Aquaculture Genome Technologies
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Chapter 7
Allozyme and Mitochondrial DNA Markers

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In the first five chapters of this section, various marker systems were discussed including restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites, and single nucleotide polymorphisms (SNP) that are all important to aquaculture genome research. Here we present two additional types of markers—the allozyme markers and the mitochondrial DNA markers. In spite of the very limited uses of these two types of markers for the purpose of genome research, they have been among the most popular markers in aquaculture and fishery research in the past.

Allozyme Markers

Genetic diversity measurements in aquaculture stocks are an essential part of an effective management strategy. Historically, these measurements relied on phenotypic or qualitative markers that were used in classical genetics. Morphological differences such as body dimensions, size, and pigmentation are some examples of phenotypic markers. Genetic diversity measurements based on phenotypic markers are often indirect, and inferential through controlled breeding and performance studies (Okumus and Ciftci 2003, Parker et al. 1998). Because these markers are polygenically inherited and have low heritabilities, they may not represent the true genetic differences (Smith and Chesser 1981). Only when the genetic basis for these phenotypic markers is known, can some of them be used to measure genetic diversity. Molecular markers were developed to overcome problems associated with phenotypic markers.

Many types of molecular markers are currently available to use in both aquaculture and population genetics. These markers can be categorized in two types: protein and DNA markers (Okumus and Ciftci 2003). In terms of variation source, on the other hand, they are classified into three groups: allozyme markers, mitochondrial DNA markers, and nuclear DNA markers. Allozymes are protein products of genes that are encoded by a single gene locus. Since they represent genes of known function, they are considered to be Type I markers (Liu and Cordes 2004).

The term "isozyme" refers to multiple biochemical forms of an enzyme having identical substrate specificity (or the same catalytic activities) within the same organism. "Allozymes" or "allelic isozymes" are the different allelic forms of the same enzymes encoded at the same locus (Hunter and Market 1957, Parker et al. 1998, May 2003). Strictly speaking, allozymes represent different allelic forms of the same gene and isozymes represent different genes whose products catalyze the same reaction. However, the two terms are usually used interchangeably. The variation detected in allozymes may be the result of point mutations, insertions, or deletions (indels). It is

believed that isozymes usually form as a result of gene duplication; however, there may be other events (hybridization, polyploidization) that lead to the formation of isozymes.

The most common use of allozyme electrophoresis is to detect genetic variation in natural populations. In the last 30 years, large amounts of allelic frequency data were collected from many fish species for management purposes. Although use of allozyme data in aquaculture appears to be limited compared to population studies in fisheries, the aquaculture industry has long used this information for its development because aquaculture and fisheries can not be separated from each other (Dunham 2004). Allozyme electrophoresis in aquaculture is used for stock identification, parentage analysis, inbreeding analysis, and limited genetic mapping (Liu and Cordes 2004).

Some Considerations Related to Allozyme Analysis

In most cases, sampling for allozyme analysis is lethal. Most commonly used tissues in allozyme electrophoresis for analyzing the full range loci are muscle, liver, eye, and heart samples that are freshly obtained from individual fish. Since enzymes are heat labile, samples are either immediately processed and analyzed or properly frozen. Sample preparation requires each tissue to be mechanically ground in a buffer, but a sonicator can also be used for this purpose. However, heat generated during the sonication process often yields poor resolution due to enzyme degradation. Because many enzyme loci are used to detect genetic variability in fisheries and aquaculture, we will not provide detailed descriptions of the allozyme assays, but rather provide a comprehensive list of the most frequently used allozymes (Table 7.1) with linkage to Internet sources containing detailed descriptions and references.

Commonly used support media in allozyme electrophoresis are starch, cellulose acetate, acrylamide, and agarose. With the consideration for cost, resolving power, and electrophoresis time, starch gels are the most often used medium of support.

There is no single buffer system that will give a desirable resolving power with starch electrophoresis. Every enzyme has its own optimal buffer systems. Interested readers are referred to three published books on isozymes (Pasteur et al. 1987, Morizot and Schmidt 1990, Richardson et al. 1986). These books are excellent sources of information related to isozyme analysis.

