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Genetic Characterization of Cultured Tilapia in Fiji Using Allozymes and Random Amplified Polymorphic DNA

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Abstract

Fiji has recognized that appropriate breeding approaches are necessary if tilapia production is to continue to meet market demands. To this end, genetic markers (allozymes and RAPDs) were developed to characterize the levels and patterns of genetic diversity in four cultured stocks of tilapia (*Oreochromis niloticus* (L.) 'Chitralada', *Oreochromis niloticus* (L.) 'Israel', *Oreochromis mossambicus* (Peters) and a Red hybrid stock) in Fiji across four generations. Although marker analyses revealed identical patterns of genetic relationship among the stocks, genetic diversity levels differed according to marker type. Allozyme analysis revealed 50 loci of which 25 were polymorphic among the stocks cultured in Fiji. RAPD electrophoresis of seven random 10mer primers resulted in 95 scorable bands of which 58 putative loci were polymorphic among the stocks.

Processes other than gene introgression among stocks have maintained relatively high levels of genetic diversity in most of the sampled stocks. Low levels of genetic diversity in *O. mossambicus* may preclude the use of this species in future breeding applications.

This study is the first characterization of genetic diversity in any tilapia stock in the Pacific region and has provided a baseline for future genetic improvement and monitoring of the gene pools of cultured tilapia stocks.

Introduction

Tilapias are becoming a major international commodity, with approximately 800,000 tonnes produced worldwide in 1996 (FAO 1998). Tilapias are comparatively easy to culture under several different systems and production is relatively low cost. Rapidly expanding markets for tilapia have been established in the USA, Japan, Singapore, Europe and many Asian countries (FAO 1998) with interest in their culture continuing to expand.

Despite many positive culture attributes, many Asian tilapiine stocks (in particular *O. niloticus*) are characterized by high levels of inbreeding and have experienced declines in levels of genetic diversity resulting from the use of small founder populations (Pullin and Capili 1988). Widespread introgression of *O. mossambicus* alleles into some cultured stocks (particularly *O. niloticus*) and poor stock management practices have been highlighted as potential causes for reductions in performance of some stocks in culture (Hulata et al. 1986, Guerrero and Tayamen 1988, Yan 1988, Eknath et al. 1991, Guerrero 1992, Hulata et al. 1993, Macaranas et al. 1995). In addition, undocumented transfers and introductions have confounded tilapia genetic resources in many regions, making accurate identification and characterization of broodstocks and hatchery populations problematic (Taniguchi et al. 1985, Macaranas et al. 1986, Pante and Macaranas 1989).

The future of tilapia stock improvement will rely on appropriate stock choice, development of sound management techniques and selective breeding. The basis of this approach is the ability to characterize and monitor tilapia genetic resources under culture, provide a sound knowledge of the genetic characteristics of each stock and to examine the effects of management practices on the gene pools of each stock. With this in mind, the current study sought to develop genetic markers in Fijian cultured tilapia stocks as a baseline for their long-term genetic improvement. Tilapia culture in Fiji is still relatively new, but is considered a viable way of addressing an increasing shortfall in protein nutrition caused by overexploitation of Fiji's inshore marine resources (Macaranas et al. 1997).

O. mossambicus from Malaysia was the first tilapia species introduced to Fiji in 1954 (Holmes 1954). Further introductions of other tilapia strains/species occurred over the next several decades with imports of the *O. niloticus* 'Israel' strain from Israel in 1972, a red hybrid (*O. niloticus* × *O. mossambicus*) stock from Taiwan in 1984, *O. hornorum* and *O. aureus* from Taiwan in 1985 and *O. niloticus* 'Chitralada' strain from Thailand in 1988 (Macaranas et al. 1997).

While the stocks have been maintained separately since their introductions, little is known on management practices since introduction have affected their relative levels and patterns of genetic diversity. Plans for future expansion of the industry within Fiji and the development of improved cultured tilapia stocks will require technologies that allow the genetic characteristics of all stocks to be identified and monitored over time.

