



UNIVERSITI PUTRA MALAYSIA

**APPLICATIONS OF DNA MICROSATELITE MARKERS
INTILAPIA CULTURE**

SUBHA BHASSU

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**APPLICATIONS OF DNA MICROSATELITE MARKERS
IN TILAPIA CULTURE**

By

SUBHA BHASSU

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in
Fulfilment of Requirement for the Degree of Doctor of Philosophy**

September 2002



To my parents (Amma and Papa).....

**Who believed that the most priceless gift one can give to their
children is a good education.**

To my dearest Rajeev....

Who believed I could achieve my goals.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**APPLICATIONS OF DNA MICROSATELLITE MARKERS
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September 2002

Chairman: Profesor Datin Dr. Khatijah Yusoff

Faculty : Science and Environmental Studies

In Malaysia, the role of aquaculture in fish production is anticipated to increase as marine fish catches have already exceeded the maximum sustainable yield. In order to address this issue, aquaculture is being developed on a commercial scale. Fish farming in Malaysia is focused on providing the fish grower with the best fingerlings, with uniformity and fast growth rates. There is great necessity for genetic evaluation, monitoring of stocks and application of appropriate breeding approaches if tilapia production is to continue to meet market demands. With the aid of microsatellite markers, stock integrity and genetic variability can be examined.

Three major experiments were carried out; a population genetic study on eight populations of tilapia, quantitative genetics study on two populations of *O. niloticus*, Taiwan A and B across two generations and heritability study on the Taiwan A population.

The primary focus of the population genetic study was to determine the relationships among eight populations which was used for breeding and to determine the viability of these populations. Microsatellite markers were used to determine the relationships among tilapia populations. The technique was optimized by varying parameters including the amount of template DNA, different thermal cyclers and others. The results showed that microsatellite markers are good markers for tilapia genetic studies.

For the population genetic study, the mean allele number and the mean heterozygosity level for the 40 loci were 43 and 0.5420 respectively. The F_{ST} value of 0.2401 among the populations suggested a moderate amount of genetic differentiation among the tilapia populations studied. Low heterozygosity levels suggest low effective population sizes, which may result in high levels of inbreeding. Most loci showed a deficiency in heterozygosity, which may be a sign of inbreeding. Most sampled populations showed significant deviations from Hardy-Weinberg equilibrium, which could result from mutation, migration or selection. This outcome could also be due to the small sample sizes examined and the high number of alleles present at individual loci.

Based on 11 loci, different genetic distance measures were applied to test the difference in the topology of the five populations examined. The topology and correlation values varied using two models, IAM and SMM. The genetic distances used in this study was selected to resolve relationships among the sampled populations as an

aid for breeding and not to look in detail at the underlying causes of differentiation, either due to genetic drift or mutation or both. The genetic distance values (Nei, 1978) among the populations ranged from 1.7 to 3.5. The phenogram showed that the Taiwan A and B populations grouped together with *O. mossambicus* as an isolated clade. *UNH 112* can be used to differentiate between *O. niloticus* and *O. mossambicus* populations as alleles for this locus can only be detected in the *O. niloticus* stocks.

In the quantitative genetic study, Taiwan A displayed the highest mean weight in both generations. Both populations in generation one showed significant correlations ($P < 0.05$) between growth and multi locus heterozygosity. The correlation value is 0.582 for Taiwan A and 0.415 for Taiwan B. Significant positive correlations were only detected in generation 1; could be due to the higher number of individuals sampled and the high levels of variation detected in generation 1 compared to generation 2.. The final study was focused on obtaining a heritability estimate for growth. However, the value obtained was negative, probably due to the low number of individuals and families used in this study.

The three experiments conducted on the Malaysian tilapia stocks will give breeders better insights and abilities to manage their tilapia breeding programs. Specifically, they can now see the value of maintaining stocks with high genetic variabilities under proper environmental conditions and the applications of good hatchery practices in the breeding systems that they use.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

APLIKASI TEKNIK MIKROSATELLITE DALAM KULTUR TILAPIA.

