Biochemical and Morphometric Approaches to Characterize Farmed Tilapias

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The first introduction of tilapias to the Philippines in the form of *Oreochromis mossambicus* failed to start a commercially successful culture industry there because this species was unattractive to consumers and its early reproduction caused overcrowding in fishponds. Tilapia culture became popular only after the introduction of Nile tilapia (*Oreochromis niloticus*) in the 1970s. But, the tilapia industry in the Philippines, as elsewhere in Asia, suffers from poor or variable fish growth performance.

Collaborative research efforts between the Marine Science Institute of the University of the Philippines (UPMSI) and ICLARM, initiated in early 1980s, have focused on genetic characterization of farmed tilapias, broodstock management practices, and their implications for the future of the Philippine tilapia industry. These studies revealed the poor genetic status of farmed strains: narrow genetic base (descendants of a few introductions of small numbers of fish, mostly through intermediate countries); poor broodstock management resulting in inbreeding; and widespread introgression of genes from undesirable feral *O. mossambicus*. The general conclusion was that any genetic improvement efforts using the existing tilapia genetic resources would start at a disadvantage and may not bear fruit.

A new base population with a wider genetic base is being established through the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT) by combining germplasm recently brought from Africa with the farmed strains in the Philippines (see genesis of the GIFT project, p. 3).

Biochemical (electrophoresis and mitochondrial DNA) and morphological analysis are important tools for characterization of strains.

Electrophoresis: Basic Concepts

Electrophoresis is the most useful technique for studying the genetic composition of individuals and populations at the level of individual genes. A gene is a specific length of DNA (deoxyribonucleic acid) occupying a position on the chromosome called a locus. One of several alternate forms of a gene constitutes an allele. Diploid organisms carry two alleles of each gene, one from each of the parents. Individuals having different alleles at one or more given loci are heterozygotes, while those with identical alleles are homozygotes. Alleles are distinguished by their protein products (usually enzymes from various tissues) in an electrical field. Different forms of the same enzyme, distinguishable by their mobility (or bands)in a starch gel medium (zymogram), are called isozymes. A locus is monomorphic if only one form of allele is known (one band on the zymogram) and polymorphic if two or more alleles (two or more bands) are found. Allelic isozymes are called allozymes. Isozyme analysis thus provides a tool for precise identification of the genotype of individuals for a given locus. Proteins can also be made to separate according to their isoelectric points through a procedure called isoelectrofocusing.

Characterization at the population or strain level essentially involves determination of the frequency of occurrence of each allele at a number of different loci. Several important estimates are used to detect relative levels of genetic variability and relationships among populations: percentage of loci that are polymorphic (a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95), average number of alleles per locus, frequency of heterozygotes (heterozygosity) and genetic distance. Genetic distance is measured in terms of the gene frequencies averaged over all loci across different populations. It is based on the concept that two populations which have diverged through time have accumulated a number of substitutions per locus. The

more distinct the differences between populations the greater the genetic distance between them. The genetic distances between populations become clear when they are layed out as a 'dendrogram' constructed by certain routine statistical procedures. These measures provide valuable insights into evolutionary processes, including mixing (introgression), genetic drift (random fluctuations in allele frequencies, particularly in small populations) mutation, migration and selection.

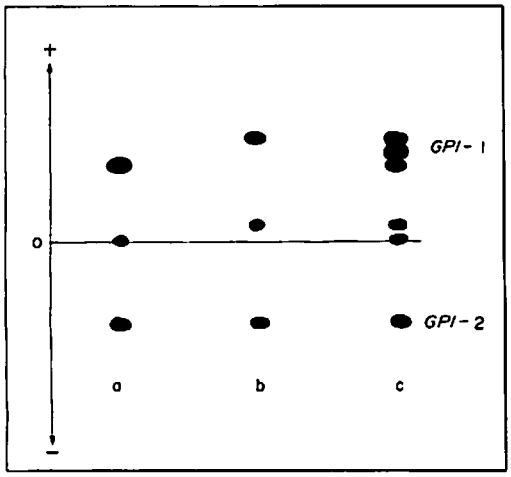

Fig. 1. ***Allozyme expression of GPI in* Oreochromis niloticus *(a),* O. mossambicus *(b) and in introgressed hybrids between both species (c). GPI-2 has a common allele for both species while GPI-1 displays a faster moving allele in* O. mossambicus *compared to* O. niloticus. *Introgressed hybrids are three-banded at GPI-1.***

Evidence for Introgression

Electrophoretic studies on Philippine tilapias, initiated by our group in 1983, provided evidence for widespread mixing (introgression) of less desirable feral *O. mossambicus* populations with farmed Nile tilapia stocks. Of the 20 loci examined, six were found to be diagnostic markers of introgression. A typical zymogram of an isozyme – glucose phosphate isomerase (GPI) is shown in Fig. 1. GPI in tilapia is controlled by two loci: GPI-1 and GPI-2. Locus GPI-2 has the same allele for both *O. niloticus* and *O. mossambicus*, but the other locus (GPI-1) has different alleles in these species. Introgressed hybrid populations exhibit both alleles.