Majority of the studies that have examined genetic diversity or genetic relationships in tilapia have used allozyme electrophoresis (McAndrew and Majumdar 1983, Taniguchi et al. 1985, Macaranas et al. 1986, De Silva and Ranasinghe 1989, Pante and Macaranas 1989, Sodsuk and McAndrew 1991, Oosthuizen et al. 1993, Macaranas et al. 1995, Agnese et al. 1997, De Silva 1997). More recently, DNA markers have also been proven successful at discriminating among species and strains (Wright 1989, Carter et al. 1991, Harris et al. 1991, Franck et al. 1992, Seyoum and Kornfield 1992, Franck and Wright 1993, Agnese et al. 1997). To date, only a few studies have used anonymous DNA methods (e.g. RAPDs and Amplified Fragment Length

Polymorphism or AFLP) to characterize genetic diversity in tilapiine fishes (Bardakci and Skibinski 1994, Naish et al. 1995, Dinesh et al. 1996).

In this study, allozyme and RAPD markers were developed to document genetic diversity within the tilapia stocks cultured in Fiji based on their ability to screen for variation in different parts of the genome. The aim of the research was to develop genetic markers and monitor parameters across parental and three generations of growout evaluation to provide the data necessary to assess the impacts of current management practices on genetic diversity and gene introgression among the tilapia stocks cultured in Fiji. In turn, it is anticipated that genetic markers developed in this study will play a valuable role in maintaining and monitoring future husbandry practices undertaken during the culture of tilapia stocks in Fiji.

Materials and Methods

Tilapia culture at Naduruloulou Research Station, Suva

Individuals from four tilapia stocks (*O. niloticus* 'Chitralada', *O. niloticus* 'Israel', *O. mossambicus* and a Red hybrid) were obtained from the Naduruloulou hatchery. Six ponds, approximately 600 m^2 in area and 1 m in depth were used for evaluation trials as described in Macaranas et al. (1997). At the beginning of each generation, hapas in each pond contained 100 fry, approximately 2 to 3 grams (100 fry·stock·hapa × 6 ponds). Final randomized sampling of each stock was undertaken after four months of growout when fish were approximately six months old. This final sampling produced the individuals used in all the genetic analyses. Individuals were typed for both allozyme and RAPD markers. Sampled individuals were not parents of the next generation but represented a random sample of the possible genotypes present in each stock that were available as parents for the next generation.

After completion of genetic sampling, 50 males and 100 females from each stock were chosen randomly from the six ponds and placed into grow out tanks. The breeding designs for generation of 100 families per stock per generation are described in Macaranas et al. (1997).

Heart, muscle and liver tissue samples for genetic analyses were removed and sent to the Queensland University of Technology in dry ice and maintained at -70^0C until required for analysis.

Allozyme electrophoresis (CAGE)

Allozyme analyses were undertaken as described in Appleyard and Mather (2000). The allozyme systems shown in table 1 were based on polymorphic systems previously identified in Fijian cultured tilapia (data not shown here, Appleyard 1998). Electrophoretic running conditions and staining procedures were as described in Appleyard (1998) and Appleyard and Mather (2000).

Enzyme nomenclature, abbreviations and allele designations of allozyme systems examined in this study conformed to the recommendations of Shaklee et al. (1990). For enzyme systems that were encoded by more than

Table 1. Protein and enzyme systems, enzyme number, number of putative loci and tissue source for enzymes screened in tilapia cultured in Fiji

Protein/Enzyme	E.C. number[a]	Locus	Tissue source
Aspartate aminotransferase (AAT)	2.6.1.1	*AAT-1**	Liver
		*AAT-2**	Liver
Acid phosphatase (ACP)	3.1.3.2	*ACP**	Liver
Adenosine deaminase (ADA)	3.5.4.4	*ADA-1**	Heart
		ADA-2*	Muscle
		*ADA-3**	Muscle
Alcohol dehydrogenase (ADH)	1.1.1.1	*ADH**	Liver
Aconitate hydratase (AH)	4.2.1.3	*AH-1**	Liver
		*AH-2**	Liver
Adenylate kinase (AK)	2.7.4.3	*AK-1**	Muscle
		*AK-2**	Muscle
Aldolase (ALD)	4.1.2.13	*ALD-1**	Muscle
		*ALD-2**	Liver
Aldehyde dehydrogenase (ALDH)	1.2.1.5	*ALDH-1**	Liver
Creatine kinase (CK)	2.7.3.2	*CK-1**	Heart
		CK-2*	Heart
		*CK-3**	Muscle
Esterase (EST)	3.1.1.	*EST-1**	Heart, Liver
		*EST-2**	Heart, Liver
Fructose-bisphophatase (FBP)	3.1.3.11	*FBP-1**	Muscle
		*FBP-2**	Muscle
Fumarate hydratase (FH)	4.2.1.2	*FH-1**	Heart
		*FH-2**	Heart
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1.2.1.12	*GAPDH-1**	Heart
		*GAPDH-2**	Heart, Muscle
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8	*G3PDH-1**	Heart
		*G3PDH-2**	Muscle
Glucose-6-phospate dehydrogenase (G6PDH)	1.1.1.49	*G6PDH**	Muscle
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	*GPI-1**	Heart
		*GPI-2**	Heart
L-Iditol dehydrogenase (IDDH)	1.1.1.14	*IDDH-1**	Liver
		*IDDH-2**	Liver
Isocitrate dehydrogenase (NADP+) (IDHP)	1.1.1.42	*IDHP**	Liver
L-Lactate dehydrogenase (LDH)	1.1.1.27	*LDH-1**	Heart
		*LDH-2**	Muscle
Malate dehydrogenase (MDH)	1.1.1.37	*sMDH-1**	Muscle
		sMDH-2*	Heart
		mMDH-3*	Muscle
		*mMDH-4**	Muscle
Malic enzyme (NADP+) (MEP)	1.1.1.40	*MEP**	Liver
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	*PGDH**	Liver
Phosphoglucomutase (PGM)	5.4.2.2	*PGM**	Muscle
Superoxide dismutase (SOD)	1.15.1.1	*SOD**	Liver
General Protein (PROT)		*PROT-1**	Muscle
		PROT-2*	Muscle
		PROT-3*	Muscle
		*PROT-4**	Muscle