Oleh

SUBHA BHASSU

September 2002

Pengerusi: Profesor Datin Dr. Khatijah Yusoff

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Di Malaysia, peranan akuakultur dalam pengeluaran ikan dijangka akan meningkat memandangkan penangkapan ikan laut telah melebihi tahap optimum. Justeru, akuakultur diperkembangkan ketahap komersial. Perternakan ikan di Malaysia bertumpu kepada penghasilan anak ikan yang baik, keseragaman dan kadar pertumbuhan yang cepat. Oleh itu, penilaian genetik, pengawasan stok dan aplikasi pendekatan pembiakan yang sesuai diperlukan jika penghasilan ikan tilapia diperlukan untuk menampung permintaan pasaran. Dengan kehadiran penanda mikrosatelit, integriti stok dan kepelbagaian genetik boleh ditentukan.

Tiga eksperimen utama telah dijalankan iaitu; kajian populasi ke atas lapan populasi ikan tilapia, kajian genetik kualitatif yang melibatkan dua populasi ikan tilapia, Taiwan A dan Taiwan B (2 generasi) dan kajian heritabiliti terhadap populasi Taiwan A.

Fokus utama dalam kajian genetik populasi adalah untuk menentukan hubungan antara lapan populasi yang digunakan untuk pembiakan dan menentukan keviabelan populasi

ini. Penanda mikrosatelit digunakan dalam kajian ini untuk menentukan hubungan antara populasi tilapia. Teknik ini dioptimumkan dengan merubah parameter seperti jumlah templat DNA, kitaran haba yang berbeza dan sebagainya. Keputusan menunjukkan bahawa penanda mikrosatelit adalah penanda yang baik untuk kajian genetik tilapia.

Dalam kajian genetik populasi, nombor min alel, dan nombor min heterozigositi untuk 40 lokus adalah 43 dan 0.5420 masing-masing. Nilai F_{st} antara populasi adalah 0.2401. Ini menunjukkan perbezaan genetik yang sederhana dalam ikan tilapia yang dikaji. Tahap heteozigositi yang rendah pula menunjukkan keberkesanan saiz populasi yang rendah, ini yang mengakibatkan tahap pembiakan sebaka yang tinggi. Kebanyakan lokus menunjukkan heterozigositi yang rendah, ini wujudnya pembiakan sebaka. Kebanyakan populasi menunjukkan penyimpangan yang jelas daripada keseimbangan Hardy-Weinberg. Ini mungkin disebabkan oleh mutasi, migrasi atau pemilihan dan juga disebabkan oleh saiz sampel kajian yang rendah dan bilangan alel yang tinggi dalam lokus individu.

Berdasarkan 11 lokus, jarak genetik yang berlainan digunakan untuk menguji perbezaan topologi antara lima populasi yang dikaji. Nilai topologi dan korelasi berbeza mengikut dua model, IAM dan SMM. Jarak genetik yang digunakan dalam kajian telah dipilih untuk mengatasi masalah hubungan antara populasi yang disampel sebagai rujukan pembiakan dan bukan untuk mengkaji secara terperinci kesan pembezaan, yang diakibatkan sama ada daripada hanyutan genetik atau mutasi atau kedua-duanya.

Nilai jarak genetik (Nei, 1978) antara populasi menjulat daripada 1.7 ke 3.5. Fenogram menunjukkan bahawa populasi Taiwan A dan Taiwan B dikumpul bersama dengan *O. mossambicus* sebagai populasi terasing. UNH 112 boleh digunakan untuk membezakan antara populasi *O. niloticus* dan *O. Mossambicus* kerana alel untuk lokus ini hanya dapat dikesan dalam stok *O. niloticus*.

Dalam kajian genetik kualitatif, Taiwan A telah mempamerkan min berat yang paling tinggi dalam kedua-dua generasi. Kedua-dua populasi dalam generasi 1 telah menunjukkan korelasi yang signifikan ($P < 0.05$) antara pertumbuhan dan heterozigositi lokus majmuk. Nilai korelasi adalah 0.582 untuk Taiwan A dan 0.415 untuk Taiwan B. Korelasi positif yang signifikan hanya dapat dikesan dalam generasi 1. Ini mungkin disebabkan oleh bilangan individu yang disampel dan variasi yang tinggi dalam generasi 1 berbanding generasi 2. Kajian terakhir adalah untuk memperoleh anggaran heritabiliti bagi pertumbuhan. Walau bagaimanapun nilai yang diperolehi adalah negatif, ini mungkin disebabkan oleh bilangan individu dan famili yang digunakan adalah rendah dalam kajian ini.

