3 Catfish

Zhanjiang Liu

The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, AL 36849, USA
e-mail: zliu@acesag.auburn.edu

3.1 Introduction

Catfish is the major aquaculture species in the United States, accounting for more than 60% of all US aquaculture production. Its global importance is increasing as several countries in Asia, such as China and Vietnam, are now heavily involved in catfish aquaculture. Of the cultured catfish in the US, channel catfish (*Ictalurus punctatus*) is the major cultured species. However, a closely related species, blue catfish (*I. furcatus*) is also important because of its ability to produce hybrid catfish with channel catfish. In addition, a number of characteristics of blue catfish make it a highly useful model for genome research.

Channel catfish belongs to the phylum Craniata, class Actinopterygii, order Siluriformes, family Ictaluridae, and genus *Ictalurus*. It belongs to a general group referred to as catfish, i.e., a group of fishes with smooth skin, large flat heads, and long barbels near the mouth. Like the carps, this group of fishes is hardy such that they are more adaptable for artificial spawning, handling, and culture. They have all the characteristics necessary for aquaculture, e.g., easy to produce seeds, ability to manipulate spawning (including artificial), easy to culture, high tolerance to low dissolved oxygen, and efficient feed conversion. Catfish is particularly adaptable for western lifestyles because it lacks small bones and can therefore be processed into fillets on an industrial scale.

Catfish culture consisted of only sporadic activities before the 1970s in the United States. Starting in the 1960s, a group of scientists at Auburn University, led by Homer Scott Swingle, started pond culture of channel catfish. Research conducted at Auburn University and elsewhere optimized pond design, aeration, nutrition, feeding strategies, hatchery production, and disease management, allowing catfish to become a significant national aquaculture industry in the United States. The industry took off in the early 1980s and has been steadily growing since then (Fig. 1). In 2006, catfish production reached 700 million pounds, becoming one of the fastest growing sectors in American agribusiness.

The major catfish cultured in the US is channel catfish. However, the closely related blue catfish is quite important, not only for research, but also for production. Channel catfish and blue catfish exhibit different phenotypes for important production and performance traits. Channel catfish is superior to blue catfish in growth rate, resistance to columnaris disease, and perhaps feed conversion efficiency since that has been found to correlate with growth rate. Blue catfish is more resistant than channel catfish to the most serious bacterial disease, enteric septicemia

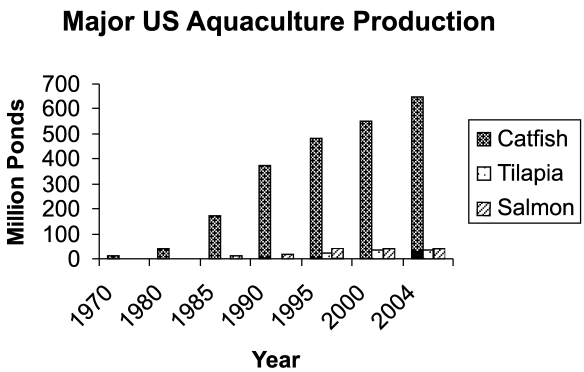

Fig. 1 Catfish is the major aquaculture species in the United States CE5

Genome Mapping and Genomics in Animals, Volume 2
Genome Mapping and Genomics in Fishes and Aquatic Animals
T. D. Kocher, C. Kole (Eds.)

of catfish (ESC) caused by *Edwardsiella ictaluri*. The bacterial pathogen can infect blue catfish in nature, but the incidence rate is much lower. Blue catfish also has a smaller head and a more uniform body shape, making it more adaptable to processing machines to provide a greater fillet yield than channel catfish. Blue catfish stays higher in the water column, making it easier to harvest by seining. One way of exploiting these desirable traits from blue catfish is through the use of an interspecific hybrid catfish. The other way is to introgress the beneficial genes into channel catfish to develop synthetic breeds.