[a]E.C. number refers to Enzyme Commission number assigned to each enzyme by the International Union of Biochemistry

a single locus, the most anodal locus was designated (1). An *O. niloticus* 'Chitralada' and an *O. mossambicus* individual were used on all gels as standards. In each system, alleles were designated according to mobility and direction from the most common 'Chitralada' (100) allele. Genotype frequencies were scored from observations of allozyme phenotypes on cellulose acetate plates. Allelic frequencies were then estimated from the presumptive genotypes assuming codominance of alleles.

RAPD electrophoresis (PAGE)

A CTAB DNA extraction method (Doyle and Doyle 1987) modified by Grewe et al. (1993) was used to extract genomic DNA from approximately 100 mg of white muscle tissue (Appleyard and Mather 2000). Genomic DNA for RAPD applications was diluted to a concentration of 50 to 70 ng·μl of which 1 μl was used in each 25 μl polymerase chain reaction (PCR).

Seven out of 20 primers from Operon Kit A (Operon Technologies, Alameda USA) were used to examine RAPD variation in each stock per generation (Table 2). PCR amplifications were performed as described in Appleyard and Mather (2000). Reactions were stored at 4^0C until electrophoresis.

Amplification products were separated using a 4% stacking and 12% resolving non-denaturing polyacrylamide gel in a Bio Rad Mini Protean II electrophoresis cell (running conditions as in Appleyard and Mather 2000). Bands were visualized using a Pharmacia LKB 2011 transilluminator and photographed using Polaroid 665 black and white film. Amplified products were scored by viewing the negatives of each gel over a light box.

Loci were classified as described in Appleyard and Mather (2000) following the estimation of loci sizes by unweighted linear regression relative to the position of a ϕX174 *HaeIII* DNA size standard. Allelic frequencies were estimated from presumptive phenotypes assuming dominance of alleles such that +/+ and +/- individuals had the (1) phenotype and -/- individuals had the (0) phenotype.

Statistical analysis

Population allozyme variation statistics (see Appleyard 1998) were calculated using BIOSYS-1 Release 1.7 program (Swofford and Selander 1989).

Table 2. RAPD primers from Operon Kit A used in screening of tilapia stocks cultured in Fiji

Primer	Sequence (5'-3')	No. scorable bands	Informative bands[a]
OPA-4	AATCGGGCTG	9	3
OPA-7	GAAACGGGTG	12	7
OPA-8	GTGACGTAGG	13	7
OPA-9	GGGTAACGCC	13	9
OPA-10	GTGATCGCAG	15	9
OPA-16	AGCCAGCGAA	17	11
OPA-18	AGGTGACCGT	16	12

[a]refers to bands absent in individuals from at least one stock

Examination of genotype conformation to Hardy-Weinberg equilibrium and linkage disequilibrium within stocks were estimated using GENEPOP vers 3.2 (Raymond and Roussett 2000). Significance of departure from equilibrium levels was tested using a Markov chain procedure, with significance levels determined after 400 batches of 4,000 iterations each.