Ketiga-tiga eksperimen yang dijalankan terhadap stok ikan tilapia Malaysia akan membantu perternak mengurus program pembiakan dengan lebih baik. Khususnya, mereka dapat melihat kepentingan nilai dalam mengekalkan stok yang mempunyai kepelbagaian genetik yang tinggi di bawah keadaan persekitaran serta penggunaan amalan penetasan yang baik bagi sistem pembiakan yang digunakan.

I certify that an Examination Committee met on 12th September 2002 to conduct the final examination of Subha Bhassu on her Doctor of Philosophy thesis entitled "Applications of DNA Microsatellite Markers in Tilapia Culture" in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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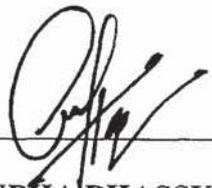
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vi
ACKNOWLEDEMENTS		ix
APPROVAL SHEET 1		x
APPROVAL SHEET 2		xi
DECLARATION FORM		xii
LIST OF TABLES		xvii
LIST OF FIGURES		xviii
LIST OF ABBREVIATIONS		xx
CHAPTER		
I	INTRODUCTION	1.1
II	LITERATURE REVIEW	2.1
	2.1 Tilapia in Culture	2.1
	2.1.1 Suitability of Tilapia as a Culture Organism	2.1
	2.2 Genetic Improvement of Cultured Tilapia	2.3
	2.3 Applications of Genetic Markers in Tilapia Stocks	2.4
	2.4 Microsatellites, their Characteristics and Distribution	2.8
	2.4.2 Nature of Polymorphism	2.9
	2.5 Current Applications of Microsatellites	2.11
	2.6 Stock Improvement	2.14
	2.6.1 Selective Breeding	2.17
	2.7 Microsatellites in Stock Improvement	2.18
	2.7.1 Applications of Microsatellites in Tilapia Breeding Programs	2.19
	2.7.2 Applications of Microsatellites in Universiti Of Malaya	2.24
III	MATERIALS AND METHODS	3.1
	3.1 Experimental Studies	3.1
	3.2 Study 1: Population Genetic Study	3.1
	3.2.1 Experimental Stocks	3.1
	3.2.2 Origin/ History and Morphology of the Eight Populations	3.2
	3.2.2.1 Chitralada and Israel Population (Red Tilapia)	3.3
	3.2.2.2 The Local Black and <i>O. mossambicus</i>	

	Populations	3.4
	3.2.2.3 Red Hybrid	3.4
	3.2.2.4 Taiwan A and B	3.5
	3.2.2.5 Philippines	3.8
3.2.3	Tissue Collection	3.8
3.2.4	DNA Isolation	3.8
3.2.5	Measurement of DNA Purity	3.9
3.2.6	Polymerase Chain Reaction (PCR) Amplification	
	3.2.6.1 Microsatellite Primers	3.9
	3.2.6.2 Optimization of PCR	3.10
3.2.7	Electrophoresis of PCR Products	3.11
3.2.8	Applied Voltage (2.5 V/cm)	3.11
3.3	Data Analysis for Population Genetic Study	3.11
	3.3.1 Band Scoring	3.11
	3.3.2 Statistical Analyses of Microsatellite Data Sets	3.12
	3.3.3 Testing for Hardy-Weinberg Equilibrium	3.13
	3.3.4 Heterozygosity	3.14
	3.3.5 Allele Frequencies	3.15
	3.3.6 Genetic Distance	3.15
	3.3.7 Mantel Test	3.18
	3.3.8 IAM based F Statistics	3.18
	3.3.9 SMM based F Statistics	3.21
	3.3.10 Estimating Migration Rates	3.22
	3.3.11 Construction of Phenograms	3.22
3.4	Study 2: Quantitative Genetic Study	3.22
	3.4.1 Sample Collections	3.22
	3.4.2 Experimental Populations	3.23
	3.4.3 Statistical Analysis of Quantitative Genetic Studies	3.23
	3.4.3.1 Weight and Length Relationship	3.24
	3.4.3.2 Microsatellite and MLH Relationship	3.24
	3.4.3.3 Heterozygosity Class Analysis	3.25
	3.4.3.4 Individual Loci Analysis	3.25
3.5	Study 3: Heritability Study	3.25
	3.5.1 Experimental Stocks	3.25
	3.5.2 Experimental Procedure	3.26
	3.5.3 Statistical Analysis	3.26
IV	RESULTS	4.1
	4.1 Study 1: Population Genetic Study	4.1
	4.1.1 The Test of Reproducibility and Reliability of Microsatellites	4.1
	4.1.2 Optimisation and the Effects of Different Reaction Parameters	4.1
	4.1.2.1 DNA Template Concentrations	4.1
	4.1.2.2 Expression Patterns of DNA from Various Tissues of an Individual	4.2
	4.1.2.3 Annealing Time and Temperature	4.2