Channel catfish female × blue catfish male interspecific hybrid catfish are superior to either parent in most production and performance traits. Experiments conducted at Auburn University using various combinations of hybrid catfish production involving channel catfish, blue catfish, white catfish, and flathead catfish demonstrated that only the female channel catfish × blue catfish male hybrid results in heterosis or hybrid vigor. All other hybrids produced by crossing channel catfish with any other tested ictalurid catfishes, or combinations of any other mating scheme, including the reciprocal hybrid of blue catfish female × channel catfish male produced hybrids that performed worse than channel catfish and, therefore, do not have any application values. The channel catfish female × blue catfish male interspecific hybrid catfish, however, is better in almost all important traits. It grows faster, has a more aggressive feeding behavior, exhibits a greater feed conversion efficiency, is more resistant to major bacterial diseases, is easier to harvest, and provides a greater fillet yield. Wide commercial application of the hybrid catfish could revolutionize the catfish industry. However, mass production of the hybrid seeds is still difficult. Due to reproductive isolation, artificial spawning must be conducted to produce the hybrid catfish fingerlings. In addition, the fertilization rate and hatching rate of the hybrid catfish is significantly lower than the natural spawning of channel catfish. Research is needed to figure out ways to mass produce the hybrid catfish fingerlings to provide sufficient sources of seed stocks for the catfish industry.

Domestic channel and blue catfish exhibit significant phenotypic and genetic variation for economic traits such as disease resistance, growth rate, feed conversion efficiency (found highly correlated with growth), environmental stress tolerance, carcass yield, seinability, and reproduction (Dunham et al. 1982, 1983, 1984, 1985, 1987a, b, 1990, 1992, 1993a, 1993b; Dunham and Smitherman 1983a, b, 1984, 1987; Bondari 1984; Hallerman et al. 1986; Cadieu 1993; Wolters and Johnson 1994; Dunham 1996; Wolters et al. 1996). Auburn University established an ongoing catfish genetics research program in 1969 to evaluate traditional selective breeding and molecular genetics for improving these quantitative traits. Growth rate and feed conversion efficiency have been improved by as much as 50% through selection (Bondari 1983; Dunham and Smitherman 1983a, 1987; Rezk 1993; Padi 1995), intraspecific crossbreeding (Bondari 1983, 1984; Dunham and Smitherman 1983b), interspecific hybridization (Dunham et al. 1990; Ramboux 1990; Dunham 1996), and genetic engineering (Dunham et al. 1992; Dunham 1996). Disease resistance has been improved primarily through interspecific hybridization (Plumb and Chappell 1978; Dunham et al. 1990, 2000; Dunham 1996; Wolters et al. 1996; Argue et al. 2003), intraspecific crossbreeding (Plumb et al. 1975), and strain selection (Dunham and Smitherman 1984; Wolters and Johnson 1994). Tolerance to low oxygen was improved primarily by interspecific hybridization (Dunham et al. 1983). Seinability can be improved by interspecific hybridization (Dunham 1996) and strain selection (Chappell 1979), and carcass yield by strain selection (Dunham et al. 1984), hybridization (Dunham 1996; Dunham et al. 2000; Argue et al. 2003), and indirect selection (Dunham et al. 1985; Rezk 1993). Heritabilities and genetic correlations have been calculated (Patino 1986; Cadieu 1993; Dunham et al. 2000; Argue et al. 2003).

It is widely recognized that aquaculture must grow rapidly to become an alternative seafood source to the world's collapsing fisheries. It is also clear that improved brood stocks must be developed to overcome the major biological and production hurdles blocking the development of an intensive, reliable, cost-effective, and sustainable aquaculture industry. The greatest obstacle to the catfish industry is the disease problems related to intensive aquaculture. In fact, a recent survey performed by the Auburn University Extension System indicated that all the participating catfish farmers listed disease problems as their top concern (John Jensen unpublished). "To keep my fish alive" is the greatest wish of catfish farmers. Diseases are the primary cause of losses of all operations (NAHMS 1997). The catfish industry suffers an annual

CE5 The scale should read "Million Pounds", not "Million Ponds"

loss of more than $100 million due to diseases. Other hurdles include low profit margins to catfish producers due to high levels of spending on feeds and the reduction of environmental stresses.