Allele frequencies at informative RAPD loci were compiled using the RAPDPLOT (Black 1993) program and analyzed using BIOSYS-1 Release 1.7 program (Swofford and Selander 1989). Dissimilarity values between and among stocks were calculated from RAPD loci. Dissimilarity values can be considered a general approximation of variation within a stock (Lynch 1990, Baruffi et al. 1995) and were calculated by comparing banding patterns between pairs of individuals (x and y) according to Nei and Li (1979). Due to the dominant nature of RAPD alleles, it was assumed that populations conformed to Hardy-Weinberg equilibrium although this assumption could not be tested. GENEPOP Version 3.2 (Raymond and Rousset 2000) was used to test for linkage disequilibrium between pairs of RAPD loci.

Chi-square tests in GENEPOP vers 3.2 (Raymond and Roussett 2000) were used to examine if allele frequencies among stocks were homogenous across the generations. Significance levels were determined after 400 batches of 4,000 iterations each using a Markov chain. An exact test was used to test temporal differentiation of each stock across generations using ARLEQUIN vers. 2.00 (Schneider et al. 2000). Significance levels were based on 100,000 steps of a Markov chain procedure. In all cases with multiple tests, significant levels were adjusted using a standard Bonferroni procedure (Lessios 1992).

F-statistics (F_{ST} and F_{DT}) among stocks were calculated in GENEPOP vers. 3.2 (Raymond and Roussett 2000) and BIOSYS-1 Release 1.7 program (Swofford and Selander 1989). F_{ST} values in the current study were estimated by a weighted analysis of variance (Cockerham 1973, Weir and Cockerham 1984).

The Analysis of Molecular Variance (AMOVA) procedure developed by Excoffier et al. (1992) in the program ARLEQUIN vers. 2.00 (Schneider et al. 2000) was used to measure the genetic variance of stock structure. The significance of the variance component was based on 16,000 re-sampling trials.

Assessment of genetic distance among stocks based on allozyme loci was measured using Nei's (1978) unbiased genetic distance (D_N). For RAPD loci, more traditional dissimilarity values were calculated to reflect genetic distance. The unweighted pair group method of analysis (UPGMA) was used to cluster all D_N estimates and dissimilarity values (BIOSYS-1, Swofford and Selander 1989, RAPDPLOT, Black 1993).

Results

CAGE analysis of 25 allozyme systems resulted in products at 50 loci. Of these, 25 loci were monomorphic for a single allele common to all stocks (across 4 generations) and 25 loci showed polymorphisms (0.95 criterion

level) among stocks. In comparison, PAGE analysis of 7 random primer systems resulted in scorable products at 95 RAPD loci (ave. 13.6 loci/primer). Of these, 37 were monomorphic in all stocks and 58 putative loci (ave. 8.3 loci/primer) were polymorphic/informative among stocks. In both analyses, the relatively high number of polymorphic or informative loci was the result of fixed allelic differences at many loci among stocks particularly between the *O. niloticus* strains and *O. mossambicus* (allozymes, 15 loci; RAPDs, 46 putative loci).

Table 3. Genetic variability measures based on 50 allozyme loci in tilapia stocks across four generations

Stock	Gen	N	Av. alleles/ locus	% poly. loci	Mean per heterozygosity locus	
					Observed[a]	Expected[b]
O. niloticus	1	10	1.32 (0.14)	18.0	0.098 (0.032)	0.090 (0.029)
'Chitralada'	2	50	1.38 (0.11)	16.0	0.087 (0.026)	0.077 (0.025)
	3	50	1.54 (0.13)	24.0	0.156 (0.038)	0.087 (0.024)
	4	50	1.34 (0.10)	20.0	0.099 (0.033)	0.064 (0.021)
O. niloticus 'Israel'	1	10	1.16 (0.06)	14.0	0.054 (0.024)	0.044 (0.018)
	2	50	1.38 (0.10)	18.0	0.061 (0.021)	0.057 (0.019)
	3	50	1.30 (0.08)	24.0	0.119 (0.036)	0.093 (0.026)
	4	50	1.36 (0.10)	20.0	0.101 (0.032)	0.091 (0.028)
O. mossambicus	1	10	1.04 (0.03)	4.0	0.026 (0.019)	0.021 (0.015)
	2	50	1.38 (0.09)	20.0	0.091 (0.026)	0.064 (0.019)
	3	50	1.42 (0.09)	20.0	0.111 (0.027)	0.068 (0.020)
	4	50	1.38 (0.09)	14.0	0.095 (0.028)	0.062 (0.020)
Red hybrid	1	10	1.54 (0.10)	44.0	0.134 (0.030)	0.200 (0.035)
	2	50	1.66 (0.11)	44.0	0.165 (0.031)	0.192 (0.033)
	3	50	1.68 (0.12)	48.0	0.187 (0.036)	0.208 (0.033)
	4	50	1.78 (0.13)	48.0	0.173 (0.032)	0.214 (0.034)