4.1.2.4	The PCR machine and the Number of PCR cycles	4.4
4.1.2.5	Effects of Different MgCl ₂ Concentrations	4.4
4.1.2.6	Different Taq polymerases, Extension time and Pre-enturation time	4.4
4.1.2.7	Concentrations of dNTPs	4.8
4.1.3	Primer Screening	4.9
4.1.4	Detection and Scoring of Polymorphism	4.9
4.1.4.1	Comparison of different Gel System	4.9
4.1.5	Variability of Microsatellite loci Within and Among 8 populations	4.14
4.1.6	Population Structure	4.14
4.1.6.1	Allele Number, Effective Allele Number	4.14
4.1.6.2	Level of Genetic Variation Among Tilapia Populations	4.14
4.1.6.3	Diagnostic and Divergent Loci between <i>O.niloticus</i> and <i>O.mossambicus</i>	4.14
4.1.6.4	Population Differentiation	4.15
4.1.6.5	Neutrality	4.15
4.1.6.6	Migration	4.15
4.1.6.7	Degree of Heterozygosity	4.17
4.1.6.8	Hardy-Weinberg Equilibrium	4.18
4.1.7	Genetic Distance	4.21
4.1.7.1	Analysis of Different Genetic Distance Measures	4.21
4.1.7.2	Analysis of Genetic Measures based on Different Number of Loci	4.23
4.1.7.3	Construction of Phenogram based on Two Different Parameters	4.23
4.2	Study 2: Quantitative Genetic Study of Taiwan A and B	4.27
4.2.1	Population Genetic Study of Taiwan A and B Across Two Generations	4.27
4.2.1.1	Observed Allele Number and Effective Allele Number	4.27
4.2.1.2	Hardy-Weinberg Equilibrium	4.28
4.2.1.3	Degree of Heterozygosity	4.28
4.2.2	Growth pattern of the <i>Oreochromis niloticus</i> Strains, Taiwan A and B for Two Generations	4.29
4.2.3	Relationship between Shape of Head, Weight and Colour	4.32
4.2.4	Sex Differences	4.33
4.2.5	Relationships between Weight and Length	4.34
4.2.6	Microsatellite and MLH Relationship	4.34
4.2.7	Heterozygosity Class Analysis	4.37
4.2.8	Individual Loci Analysis	4.37
4.2.9	Weight Class Analysis	4.38

4.3	Study 3: Heritability Studies	4.40
V	DISCUSSION	5.1
5.1	Study 1: Population Genetic Study	5.1
5.1.1	The Test of Reproducibility and Reliability of Microsatellites	5.1
5.1.2	Genetic Variation revealed by Microsatellite Markers in the 8 populations of <i>O. niloticus</i> and <i>O. mossambicus</i> Stocks	5.4
5.1.3	Variability of Microsatellite Loci Within and Among Populations.	5.5
5.1.4	Characteristics of Individual Microsatellites	5.6
5.1.5	Degree of Heterozygosity	5.8
5.1.6	Hardy-Weinberg Equilibrium	5.11
5.1.7	Applications of Different Genetic Measures	5.12
5.1.8	Evaluation of Genetic Distance	5.15
5.1.9	Application of Genetic Variation to the Tilapia Stocks	5.17
5.2	Study 2: Quantitative Genetic Study for Taiwan A and B Populations	5.18
5.2.1	Comparisons of microsatellite Variability between Generations	5.19
5.2.2	Correlation Studies in Taiwan A and B Populations	5.17
5.3	Study 3: Heritability Studies	5.30
VI	CONCLUSIONS	6.1
	REFERENCES	R.1
	APPENDICES	A.1
	BIODATA OF THE THOR	B.1