Genetic improvement of catfish is a proven method of addressing these problems. Previous research in selective breeding and molecular genetics has resulted in genetically improved catfish (Dunham and Smitherman 1983a, b, 1987; Dunham et al. 1990, 1992; Dunham 1996) and four releases of genetically improved catfish to the industry. The catfish industry has reached a new milestone based upon research with the establishment of the first few viable breeding companies in the industry within the past 10 years, as well as the increased use of genetically improved catfish and breeding principles by catfish farmers over the past 20 years. An understanding of the chromosomal location of economic trait loci (ETL) is required for further improvement in disease resistance, growth, and carcass yields and other traits using marker-assisted selection (MAS), genetic engineering, or introgression of genes from both channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*). In many cases, the selected traits could be counterproductive to one another. For instance, a recent release of a line referred to as the USDA103, a genetically improved catfish line with enhanced growth rate and feeding behavior by USDA-ARS, has resulted in severe disease occurrence in the catfish industry, demonstrating that it is important to not just concentrate on a single trait, but rather to consider most, if not all, important traits.

In spite of the progress made by traditional selection programs, further progress is limited by lack of genome information. The rationale for creating genetic maps of catfish is to increase the efficiency of selection. Breeders wish to find molecular markers correlated with genetic loci controlling economic traits and use these markers to select superior brood stocks (Waldbieser et al. 2001). Traits such as growth rate are relatively easy to measure and select using phenotypic information alone. A genetic map will be more useful to select fish for traits for which measurement is difficult or expensive (e.g., disease resistance) or lethal to brood stocks (e.g., carcass composition). A genetic map will also be beneficial for introgression of alleles into channel catfish from other species with which hybrid production is feasible, such as blue catfish. In addition, a gene map would provide a guide to selection on multiple traits. For instance, fast growth is often correlated with low reproductive capacity. A gene map would allow both traits to be mapped such that brood stocks can be obtained to harbor superior traits on both growth and reproduction traits.

Catfish genome studies started in the 1980s. However, due to limitations in technology, very limited genome characterization was conducted. Channel and blue catfish both have 29 pairs of chromosomes and genomic sizes of approximately 1.0×10^9 bp per haploid genome (LeGrande et al. 1984; Tiersch et al. 1990, Tiersch and Goudie 1993). The recombination genomic size is still not well known but is estimated to be 3,000–4,000 cM. Several studies on linkage analysis were conducted in early stages of catfish genome research. Hallerman et al. (1986) found that 9 of 13 polymorphic allozyme loci changed their frequency in response to selection for growth rate, indicating these loci were linked with growth trait. Three electrophoretic studies (Dunham and Smitherman 1984; Hallerman et al. 1986; Carmichael et al. 1992) have documented extensive genetic variability within and between blue and channel catfish at 70 isozyme loci. This genetic variability was first used in gene mapping to estimate gene–centromere distances for six loci in gynogenetic channel catfish (Liu et al. 1992) and for additional polymorphic loci in blue–channel hybrids. The first *Ictalurus* linkage group, comprised of loci coding for glutathione reductase and phosphoglucomutase (Morizot et al. 1994), was identified by studies of segregation in intraspecific channel catfish crosses and interspecific F_1 (channel × blue) × channel catfish backcrosses. Genetic segregation analysis using allozyme markers demonstrated that the marker inheritance from the interspecific hybrid backcrossing was all normal (Liu et al. 1992; Morizot et al. 1994), as indicated by karyotype studies (LeGrande et al. 1984). This early genome-related research paved the way for large-scale catfish genome research. Here in this chapter, I will summarize the recent progress made in catfish genome research and also briefly introduce the National Aquaculture Genome Project in the United States.

3.2 Construction of Genetic Maps

The US National Aquaculture Genome Project is a part of the National Animal Genome Re-

search Program (NAGRP). Details of the NAGRP can be found at http://www.csrees.usda.gov/nea/animals/in_focus/an_breeding_if_nagrp.html.
The Aquaculture Genome Project was launched officially in 1997 when the first Aquaculture Genome Workshop was held in Dartmouth, Massachusetts. The workshop, organized by Acacia Warren of Tufts University, marked the start of large-scale aquaculture genomic research. The US aquaculture genome project was initially established as a regional project (NE-186), and in 2003 joined the National Animal Genome Project (NRSP-8). Under the umbrella of the US National Aquaculture Genome Project, six species groups are listed: catfish, salmonids, tilapia, shrimps, oysters, and striped bass. For more information on the US Aquaculture Genome Project, visit http://www.animalgenome.org/aquaculture/. Below, I will summarize research progress in catfish genomics.