Extensive studies encompassing Nile tilapia populations from commercial farms, experimental stations, and government hatcheries in Luzon, Visayas and Mindanao indicated well-established introgression with *O. mossambicus*. In some populations, the degree of introgression was very high, that is they had very small genetic distance from *O. mossambicus*. It also became apparent that the genetic variability observed in Nile tilapia populations was caused primarily by introgression of *O. mossambicus*.

Characterization of Strains

We analyzed progeny from the eight available *O. niloticus* strains, four African and four Philippine (see p. 3), at 30 loci. All strains shared alleles at 14 monomorphic and 16 polymorphic loci. A dendrogram constructed from genetic distance values (Fig. 2) reflects the close identity of the Philippine strains with those from Egypt and Ghana – confirming that the origin of Philippine strains is Egypt and Ghana. The wider separation of Kenya strain supports its recognition by Dr. E. Trewavas as a different subspecies (*O. niloticus vulcani*) from all others tested (*O. niloticus niloticus*).

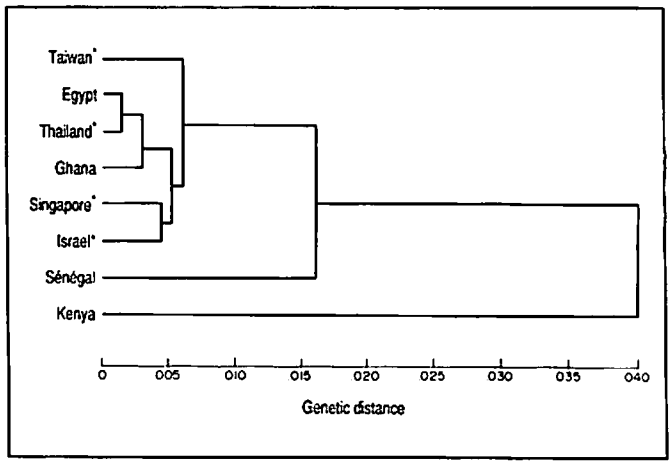

Fig. 2. ***Dendrogram constructed from genetic distance values shows three separable groups amongst eight tested strains of* O. niloticus *- a cluster of Philippine farmed strains (marked with asterisks; showing countries of origin of stocks used in the Philippines) with the Egypt and Ghana strains, Sénégal strain and the Kenya strain.***

Mitochondrial DNA (mtDNA)

Although protein electrophoresis has been a successful technique for describing

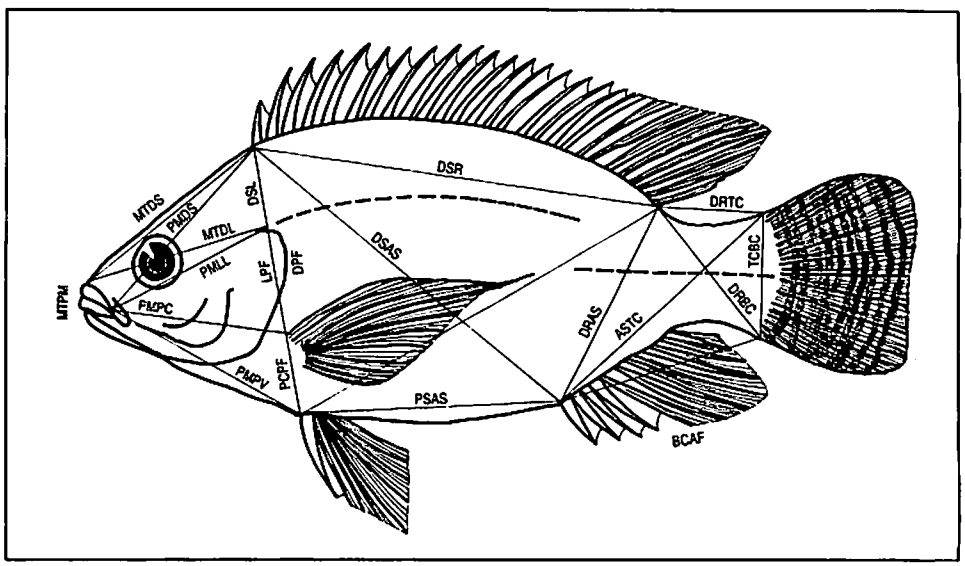

Fig. 3. ***Truss network of 21 landmark points on the body outline measured during morphometric characterization of the eight tilapia strains.***