LIST OF TABLES

Table	Page
2.1 Applications of microsatellites	2.15
3.1 Source of populations studied and sample sizes used	3.2
4.1 The summary of the population structure for the 8 populations based on the 40 loci.	4.15
4.2 Chi square analysis for the eight populations	4.18
4.3 Correlation value between five measures of genetic distance	4.20
4.4 The mean observed and effective number alleles for the two populations over two generations	4.26
4.5 The mean observed heterozygosities observed in Taiwan A and B over two generations	4.27
4.6 The mean weights and length measurements of the Taiwan A and B for two generations at different periods of time.	4.28
4.7 Relationship between shape of head, colour and weight	4.31
4.8 Sex ratios observed in each population at final harvesting	4.32
4.9 Mean weight of male and female fishes	4.33
4.10 Analysis of variance of effect of heterozygosity class on individual body weights of Taiwan A and B.	4.36
4.11 Relationship between individual weight and MLH scores by weight classes	4.39

LIST OF FIGURES

Figure		Page
2.1	Schematic Diagram of the Genome	2.7
2.2	Detection of Microsatellites	2.10
3.1	The photo of <i>O. niloticus</i> (Taiwan B)	3.5
3.2	The photo of <i>O. niloticus</i> (Taiwan A)	3.5
3.3	The photo of <i>O. mossambicus</i>	3.6
3.4	The photo of <i>O. niloticus</i> (Chitralada)	3.6
3.5	The photo of <i>O. niloticus</i> (Philippines)	3.6
3.6	The photo of <i>O. niloticus</i> (Hybrid)	3.7
3.7	The photo of <i>O. niloticus</i> (Local Black)	3.7
3.8	The photo of <i>O. niloticus</i> (Israel)	3.7
4.1	DNA template concentrations on the banding resolution	4.3
4.2	Microsatellite banding pattern generated by various tissues and different annealing times	4.3
4.3	The effect of different PCR machines and PCR cycles on the banding resolution	4.5
4.4	Microsatellite banding pattern generated by different MgCl ₂ concentrations	4.6
4.5	Types of Taq polymerase, extension time and predenaturation time on the banding resolution	4.7
4.6	Banding resolution resulted from different predenaturation time and extension time	4.7
4.7	The effects of dNTPs and MgCl ₂ on the banding resolution	4.8
4.8(a)	Banding pattern generated by primer UNH 155, tested on 10 individuals of Taiwan B	4.10
4.8 (b)	Schematic representation of the same figure of Figure 4.8(a)	4.10

4.9(a)	Banding pattern generated by primer UNH 155, tested on 10 individuals of Taiwan	4.11
4.10(a)	Banding patterns generated by primer UNH 155, tested on 10 individuals of Taiwan B using 6% polyacrylamide gel system	4.12
4.10(b)	Banding patterns generated by primer UNH 155, tested on 10 individuals of Taiwan B using Nusieve gel system	4.12
4.11	Phenogram on five different genetic measures, clustered by UPGMA	4.21
4.12(a)	The UPGMA phenogram based on 40 loci	4.23
4.12(b)	The UPGMA phenogram based on 29 loci	4.24
4.12(c)	The UPGMA phenogram based on 11 loci	4.23
4.13	The NJ phenogram based on 29 loci	4.25
4.14	The weight and length for Taiwan A and B populations across two generations	4.29
4.15	Mean weight measurements across two generations for two populations of <i>O.niloticus</i>	4.30
4.16	Mean length measurements of Taiwan A and B for two generations	4.31
4.17	The relationship between weight(g) and standard length (cm) in the stocks for both generations	4.32
4.18	Correlation between weight of tilapia stocks and heterozygosity (combined male and female)	4.35
4.19	Scatter plot on the relationships of heterozygosity (pooled loci) and body weights; and weight class and heterozygosity for Taiwan A and Taiwan B in generation 1	4.38