Genotyping of allozyme gels can be complicated at times. Individual genotypes at each locus are inferred from the banding patterns observed on the gels. Allele nomenclature and the locus identification in allozyme electrophoresis used in aquaculture are based on the relative mobility of the proteins (Shaklee et al. 1990). Before using electrophoretic data for genetic variation analysis, breeding data must be used to verify observed variation (Wolf et al. 1970). The variation detected by electrophoretic data may not be limited to true genetic variation. In some exceptional cases, patterns on the gels do not always fit the simple Mendelian inheritance. One of the complications is the presence of null alleles (Stoneking et al. 1981). The second one is the sample artifact due to improper storage and processing. Some enzyme systems may give different banding patterns due to pathological or environmental differences. The pattern detected after gel staining depends on the quaternary structure of the enzyme. Diploid organisms have two copies of each gene—one maternal, the other paternal. However, in some cases there may be multiple copies of the same enzyme

Table 7.1. List of enzymes or other protein loci used in fish genetic research. The most commonly used enzymes were indicated with gray shading. The list was compiled from Shaklee et al. 1990 and BRENDA enzyme database (http://brenda.bc.uni-koeln.de/) (Schomburg et al. 2004). *Gene functions are described in the Gene Ontology Web page (http://www.geneontology.org/). By using either the E. C. number or the enzyme name, one can obtain more information including related literature.

Enzyme or protein name	E. C. number	Abbreviation	Gene ontology number*
beta-N-acetylgalactosaminidase	3.2.1.53	bGALA	-
beta-N-acetylhexosaminidase	3.2.1.30	bGLUA	16231
Acid phosphatase	3.1.3.2	ACP	3993
Aconitate hydratase	4.2.1.3	AH	3994
Adenine phosphoribosyltransferase	2.4.2.7	APRT	3999
Adenosine deaminase	3.5.4.4	ADA	46936
Adenosine kinase	2.7.1.20	ADK	4001
Adenylate kinase	2.7.4.3	AK	4017
Alanine transaminase	2.6.1.2	ALAT	4021
Alcohol dehydrogenase	1.1.1.1	ADH	4025
Alkaline phosphatase	3.1.3.1	ALP	4035
alpha-Amylase	3.2.1.1	aAMY	4556
alpha-N-arabinofuranosidase	3.2.1.55	aARAF	46556
Aspartate aminotransferase	2.6.1.1	AAT	4069
Carbonate dehydratase	4.2.1.1	CAH	4089
Carboxylesterase	3.1.1.1	ESTD	16789
Catalase	1.11.1.6	CAT	4096
Creatine kinase	2.7.3.2	CK	4111
6,7-dihydropteridine reductase	1.5.1.34	DHPR	4155
Dipeptidase	3.4. -.-	PEPA	
Esterase	3.1.1. -	EST	-
Fructose bisphosphatase	3.1.3.11	FBP	42132
Fructose-bisphosphate aldolase	4.1.2.13	FBALD	4332
alpha-L-Fucosidase	3.2.1.51	aFUC	4560
Fumarate hydratase	4.2.1.2	FH	4333
Galactokinase	2.7.1.6	GALK	4335
alpha-Galactosidase	3.2.1.22	aGAL	4557
beta-Galactosidase	3.2.1.23	bGAL	9341
General (unidentified) protein	-	PROT	-
Glucose 1-dehydrogenase	1.1.1.47	GDH	47936
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	G6PDH	4345
Glucose-6-phosphate isomerase	5.3.1.9	GPI	4347
alpha-Glucosidase	3.2.1.20	aGLU	4558
beta-Glucosidase	3.2.1.21	bGLU	8442
beta-Glucuronidase	3.2.1.31	GUS	4566
Glutamate dehydrogenase	1.4.1.-	GLUDH	-
Glutamate-ammonia ligase	6.3.1.2	GLAL	4356
Glutathione-disulfide reductase	1.8.1.7	GR	4362
Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	1.2.1.12	GAPDH	8943
Glycerate dehydrogenase	1.1.1.29	GLYDH	8465

(*Continued*)

Table 7.1. (*Continued*)