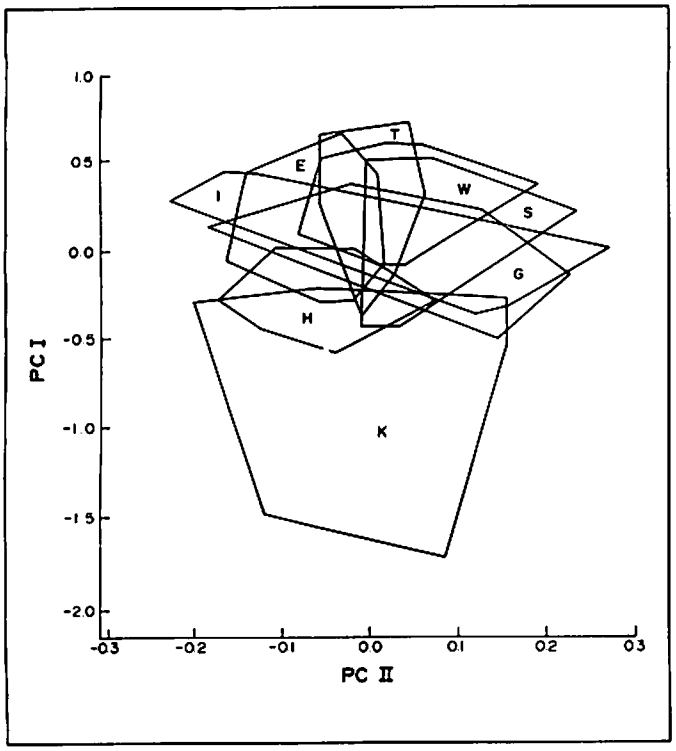

Fig. 4. *Plot of Principal Component I (PC I) and residuals of Principal Component II (PC II) of 21 morphometric characters of male and female* O. niloticus. *K-Kenya; H-Ghana; E-Egypt; S-Sénégal; I-Israel; G-Singapore; W-Taiwan; T-Thailand.*

the genetic structure of populations, it cannot detect all the genetic variation that may be of value. Its application is restricted to the part of the genome that codes for soluble enzymes. It resolves only the existing genetic variability and in many instances has failed to identify genetically discrete stocks.

Working at the DNA level (rather than the products of the genes) can tremendously increase our understanding of genetic variation and get a magnified view of the genetic distances between populations. About 99% of the DNA resides within the nucleus. Of the remaining 1%, it is the mtDNA that is of importance in quantifying genetic differences. An important property of mtDNA is its mode of inheritance which is strictly maternal. This property makes mtDNA a valuable tool for tracing female lineages within and among populations. The principal tools in mtDNA analysis are the restriction endonucleases – enzymes which break up the mtDNA into fragments of different lengths. These fragments are separated by electrophoresis and the patterns compared.

For the past 18 months, we have concentrated on standardizing procedures for extracting mtDNA. A technique devised by Chapman and Powers which makes use of phenol to extract protein from isolated mitochondria and ethanol to precipitate the mtDNA was found most suitable: mtDNA precipitates were obtained from fresh gonad and liver samples. Work is in progress using an array of restriction enzymes to induce fragmentation.

Morphometric Analysis: Basic Concepts

Morphometric characterization of strains of the same species often involves detection of subtle differences in variations of shape, independent of size. The truss network method (truss morphometrics) is a powerful technique to do this. The biases of traditional measurements (standard and total length, total height, etc.), namely, dense measurements in some areas of the body and a paucity elsewhere, is overcome in truss morphometrics by measuring distances between homologous (or landmark) points along the body (Fig. 3). The measurements, after appropriate data manipulation, are subjected to multivariate statistical analysis: discriminant analysis or principal component analysis (PCA). Discriminant analysis categorizes individuals based on *a priori* recognition of groups, e.g., sex or species. PCA, on the other hand, does not require *a priori* recognition of groups, and if there are several groups, data are pooled irrespective of groups. PCA constructs principal components (PC) which are linear combinations of the variables that describe the shape variations in the pooled sample.

Characterization of Tilapia Strains

In the GIFT project (see p. 3), a truss network of 21 landmark points on the body outline was used, aided by a digitizing tablet linked to a microcomputer. A computer program 'Computer Aided Monoscopic Analysis' (CAMA) was developed to calculate coordinates for these landmark points. PCA was performed using the statistical package SAS (Fig. 4). The Kenya strain, which has a relatively shorter and more streamlined mid-body region, separated out from the rest of the strains. Overall, however, the results indicate very little morphological differences among these eight strains.

Future Activities

Genetic characterization work will continue through the GIFT project. The emphasis in future will be on conservation and gene banking of potential genetic resources. DNA fingerprinting is also being contemplated because of its potential usefulness, for example in estimating inbreeding rates, in pedigree analysis and in detecting divergence of selected populations from the founder populations.

Further Reading

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