impact of selection on diversity in the SDAM line. As indicated earlier, the SDAM Line originated from families from the original GDAM population and was selectively bred from 8 tanks over six generations across five years by methodically transferring all males from one tank to females from other tanks in each generation to achieve appropriate levels of genetic mixing among
This practice would appear to have had a positive outcome unlike some other selective breeding programs that have produced reductions of genetic diversity in hatchery reared populations over time (Sekino et al. 2002; Was and Wenné 2002). Being however, an admixed group of randomly selected individuals representing eight tank populations; it would be unlikely that the admixed stock would conform to HWE until the population had reached equilibrium following an extended period of random mating.

SDAM Line showed higher A and Ar estimates compared with the founder GDAM Line. Selective breeding carried out on the SDAM Line was directed at improving and important quantitative trait (individual growth rate) and in parallel conserving genetic diversity relative to the source population.

**Comparison of this study to others**

Estimates of A and Ar in the Udawalawa broodstock population (UDA) were higher than GDAM or SDAM indicating that more genetic diversity remained in this line. Sukmanomon et al. (2012) applied 14 microsatellite markers (8 of which were common to those screened in the current study) to examine diversity in the UDA Line except that he screened the 9th generation where Ar was lower (7.14). Potentially, the difference may have resulted from the smaller sample size examined. Studies of Rutten et al. (2004) based on 14 loci and McKinna et al. (2010) based on 4 loci also showed lower genetic diversity estimates (7.5 and 7.1) for the 9th and 6th generations of GIFT populations respectively than was evident in the UDA Line here, but the identity and number of loci screened were different and so direct comparison are not possible.

The three GIFT lines screened here had lower A estimates when compared with other GIFT (4.37) and GIFT derived populations (4.17-5.42) Sukmanomon et al. (2012). The founding size of each line and the impact that different management practices in combination with effects of selective breeding (SDAM Line), all may have contributed to the relatively low diversity estimates observed in these lines.

**Significance and further studies**

Molecular studies of diversity in cultured tilapia broodstocks in Sri Lanka have not being conducted since the first introduction of tilapia species to the country. Therefore comparative studies on genetic status of present and past lines is not possible. The UDA and GDAM lines do not however, show substantial loss of genetic variation which could be considered as an indication of a positive outcome from applying cautious management practices. A comparative study of relative growth performances of the GDAM and SDAM Lines is warranted however, to determine the best performing stock under production environments in Sri Lanka. This would provide a basis for development of a suitable broodstock management scheme for the culture industry.

High levels of genetic diversity in general, enhance the production of quality seed with respect to both fitness and adaptability (Taniguchi 2003). Sri Lanka mainly has culture based fishery depending largely on stocking fingerlings in reservoirs. Limited tilapia aquaculture currently takes place. Fingerlings produced from the broodstock Lines screened here appear sufficiently robust to survive and grow well in large reservoirs and seasonal tanks where they stocked. Long-term maintenance of their relative genetic quality however, remains an important issue and attention in the future should be directed at conserving diversity in the lines.
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