[a]Direct count estimate, [b]Unbiased estimate Nei (1978)
Standard errors in parentheses

Table 4. Genetic variability measures based on 95 RAPD loci in tilapia stocks across four generations

Stock	Gen	N	% poly. loci[a]	Dissimilarity values
O. niloticus 'Chitralada'	1	10	19.0	0.112
	2	10	13.0	0.094
	3	10	16.0	0.096
	4	20	20.0	0.089
O. niloticus 'Israel'	1	10	24.0	0.145
	2	10	10.0	0.073
	3	10	15.0	0.103
	4	20	26.0	0.138
O. mossambicus	1	10	9.0	0.049
	2	10	26.0	0.107
	3	10	13.0	0.078
	4	20	23.0	0.066
Red hybrid	1	10	46.0	0.296
	2	10	48.0	0.349
	3	10	48.0	0.283
	4	20	54.0	0.311

[a]0.95 criterion level

Levels of genetic diversity within stocks

Gene frequency data for each stock across the four generations are available in Appleyard (1998). Tables 3 and 4 list the estimates of genetic variability based on 50 allozymes and 95 RAPD loci for each of the stocks across four generations.

The two *O. niloticus* strains were the most polymorphic at allozyme loci of the three non-hybrid tilapia stocks. The Red hybrid displayed the highest percentage of polymorphic loci in all generations (44.0 to 48.0%). *O. mossambicus* populations generally displayed the lowest levels of observed heterozygosity and ranged from 0.091 to 0.111 (Generations Two-Four) while the Red hybrid populations displayed the highest levels of observed heterozygosity ranging from 0.165 to 0.187 (Table 2).

Results of RAPD analyses also showed that the Red hybrid stock displayed the highest percentage of polymorphic RAPD loci in all generations (46 to 54%). The lowest average RAPD values of within stock dissimilarity were observed for individuals within the *O. mossambicus* stock (Table 4).

Only 4% of the allozyme comparisons across the four stocks and four generations did not conform to Hardy-Weinberg expectations (data not shown). Generally, where loci did not conform to Hardy-Weinberg expectations, a significant lack of heterozygotes was observed. We can attribute the lack of heterozygotes at some loci to scoring difficulties, in particular at *EST-1** (liver) and *MEP**. Richardson et al. (1986) states that the isozyme products of these loci can exhibit complex banding patterns and uninterpretable variation. Scoring at the *ALDH-2** locus was also difficult as this system was sensitive to the amount of acetylaldehyde (which is highly volatile) added to the stain which in turn affected resolution.

Highly significant (after Bonferroni correction) disequilibrium associations were detected between *AAT-2** and *GAPDH-2** (muscle) (P=0.000) in the Red hybrid and *GAPDH-2** (muscle) and *CK-3** (P=0.000) in *O. mossambicus* but the associations were not consistent across generations and were not observed in other stocks. Such associations could be tested further using direct crossing experiments. Since *AAT-2**, *GAPDH-2** and *CK-3** provided only a limited contribution to stock differentiation, they were not considered further.

No RAPD loci showed significant linkage disequilibrium (after Bonferroni correction) in any of the four stocks across generations. It was therefore assumed that allelic variation at allozyme and RAPD loci could be considered independent.

Tests of homogeneity of allele frequencies within each tilapia stock across the generations

Generally, the four tilapia stocks did not show evidence of significant heterogeneity across the generations at either polymorphic allozyme or RAPD loci (Table 5), and no significant overall heterogeneity was detected between Generations One and Four in any of the stocks.

At individual loci, significant allele frequency differences (between Generations One and Four, after Bonferroni correction, $P = 0.000$) were observed at three loci in the 'Chitralada' stock and at four loci in the 'Israel stock'. One out of 11 polymorphic allozyme loci in *O. mossambicus* and two of the 24 polymorphic loci in the Red hybrid stock displayed significant heterogeneity in allele frequencies ($P = 0.000$). Of the significant heterogeneous allele frequencies observed in each tilapia stock, the majority of them (90%) were observed at either EST or MEP loci.