Enzyme or protein name	E. C. number	Abbreviation	Gene ontology number*
Glycerol-3-phosphate dehydrogenase (NAD+)	1.1.1.8	G3PDH	4367
Guanine deaminase	3.5.4.3	GDA	8892
Guanylate kinase	2.7.4.8	bGUK	4385
Hekokinase	2.7.1.1	HK	4396
Hemoglobin	-	HB	-
Hydroxyacylglutathione hydrolase	3.1.2.6	HAGH	4416
3-Hydroxybutyrate dehydrogenase	1.1.1.30	HBDH	3858
Hypoxanthine phosphoribosyltransferase	2.4.2.8	HPRT	4422
Inorganic pyrophosphatase	3.6.1.1	PP	4427
Lactoylglutathione lyase	4.4.1.5	aLGL	4462
L-iditol dehydrogenase	1.1.1.14	sIDDH	3939
L-Lactate dehydrogenase	1.1.1.27	LDH	4459
lsocitrate dehydrogenase (NADP+)	1.1.1.42	IDHP	4450
Malate dehydrogenase	1.1.1.37	MDH	30060
Malate dehydrogenase (decarboxylating)	1.1.1.39	ME	4471
Malate dehydrogenase (oxaloacetate-decarboxylating)	1.1.1.38	ME	16619
Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)	1.1.1.40	MEP	4473
Mannose-6-phosphate isomerase	5.3.1.8	MPI	4476
alpha-Mannosidase	3.2.1.24	sMAN	4559
Nucleoside-phosphate kinase	2.7.4.4	NPK (UMPK)	50145
Nucleoside-triphosphate diphosphatase	3.6.1.19	NTP	47429
Octanol dehydrogenase	1.1.1.73	ODH	4552
Ornithine carbamoyltransferase	2.1.3.3	OTC	9348
Parvalbumin	-	PVALB	-
Peptidase-C	3.4. -. -	PEPC	
Peptidase-S	3.4. -. -	PEPS	
6-phosphofructokinase	2.7.1.11	PFK	3872
Phosphoglucomutase	5.4.2.2	PGM	4614
Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	6PGDH	4616
Phosphoglycerate kinase	2.7.2.3	PGK	4618
Phosphoglycerate mutase	5.4.2.1	PGAM	46538
Phosphoglycolate phosphatase	3.1.3.18	PGP	8967
Phosphopyruvate hydratase	4.2.1.11	ENO	4634
Proline dipeptidase	3.4.13.9	PEPD	4251
Purine-nucleoside phosphorylase	2.4.2.1	PNP	4731
Pyruvate kinase	2.7.1.40	PK	4743
Superoxide dismutase	1.15.1.1	SOD	16954
Thymidine kinase	2.7.1.21	TK	4797
Transferrin	-	TF	-
Triose-phosphate isomerase	5.3.1.1	TPI	4807
Tripeptide aminopeptidase	3.4.11.4	PEPB	45148
Tyrosine transaminase	2.6.1.5	TAT	4838
UDP-glucose-hexose-1-phosphate uridylyltransferase	2.7.7.12	UGHUT	8108

gene due to genome or gene duplication (especially in fish). An active allozyme may have more than one subunit, and both allelic forms may result in polymeric bands. These pose a great challenge in gel scoring. Assuming the subunits of enzymes detected combine in a random fashion (Utter et al. 1974), the simplest allozyme pattern with a single polypeptide chain (monomer) yields three possible genotypes: AA, AB, and BB. However, interpretation becomes much more complex when multimeric enzymes composed of two or more subunits are involved (Figure 7.1). Glucose-6-phosphate isomerase (GPI) enzyme in brook trout *Salvelinus fontinales* is a good example of tetraploidization and the confusing nature of gel scoring. Although GPI is a dimeric enzyme, the banding pattern in gel presented in Figure 7.2 does not fit the