LIST OF ABBREVIATIONS*Chemicals*

EDTA	Ethylenediamine tetraacetate
KCL	Potassium chloride
MgCl ₂	Magnesium chloride
NACl	Sodium chloride
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA buffer
Tris	Trisma base
TA	Taiwan A
TB	Taiwan B

Units

Bp	base pair
° C	Degree of celcius
h	hour
kb	kilobase
min	minute
mM	millimolar
ng	nanogram
nmole	nanomole
OD	optical density
pmole	picomole
s	second

CHAPTER 1

INTRODUCTION

Aquaculture is currently being viewed as a source of relief for human population that face protein shortage in their daily lives. This situation has arisen because many countries have limited resources of marine fishes due to excessive fishing. It is clear that the demand for food resources have grown over the years and, therefore, a balance must be struck to ensure that mankind can produce the required amount of protein to an optimal level while preserving environmental quality and integrity. Asia accounts for 80% of the world's aquaculture production (FAO, 1997), giving an impression that fish farming is only of real benefit to developing countries. This could be due to the lack of understanding of the potential benefits provided by aquaculture (Pillay, 1994).

Over the last few decades, aquaculture has made remarkable growth in the economic sector. For example, the most recent estimate of the global value of tilapia is US \$3 billion, a figure which has almost trippled since 1984. According to FAO data, the annual world production of tilapia for 1989 was 366,000 tonnes and this had increased to 473, 477 tonnes in 1992 and 675,000 tonnes in 2000 (FAO, 2000). Demand for tilapia in the US is expected to increase to 400,000 tonnes with an estimated value of US \$2 billion by the year of 2005. The most important species of tilapia in terms of



production by weight is *Oreochromis niloticus* (64%), followed by *O. mossambicus* (10%) and *O. aureus* (3.6%). The primary objective for aquaculture in any developing country is to provide an alternative source of protein in the diet of an increasing population (Pullin, 1993). This is different to the objectives in developed countries where the focus has been on output of high quality food products that meet certain dietary requirements (Barnabe, 1994).

In Malaysia, the role of aquaculture in fish production is anticipated to increase significantly as marine fish catches have already exceeded their maximum sustainable yield. Aquaculture is being developed on a commercial scale as a sustainable industry under the aquaculture action development plan by the Department of Fisheries which aims to meet the requirements of dietary protein by the year 2010 (NAP, 1992). Fish farming in Malaysia has focused on providing tilapia growers with the best fingerlings, of uniform quality and showing fast growth rates. This would enable farmers to market all the fish over a short period of time and then restock the ponds or tanks to grow the next batch of fish, thus maximizing the total weight of tilapia that can be grown per year with the limited space available. This requires low levels of management time, handling and pond construction cost. Faster growth rates will shorten the time between the farmer's investment and the time when the stocks begin to return income from the marketable fish. Good feed conversion of available feed resources is another major goal because feed is generally the highest single cost in culture.

Fish farming in Malaysia has focused on high selection intensities in order to provide the farmers with economic gains. In order to achieve this goals, breeding programs should aim to produce high survival of fries to fingerling and to market size, lower breeding cost, improved feed conversion ratio and predictability of growth for feed management and marketing. The fish also should have an appearance and presentation suitable for the intended market; that includes good color and shape, which in some markets make the difference between high or low price. They should have good tolerance to a range of water conditions, allowing the farmer to use the available water sources without spending extra cost on water treatment, and ease of breeding to ensure a reliable supply of fingerlings.

Recently, however, the tilapia industry in Malaysia has seen a reduction in growth rates, a familiar scenario to that is seen in the tilapia industry worldwide. Cultured stocks also suffer from widespread poor and variable fish performance and in some cases due to the presence of gene introgression from poor performing stocks (Pante and Macaranas, 1985). The relatively high fecundity of tilapia allows breeders to apply high selection intensity and to derive their grow-out stock from a small number of parents. This means a small number of individuals contribute to the genetic make-up of the subsequent generation. If done continuously, this may eventually lead to fixation of alleles, which could express advantageous traits, but also unfavourable traits causing inbreeding depression. Loss of genetic variability will also be evident (Silliman, 1975, Harvey, 1986,). High reproduction, on the other hand, leads to overcrowding resulting in large quantities of small sized fish (Herpher and Pruginin, 1982). In addition,