Only one of the variable RAPD loci in each of the stocks displayed significant heterogeneity (after correction) between Generations One and Four, although the same locus was not significant in all four stocks. Generally, in each stock however, frequencies at the most common allozyme and RAPD alleles were consistent across all generations.

Diagnostic loci and genetic differentiation among stocks

Phenotypic comparisons of morphological and meristic variation have generally not been very efficient at separating tilapia stocks, especially closely related strains (McAndrew and Majumdar 1983, Basiao and Taniguchi 1984, Macaranas et al. 1986, Van der Bank and Ferreira 1987, Pante and Macaranas 1989, Oosthiuzen et al. 1993). Both allele frequencies at polymorphic allozyme loci and informative RAPD loci in each generation were therefore used to test for differentiation among stocks. In majority of the polymorphic allozyme loci, significant differences in allele frequencies were detected among stocks ($P < 0.002$). These differences were consistent across generations (Generations One and Two; 96% of polymorphic loci and Generations Three and Four; 92% of polymorphic loci were heterogeneous among stocks). Allele frequencies at most informative RAPD loci were also different among the four stocks. This was especially evident between *O.*

Table 5. Global exact tests of homogeneity (*P* values) among the same tilapia stock across four generations of evaluation (above diagonal, RAPDs; below diagonal, allozymes)

Stock	Gen	1	2	3	4
O. niloticus 'Chitralada'	1	——-	1.000	1.000	1.000
	2	0.645	——-	1.000	1.000
	3	1.000	0.546	——-	1.000
	4	0.541	0.120	0.500	——-
O. niloticus 'Israel'	1	——-	0.485	1.000	1.000
	2	0.547	——-	0.497	0.512
	3	0.721	0.012	——-	1.000
	4	0.771	1.000	0.020	——-
O. mossambicus	1	——-	0.407	0.722	0.540
	2	0.953	——-	0.218	0.307
	3	0.024	0.004*	——-	0.276
	4	0.151	0.051	0.497	——-
Red hybrid	1	——-	1.000	1.000	1.000
	2	1.000	——-	1.000	1.000
	3	1.000	1.000	——-	1.000
	4	1.000	1.000	1.000	——-

*significant after Bonferroni correction for multiple pairwise tests

niloticus and *O. mossambicus* populations. In many instances RAPD primers generated banding patterns that were stock-specific (Generations One and Four; 93% of informative loci and Generations Two and Three; 95% of informative loci were different among stocks).

A large number of diagnostic allozyme and RAPD loci (up to 64% of polymorphic allozyme loci and 80% of informative RAPD loci) were fixed for alternative alleles in *O. niloticus* and *O. mossambicus* populations across generations. In many instances, individual RAPD primers amplified stock specific loci that could be used to differentiate unambiguously between stocks.

Allelic differentiation was not assessed between the Red hybrid and the two "parental" stocks – *O. niloticus* and *O. mossambicus*, although genetic distance estimates suggested that the hybrid had a mixture of genes from the two species but was more closely related to *O. niloticus*.

No diagnostic allozyme or RAPD loci were found that could discriminate between the two *O. niloticus* strains. Divergent allele frequencies were however detected at four allozymes and six RAPD loci in the two *O. niloticus* strains. A combination of allozymes and RAPD loci could be used together to distinguish individuals of these two closely related *O. niloticus* strains with reasonable confidence.

High F_{ST} values (not shown) were obtained for most polymorphic allozyme loci and in majority of the cases, values were significantly different from zero ($P < 0.05$). In each generation, the high F_{ST} values at individual loci among stocks and indeed the high overall mean F_{ST} values (0.501 to 0.598) indicated that there was little evidence of introgression among stocks. Likewise, overall mean F_{DT} values obtained using RAPD loci (Swofford and Selander 1989) were high (0.652 to 0.670). High mean F_{ST} and F_{DT} values suggest that there has been little, if any, gene exchange between stocks in each generation and that stock integrity across the generations had been maintained.

AMOVA undertaken in all generations demonstrated that between 58 to 73% of the variations observed (either allozyme or RAPD) could be attributed to significant ($P = 0.000$) among population differences (ϕ_{ST} allozymes: 0.576 to 0.647; ϕ_{ST} RAPDs: 0.716 to 0.728). These results reflected low levels of gene flow among populations and that sampled populations belong to discrete gene pools.