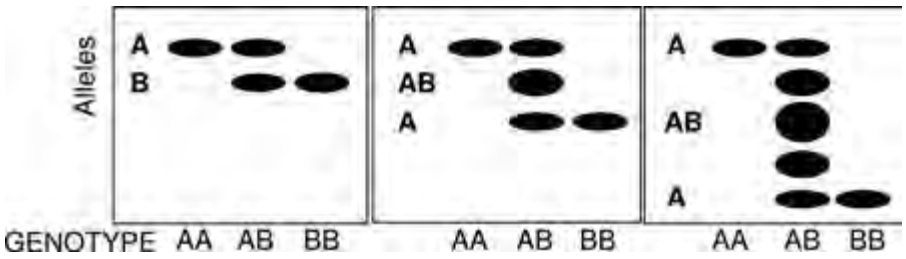

Figure 7.1. Allozyme patterns commonly observed in fishes showing a co-dominant fashion of diallelic forms. In a monomeric case, a homozygous sample with allele A should produce one band (genotype AA); similarly, a homozygous sample with allele B also produces one band (genotype BB); however, the heterozygous sample should produce two bands (genotype AB). In a dimeric case, the homozygous samples produce only one band (AA or BB), but a heterozygous sample produces three bands (AA homodimer, BB homodimer, and AB heterodimer). In a tetrameric case, the situation is more complex: homozygous samples produce only one band, AA or BB; heterozygous samples produce five bands with various intensities depending on their proportion in the sample: A4 homotetramer, A3B heterotetramer, A2B2 heterotetramer, AB3 heterotetramer, and B4 homotetramer.

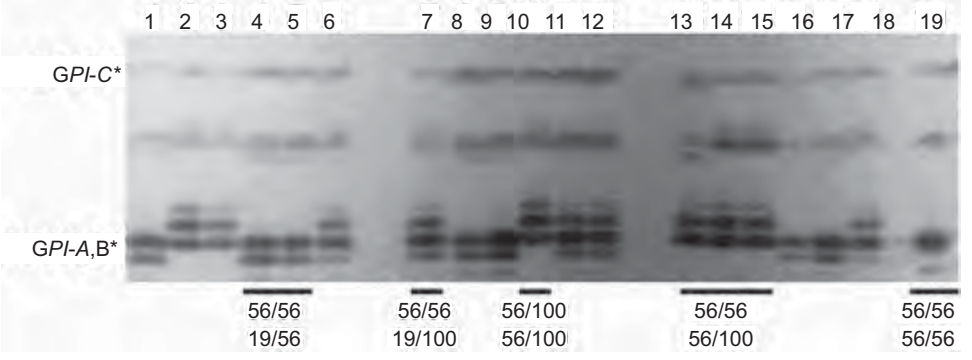

Figure 7.2. Glucose-6-phosphate isomerase (GPI) enzyme in brook trout *Salvelinus fontinales.* Sample #19 is homozygous at both loci, #13, #14, and #15 are homozygous at one and heterozygous at the other loci, and #10 is heterozygous at both loci. Note the hybrid band between duplicated loci and GPI-C*.

simple three banding pattern for a dimeric enzyme. On the other hand, a careful examination of this gel shows the duplicated nature of the enzyme. Therefore, great caution should be exercised in scoring allozyme gels.

The frequency of alleles can be calculated from the allozyme banding patterns. Allele and genotype frequencies and the relevant descriptive statistics are calculated using a variety of computer programs. BIOSYS (Version 1.7) (Swofford and Selander 1981) is the most commonly used program to analyze allozymes data in fish. The program can be used for allele frequency and genetic variability computations to test for Hardy-Weinberg equilibrium to determine substructuring, for linkage disequilibrium, calculations for F-statistics (Weir and Cockerham 1984), similarity and distance analysis (Nei 1978), and for construction of phenograms using cluster analysis (Rogers 1972). There are many other programs available to use in analyzing allozymes data. An extensive list of programs that are used for population genetics can be found at http://evolution.genetics.washington.edu/phylip/software.html.