3.2.1 Development and Evaluation of Molecular Markers

Facing a genome about which there was little information available, our first task was to develop molecular markers for marking the catfish genome. The initial effort in catfish genomics was devoted to the development of polymorphic markers and evaluation of their applications in catfish. Because we did not have any previous knowledge, we first evaluated marker systems which required no prior molecular information. Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers do not require any specific probes or sequence information. They are applicable to species where there is no known information. Therefore, they were adoptable to catfish. RAPD polymorphisms were abundant using the channel catfish × blue catfish hybrid system but were relatively infrequent among channel catfish (Liu et al. 1998a). A total of 142 primers were tested for their application in genetic studies of catfish (Liu et al. 1999a). RAPD markers were highly useful for hybrid identification in catfish but were not most suitable to genome mapping because of their relatively low reproducibility.

AFLP is similar to RAPD in its inheritance as dominant markers (Liu et al. 1998b). However, several features of AFLP make it one of the most preferred markers in catfish. First, it is a highly robust marker system, allowing multi-locus analysis to be conducted in a single analysis. Second, polymorphism rates are high, especially for analyses using the channel catfish × blue catfish hybrid resource families. In contrast to the low reproducibility of RAPD, AFLP is highly reliable. More than 3,000 polymorphic AFLPs were identified using 64 primer combinations (Liu et al. 1999b). AFLP markers were not only very useful for genome mapping in catfish (Liu et al. 2003), but also highly useful for population studies (Mickett et al. 2003; Simmons et al. 2006).

As progress was made in catfish genomics, microsatellite markers were demanded. Microsatellites are very useful markers in catfish. Their major strengths lie in their high polymorphism, codominant inheritance, high abundance, even distribution in the genome, and small locus sizes facilitating genotyping using PCR. Their major drawback is the cost and effort involved in the development of the markers. Like RAPD and AFLP, most microsatellites are type II markers that prohibit information communication among different species through the evolutionary spectrum. Several hundred microsatellite markers were developed in catfish through microsatellite-enriched libraries and analysis of expressed sequence tags (ESTs) (Waldbieser and Bosworth 1997; Liu et al. 1999c, 2001a; Tan et al. 1999; Karsi et al. 2002a; Kocabas et al. 2002b). Very recently, several thousands of microsatellites have been identified from BAC end sequences (Xu et al. 2006).

3.2.2 Development of Type I Markers

One of the lessons learned from the initial efforts of catfish genomics is that not enough attention was paid to the development of type I markers. Type I markers are associated with known genes. Like type II markers, type I markers are useful for genetic linkage and QTL mapping. However, additional benefits of being able to conduct comparative genome mapping, to study genome evolution, to allow interspecies information exchange, and to enhance interlaboratory communications can only be offered by type I markers. Three approaches have been taken to develop type I markers in catfish. The first approach

was to identify microsatellites within cDNAs through bioinformatic mining of microsatellites from EST sequences (Serapion et al. 2004a). While the coding regions of these genes allow us to identify the sequences, microsatellites offer high polymorphism to the sequences. Polymorphic microsatellites within genes of known functions make highly informative type I markers. It appears that catfish ESTs are rich in microsatellites. About 9% of ESTs deposited in GenBank contain microsatellites, twice the rate of zebrafish and seven times the rate in mammals (Liu 2003).

The second approach was to identify single nucleotide polymorphisms among expressed sequences for expressed single nucleotide polymorphisms (eSNPs). In this effort, we have taken advantage of the channel catfish × blue catfish interspecific hybrid system. Comparative analysis of expressed sequence tags (ESTs) has proven to be a very effective way for development of type I SNPs. He et al. (2004) analyzed a total of 86,603 bases from 159 genes, of which 63,537 bp were analyzed from 131 known genes. Among the 131 known genes, a total of 840 eSNPs were identified, i.e., 1.32 eSNP per 100 bp of known genes. The vast majority of the genes harbor at least one SNP between channel catfish and blue catfish.