Genetic distance estimates from both sets of markers (not shown) indicate that *O. niloticus* 'Chitralada' and *O. niloticus* 'Israel' are closely related. *O. mossambicus* was most distantly related to *O. niloticus* while the Red hybrid shared more *O. niloticus* alleles than *O. mossambicus* alleles. Figure 1 contains an example of a characteristic unweighted pair group method of analysis (UPGMA) dendogram showing genetic relationships among the four

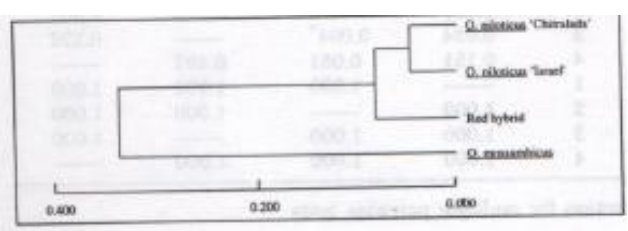

Fig. 1. Dendogram showing cluster analysis using UPGMA based on allozyme data and Nei's (1978) unbiased genetic distance estimates for tilapia stocks in Generation Four cultured in Fiji

tilapia stocks cultured in Fiji (based on allozyme data). Results for other generations showed identical topologies using either allozyme or RAPD data.

Discussion

Development of genetic markers

The major objective of this study was to characterize the levels and patterns of genetic diversity in tilapia stocks cultured in Fiji across four generations of growout and to use these data to evaluate their genetic integrity. Comparable patterns of genetic diversity among the stocks were detected with both genetic techniques; however in general, genetic variation was higher at RAPD loci. Genetic differentiation estimates among stocks calculated using RAPD data were also greater than those calculated using allozyme data. This may be attributed to the ability of RAPD markers to detect more polymorphisms and to screen for a larger number of marker loci (Liu and Furnier 1993, Peakall et al. 1995, Szmidt et al. 1996). This results from the likelihood that many RAPD loci were screened for variation in non-coding areas of the nuclear genome and thereby are probably less affected by functional constraints.

The large number of diagnostic allozyme and RAPD markers developed in this study are now available for use as genetic tags in future tilapia culture and selection programs in Fiji and elsewhere. In the present study, 16 diagnostic allozyme loci capable of differentiating *O. niloticus* and *O. mossambicus* stocks were identified. Similarly, most primers amplified RAPD markers (loci) that could differentiate among stocks. The large percentage of diagnostic markers observed here may reflect the relatively non-introgressed state of the cultured tilapia stocks in Fiji.

In general, *O. mossambicus* displayed the lowest levels while *O. niloticus* 'Chitralada', the highest levels of genetic variation among the non-hybrid tilapia stocks. In all generations, *O. mossambicus* was also the most divergent stock. The Red hybrid was more closely related to the *O. niloticus* stocks than to *O. mossambicus*. This relationship probably indicates directional backcrossing following the time the Red hybrid was developed from *O. niloticus* and *O. mossambicus* parents.

Significant differences may have existed in some allele frequencies within stocks across generations; however estimated genetic distances and dissimilar values among stocks and resulting dendograms for each generation were highly similar. Overall, stock relationships were unaffected by the different genetic markers and all resulted to be consistent among stock relationships across four generations.

Genetic variability in Fijian tilapia stocks

Levels of genetic variability are generally higher in the tilapia stocks cultured in Fiji compared with those observed in many other tilapia stocks

cultured elsewhere. Allozyme frequencies revealed little or no gene introgression by *O. mossambicus* into *O. niloticus* stocks or vice versa. The overall low level of significant Hardy-Weinberg deviations coupled with the observation of fixed alleles at many loci supports the inference that the tilapia stocks cultured in Fiji are relatively non-introgressed. This result is in stark contrast to reports from many Asian countries where significant *O. mossambicus* gene introgression has been detected in many cultured *O. niloticus* stocks (Taniguchi et al. 1985, Macaranas et al. 1986, De Silva and Ranasinghe 1989, Macaranas et al. 1995, De Silva 1997). Rognon et al. (1996) detected no gene introgression in cultured *O. niloticus* stocks from the Ivory Coast (Bouake strain) although observed heterozygosity levels were much lower than those observed in Fijian *O. niloticus* stocks.