Applications and Limitations of Allozyme Markers

Allozymes have wide applications in fisheries and aquaculture including population analysis, mixed stock analysis, and hybrid identification. In spite of limited numbers of loci, compared to other genetic tools, allozyme analysis had the most profound effect on fisheries science (May 2003). For example, inbreeding is the major concern for the aquaculture industry. Using genetic variation measured by the allozyme electrophoresis, relative degrees of inbreeding can be estimated by average heterozygosity comparisons in different broodstocks (Allendorf and Phelps 1981, Liu and Cordes 2004). Although allozyme studies did not find common application in marker assisted selection, correlations between certain allozyme markers and performance traits has been reported (Hallerman et al. 1986). Similarly, due to the limited number of polymorphic loci available, use of these markers in linkage mapping in fish is limited (Pasdar et al. 1984, May and Johnson 1993, Morizot et al. 1994). Allozyme data are also used in hybrid systems. First generation hybrids (F1) and later generation hybrids can be identified with a certain degree of probability if there are enough markers available (Avise and Van den Avyle 1984). One of the most common applications of allozyme data has been the use of mixed stock analysis, especially in salmonids (May 2003, Koljonen and Wilmot 2005). The effects of hatchery reared stocks of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) with the wild populations from which they were originated was studied by comparing the allozyme variation by Ferguson and others (1991). Use of allozyme markers in aquaculture also includes the genetic monitoring at the hatchery populations by establishing a monitoring program (Pérez et al. 2001).

The major drawback of allozyme analysis is the necessity for a large amount of fresh or frozen tissue samples. This often requires lethal sampling especially if the full array of allozyme markers are to be studied. Furthermore, although allozymes represent actual gene products, they often measure a very small portion of the genomic variation because a limited number of loci are involved (Utter et al. 1987). Mutation at the DNA level that causes a replacement of a similarly charged amino acid may not be detected by allozyme electrophoresis. Although very cheap and technically easy, the number of allozyme loci and the polymorphism is low (Agnese et al. 1997). These drawbacks will seriously limit the applications of allozymes for genome studies.

Mitochondrial DNA Markers

Mitochondria are the energy factory of living cells. Unlike the rest of the cellular functions that are determined by the nuclear DNA, these organelles have their own double-stranded circular DNA, known as mitochondrial genome (mtDNA). The size of the mitochondrial genome in animals varies among different organisms, but typically the number is around 16,000–19,000 base pairs (bp) in teleost fish (Table 7.2). Animal mitochondrial genome encodes 13 proteins, 2 ribosomal RNAs, and 22 transfer RNAs (Boore 1999). These genes are highly packed on the mitochondrial genome without introns (Burger et al. 2003). In addition to these genes, a highly variable control region, known as the D-loop (displacement loop) serves as the heavy-strand replication origin (Harrison 1989, Parker et al. 1998).

Table 7.2. List of selected aquaculture species whose mtDNA is sequenced, mtDNA size, and GenBank accession numbers.

Fish name	Size bp	Accession #	Reference
Anguilla japonica (Japanese eel)	16,685	NC_002707	Inoue et al. 2001
Conger myriaster (conger eel)	18,705	NC_002761	Inoue et al. 2001
Ictalurus punctatus (channel catfish)	16,497	NC_003489	Waldbieser et al. 2003
Cyprinus carpio (common carp)	16,575	NC_001606	Chang et al. 1994
Carassius auratus (goldfish)	16,579	NC_002079	Murakami et al. 1998
Danio rerio (zebrafish)	16,596	NC_002333	Broughton et al. 2001
Salmo salar (Atlantic salmon)	16,665	NC_001960	Hurst et al. unpublished
Oncorhynchus mykiss (rainbow trout)	16,642	NC_001717	Zardoya et al. 1995
Gadus morhua (Atlantic cod)	>10,000	Not available	Johansen et al. 1990
Oncorhynchus tshawytscha (chinook salmon)	16,644	NC_002980	Bernales et al. unpubished
Salvelinus alpinus (arctic char)	16,659	NC_000861	Doiron et al. 2002
Crassostrea virginica (eastern oyster)	17,242	NC_007175	Milbury and Gaffney 2005
Oreochromis mossambicus (Mozambique tilapia)	16641	NC_007231	Chen et al. unpubished
Tetraodon nigroviridis (green-spotted freshwater pufferfish)	16462	NC_007176	Yue et al. unpubished
Penaeus monodon (tiger prawn)	15984	NC_002184	Wilson et al. 2000
Scomber scombrus (Atlantic mackerel)	16560	NC_006398	Takashima et al. unpublished
Oryzias latipes (Japanese medaka)	16714	NC_004387	Miya et al. 2003
Esox lucius (northern pike)	16695	NC_004593	Ishiguro et al. 2003
Pagrus major (red seabream)	17031	NC_003196	Miya et al. 2001
Plecoglossus altivelis (ayu-fish)	16,537	NC_002734	Ishiguro et al. 2001
Salvelinus fontinalis (brook trout)	16,624	NC_000860	Doiron et al. 2002
Coregonus lavaretus (common whitefish)	16,737	NC_002646	Miya and Nishida (2000)
Takifugu rubripes (pufferfish)	16,442	NC_004299	Elmerot et al. 2002