The third approach is to identify microsatellites within introns. Catfish introns are rich in microsatellite sequences. PCR primers were designed from adjacent exons of selected genes. The intron sequences were amplified and sequenced for the existence of microsatellites (Serapion et al. 2004b) or length polymorphism. It appears that this approach is also effective for the development of type I markers in catfish. Among the three approaches, the largest effort to date is being devoted to the first approach. A recent EST project sequenced more than 20,000 more channel catfish ESTs and more than 10,000 blue catfish ESTs (Li et al. 2007). Bioinformatic mining of microsatellites from these ESTs allowed the identification of several thousands of EST-derived microsatellites.

In spite of the importance of type I microsatellites, it is clear that SNPs will soon serve as the predominant marker type because of their high abundance. With the recent EST project conducted at the Joint Genome Institute, 400,000 channel catfish ESTs will be produced, and 200,000 blue catfish ESTs will be produced. These resources will allow the identification of a large number of SNPs. Obviously, once the whole genome sequence is produced, SNP markers will play an even greater role in genome studies of catfish.

Recent progress in sequencing the ends of bacterial artificial chromosome (BAC) clones (Xu et al. 2006) has also generated many microsatellite markers that can be used not only for genome mapping of catfish, but also for integration of the genetic linkage maps based on meiosis and the BAC-based physical map (Xu et al. 2007). By assigning a common set of markers to both BACs and to the meiotic map, the two maps are merged together, providing greater resolution.

3.2.3 Resource Families and Linkage Maps

Linkage maps of catfish were constructed using both interspecific hybrid resource families (Liu et al. 2003) and intraspecific channel catfish resource families (Waldbieser et al. 2001). Each of the two mapping populations has its own advantages. By using the channel catfish intraspecific resource families, recombination frequency is more natural, and thus the genetic distances between markers are not distorted. Practical objectives of the map may be toward fine-mapping of performance traits showing variation among various strains/lines of channel catfish. A genetic linkage map has been constructed using the channel catfish resource families. To date, some 270 microsatellites have been mapped in 32 linkage groups (Waldbieser et al. 2001). In contrast, the use of the interspecific hybrid resource families allows exploitation of an experimental system where maximum polymorphism can be created for markers of various kinds. In this regard, most markers should be species markers, and thus data is most likely transferable among different interspecific resource families.

Because of the high polymorphism between the channel catfish and blue catfish, the hybrid system should allow mapping of various markers, such as RAPD, AFLP, microsatellites, and SNP markers. The practical objectives of using the interspecific hybrid system are to construct synthetic catfish breeds through introgression. As mentioned above, while channel catfish are different from and superior to blue catfish in growth rate, feed conversion efficiency, and resistance to columnaris disease (caused by *Flavobacterium columnare*, the most common bacterial disease

in catfish), blue catfish are different from and superior to channel catfish in resistance to enteric septicemia of catfish (ESC, the most severe bacterial disease in catfish), harvestability, and processing yield. This interspecific system, therefore, provides a model system for analysis of major QTLs involved in disease resistance and disease defenses.

3.2.4 A Summary of the Linkage Map

A genetic linkage map has been constructed using the interspecific hybrid resource families. A total of 418 AFLP markers have been mapped to 44 linkage groups (Liu et al. 2003). Another genetic linkage map was constructed using microsatellite markers (Waldbieser et al. 2001) using the intraspecific resource families. In this linkage map, a total of 293 microsatellites were mapped in 35 linkage groups.

A key demand of catfish genetic mapping is the construction of a gene-based linkage map. To that end, more than 300 type-I microsatellite markers have been genotyped in the interspecific resource family. This gene-based linkage map will serve as the base for comparative genome analysis.

A second demand of catfish genetic mapping is the integration of the various genetic maps. Genotyping the same markers across multiple resource families should allow integration of the maps and provide higher map resolution. Besides, it should be interesting to compare maps constructed using different resource families.

3.3 QTL Mapping and Identification of Candidate Genes

Catfish offer unique advantages for analysis of QTLs. Thousands of individuals can be produced per spawn. The use of large full-sib families for analysis of quantitative traits should minimize any variation due to the use of different families. However, phenotypic evaluations of aquatic animals can be challenging. Marking fish is often difficult and intrusive and causes wounding or stress of fish that interferes with phenotypic evaluations and measurements. For instance, labeling fish, no matter whether it is heat branding or PIT tagging, often leads to wounding that may stress the fish and result in infections by bacterial pathogens. Traits important for aquaculture include growth rate, feed conversion efficiency, disease resistance, body conformation and processing yield, meat quality, stress response, tolerance to low dissolved oxygen, and tolerance to low water quality. Feed conversion efficiency is very important, because feed accounts for more than 50% of the variable production costs. Diseases can cause up to 30% of annual losses. Under intensive aquaculture conditions, disease problems have been one of the top concerns of catfish producers. Faster growth means shortening of culture period, thereby reducing cost of production.