Mean allozyme heterozygosity levels in the *O. niloticus* strains cultured in Fiji were greater than those reported for farmed *O. niloticus* stocks in the Philippines (Taniguchi et al. 1985, Macaranas et al. 1995) and wild *O. niloticus* populations in Africa (Agnese et al. 1997). It must be remembered however, that this estimate is greatly affected by the number of loci sampled. Founding populations of *O. niloticus* 'Chitralada' and 'Israel' used at the Naduruloulou hatchery in Fiji must have been characterized by relatively high levels of allelic variation. It appears that the levels of genetic diversity have been maintained successfully after many generations in culture.

Basiao and Taniguchi (1984) described genetic diversity (at allozyme loci) in cultured *O. niloticus* stocks in Japan. Observed heterozygosity level in their *O. niloticus* stock was 0.091, which is comparable with the levels observed in the Fijian *O. niloticus* 'Chitralada' stock. The 'Chitralada' stock in Fiji came from Thailand in 1988. The Thai stock in turn was founded from 50 individuals taken from a Japanese *O. niloticus* stock in 1965 (Basiao and Taniguchi 1984). On transfer to Thailand, the stock was isolated from other tilapias and kept in the palace pond in Bangkok (Basiao and Taniguchi 1984). The *O. niloticus* 'Chitralada' stock being cultured in Fiji came directly from this source in Thailand.

Results of RAPD marker analyses have indicated a more distant relationship between the *O. niloticus* and *O. mossambicus* stocks cultured in Fiji than was detected using allozyme markers. This result is at variance with studies by Bardakci and Skibinski (1994) and Dinesh et al. (1996), which suggested closer relationships between *O. niloticus* and *O. mossambicus* based on RAPD results. These differences can be attributed to the use of different sets of primers, visualization procedures and different origins for the respective *O. niloticus* and *O. mossambicus* stocks. The *O. mossambicus* individuals used in Bardakci and Skibinski (1994) study were from an aquarist stock while the *O. niloticus* stocks were African in origin. The *O. niloticus* and *O. mossambicus* individuals examined by Dinesh et al. (1996) were from stocks maintained at the National University of Singapore for about 15 years. Dinesh et al. (1996) suggested that the low levels of genetic variation detected in their tilapia strains were suggestive of inbreeding due to the relatively small effective population size of each stock.

The *O. mossambicus* stock displayed the lowest average levels of heterozygosity (0.080) among the tilapia stocks cultured in Fiji. This level was much higher however than that reported by McAndrew and Majumdar (1983) (0.002 - based on 16 allozyme loci) for 40 *O. mossambicus* individuals (Mozambique, aquarist stock). It is likely that the *O. mossambicus* stock in Fiji was established from a small founder population, which is typical of *O. mossambicus* stocks in Asia and Africa (McAndrew and Majumdar 1983, Mather and Arthington 1991) and has subsequently been pushed through a series of genetic bottlenecks before it was used in culture. However, the Fijian *O. mossambicus* stocks have retained significantly higher levels of genetic variation than many other cultured *O. mossambicus* stocks in the region.

Impacts of husbandry practices

In a relative sense, genetic variation in the stocks (excluding *O. mossambicus*) may have been higher at the time of their initial introductions to Naduruloulou. Macaranas et al. (1997) suggested that the decline of breeding performance relative to the time of introduction of *O. niloticus* 'Chitralada' and 'Israel' and the Red hybrid stocks in Fiji maybe attributed to past traditional management practices. Such practices, including the use of only a few breeders for fry generation, may have constricted the gene pools of broodstocks and decreased levels of genetic variability to those observed now. A rigorous evaluation of the impacts of previous management practices (or lack thereof) on gene pools of the original introductions of tilapia stocks in Fiji is however, not possible. The current genetic study has demonstrated however, that since the inception of modern management practices, levels of genetic diversity have been maintained successfully. This indicates that current stock management practices are sound and that genetic variation is not being lost due to unrepresentative sampling of each generation.

Conclusion

A platform for future improvement of tilapia stocks in Fiji has been established, based on relatively high levels of genetic variation in the stocks. The levels of genetic variation present in the stocks should be sufficient to allow a response to genetic improvement via selective breeding in the future. The success of this approach would depend on the extent of gene expression, genetic control of quantitative traits and the influence of culture environments. The genetic markers generated in this study provide a valuable resource for monitoring the levels of genetic variation and possible impacts that selective breeding may have on gene pools of the cultured stocks in the future. The impact of gene introgression from feral *O. mossambicus* stocks in the country can also be evaluated.

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