Background and Principles

Mitochondrial genome evolves more rapidly than the nuclear genome. The rapid evolution of the mtDNA makes it highly polymorphic within a given species. The polymorphism is especially high in the control region (D-loop region), making the D-loop region highly useful in population genetic analysis. Since recombination is a rare event in mtDNA, common ancestry studies could be done with mtDNA analysis (Avise 1994). However, recent reports have indicated that recombination does occur in mtDNA (Harrison 1989, Rokas et al. 2003, Guo et al. 2006), but new genotypes could not be observed because recombination of identical mtDNA molecules should produce the same molecules (homoplasmy) (Maoguolas 2005). Therefore, mutations in mtDNA can be detected, but recombination of mtDNA most often cannot be noticed.

Mitochondrial DNA is maternally inherited for the most part, but there are reports of paternal leakage during fertilization (Birky et al. 1989). Each cell contains a variable number of mitochondria ranging from a few hundred to more than 10,000 mitochondria per cell depending on the cell types. The cells of the brain, the skeletal and heart muscles, and the eye contain the highest number of mitochondria (as many as 10,000 per cell) while the skin cells, which do not require much energy, contain only a few hundred of them. In spite of a large variation of mitochondria per cell, each mitochondrion contains a constant number of mtDNA molecules (Robin and Wong 1988). Since there are multiple copies of mtDNA per cell, many copies of them are transmitted to each offspring.

A variant mtDNA molecule transmitted to the offspring will cause heteroplasmy, or presence of two types of mtDNA in an organism. Variations in mtDNA are caused by mutations. Partitioning of different types of mtDNA into a single oocyte would result in heteroplasmy. For example, heteroplasmy detected in Milkfish (*Chanos chanos*) is attributed to both the 41 bp tandem repeat structure and the 48 bp indel at the control region of the mtDNA (Ravago et al. 2002). In addition to the heteroplasmy caused by mutations, high rates of heteroplasmy can also be caused by paternal mtDNA leakage (Kaneda et al. 1995, Ballard et al. 2005).

Due to the high levels of polymorphism and the ease of mitochondrial DNA analysis, mtDNA has been widely used as markers in aquaculture and fisheries settings. As discussed above, the non-Mendelian inheritance greatly limits the applications of mtDNA for genome research. However, as an identification tool often used in aquaculture, mtDNA can be used as a supplemental tool for aquaculture genome research. Here we will briefly describe the principles behind the wide application of mtDNA markers.

Mitochondrial DNA analysis is actually a restriction fragment length polymorphism (RFLP) analysis, as discussed in Chapter 2, except that the target molecule is mtDNA rather than nuclear genomic DNA (Liu and Cordes 2004). The high levels of polymorphism, the maternal inheritance, and the relatively small size of mtDNA make the RFLP analysis using mtDNA one of the easiest methods for many population studies (Okumus and Ciftci 2002, Liu and Cordes 2004, Billington 2003). The RFLP polymorphism detected in mtDNA is usually caused by a gain or loss of restriction sites. For example, striped bass (*Morone saxatilis*) exhibits a site loss or gain at *Xba* I restriction site, causing an RFLP polymorphism that could easily be detected with polymerase chain reaction (PCR) amplification of the polymorphic region followed by *Xba* I restriction digestion. However, polymorphism could also be caused by insertions or deletions leading to a length variation of mtDNA (Ravago et al. 2002). In this case,

electrophoresis of PCR products in the region should provide information on mtDNA haplotypes (Billington 2003).

mtDNA Analysis

Analysis of mtDNA polymorphism has become a useful genetic tool for studies of genetic divergence within and among populations (Avise 1994). Because mtDNA shows considerable variation among individuals, it is regarded as an effective marker for population structure and geographic variations. Distinct mtDNA lineages have been detected in many freshwater fishes in different parts of their species ranges. Because only half of the population (assuming 1:1 sex ratio) passes on their mtDNA to their offspring, effective population size for mtDNA is smaller than that of nuclear DNA (Harrison 1989).

Early studies using mtDNA analysis relied on purified mtDNA before the invention of PCR. Back in the 1970s and 1980s, the available molecular markers included allozymes, few RFLP, and mtDNA. In aquaculture species, RFLP markers were quite rare at that time. Most studies of natural populations have found greater genetic diversity in mtDNA compared to that revealed by allozyme electrophoresis. Lack of diversity with allozyme markers in striped bass, walleye, and many other fish species was the driving force for different laboratories to use mtDNA analysis to search for more genetic diversity in a variety of fish in the United States in the 1970s to 1980s (Wirgin et al. 1991, 1997; Billington and Herbert 1988). Initial studies done with mtDNA analysis were performed with a whole mtDNA molecule.

Mitochondrial DNA isolation was carried out by first purifying the mitochondria from the tissues containing high amounts of mitochondria, usually liver and gonadal tissues, and extraction of mtDNA from tissue lysates using density gradient centrifugation (Chapman and Brown 1990, Billington and Herbert 1988). A number of 4 and 6 base-cutter restriction endonucleases were used to digest the whole mtDNA molecule to search for fragment length polymorphisms using electrophoresis. The number and size of fragments obtained after digestion and electrophoresis produced a "haplotype." The comparison of haplotypes from several individuals is considered to be representative of the nucleotide differences of their whole mtDNA sequences because of the inferred restriction site loss at certain regions due to mutations (Maogulas 2003).

After the availability of PCR technology, RFLP analysis of the PCR-amplified regions of the mtDNA, rather than using the whole mtDNA, greatly improved mtDNA analysis. There is no need to purify the mtDNA for PCR; a simple total DNA extracted with a commercial kit is sufficient. Most often, the D-loop region is amplified by PCR, and then analyzed by RFLP. In some cases, other coding regions such as ND3, ND4, ND5, ND6, 12S, and 16S RNA regions of the mtDNA were also used (Merker and Woodruff 1996, Nielsen et al. 1998, Wirgin et al. 1997). Whether or not the PCR products need to be digested depends on the nature of the mtDNA RFLP. If the polymorphism is caused by length difference due to insertion or deletion, no restriction digestion is needed. After PCR amplification, the amplicon is analyzed directly by gel electrophoresis. If the polymorphism is caused by gain or loss of restriction site, the PCR amplicon needs to be digested by the restriction enzyme, and then analyzed by gel electrophoresis.

Applications, Data Analysis, and Limitations

Mitochondrial DNA markers have been used extensively to analyze genetic variation in several different aquaculture species including striped bass (Wirgin et al. 1991, Garber and Sullivan 2006), channel catfish (Waldbieser et al. 2003), walleye (Merker and Woodruff 1996), salmonids (Nielsen et al. 1998, Crespi and Fulton 2004), red snapper (Pruett et al. 2005), and bluegill (Chapman 1989). Data analysis in mtDNA studies include determining the number of mtDNA haplotypes, calculating the haplotype frequencies, and nucleotide diversity. Various computer programs such as Arlequin (Schneider et al. 2000), (http://anthro.unige.ch/arlequin/) and TFPGA (Miller 1997) (http://www.marksgeneticsoftware.net/tfpga.htm) are available to perform these analyses. A review by Labate (2000) describes the attributes of several software applications for population genetic analysis.

There are two major drawbacks of mtDNA markers. One is the non-Mendelian inheritance of mtDNA, and the second is the proportion of the total genomic variation one can observe with mtDNA alone. Additionally, mtDNA markers are subject to similar problems that exist for other DNA-based markers. For example, in back mutation cases, nucleotide sites that have already undergone substitution are returned to their original state. Mutations taking place at the same site on the mtDNA in independent lineages and unparallel rates of heterogeneity at the same region (Liu and Cordes 2004) can place limitations on the validity of using mtDNA for genetic studies.

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