Selective genotyping is a very effective approach for initial QTL analysis in catfish. Because families are large, phenotypic extremes can be selected for genotyping. For certain markers such as SNPs, selective genotyping coupled with pooling of DNA samples has proven to be very efficient for initial identification of markers linked to performance traits. To date, three markers have been tentatively linked to feed conversion efficiency, and several markers are being evaluated for their linkage with resistance to ESC (e.g., Fig. 2). QTL projects are ongoing at Auburn University. Our most important objective is to identify DNA markers linked to disease resistance. The interspecific hybrid system is currently being used for the assessment of major genes affecting resistance to ESC disease.

3.4 Landscape of the Catfish Genome

An understanding of the basic genomic landscape is very important for decision making related to marker development, linkage mapping, physical mapping, and whole-genome sequence assembly. In this regard, we have given major attention to identifying repetitive elements in the catfish genome. Our recent BAC-end sequencing experiments suggest that about 10% of sequences in the catfish genome are highly repetitive. By using the Repeatmasker web server (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker) and the zebrafish repeat database, about 8% of the catfish sequences were

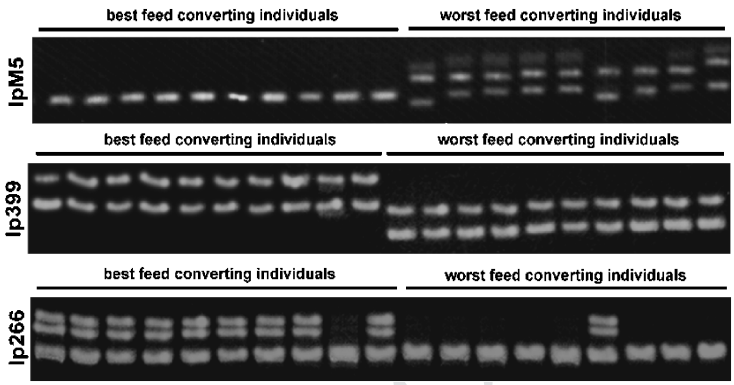

Fig. 2 Putative microsatellite markers associated with feed conversion efficiency. Shown on the *left* are the best performers and on the *right* are worst performers of feed conversion. Note the great differences in allele usages. Marker names are marked on the *left margin*

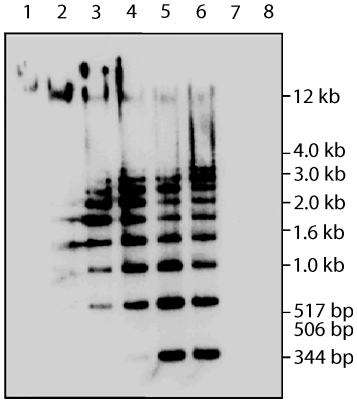

Fig. 3 Southern blot analysis of the Xba elements revealed its head-to-tail tandem arrangements. *Lanes 1–6*, catfish genomic DNA was digested with incremental amount of *Xba*I endonuclease, generating a ladder of monomer, dimmer, trimer, etc. *Lane 7*, negative control, and *lane 8*, molecular weight marker

masked. Additional use of Fugu repeat databases allowed masking of another 2% of the catfish sequences. The largest group of the repeats contains transposons accounting for approximately 4% of the catfish genome. Simple sequence repeats also account for a significant amount of the catfish repeats in the genome (Xu et al. 2006).

Several repetitive elements have been identified and characterized in catfish. The *Xba* elements are highly repetitive, accounting for about 5% of the catfish genome. This element was initially identified by the observation of prominent bands after catfish genomic DNA was digested with restriction endonuclease *Xba*I. Sequencing analysis revealed that it is about 330 bp in size and highly A/T-rich. Further analysis using Southern blot hybridization indicated that the *Xba*I element was arranged in head-to-tail arrays because incremental amounts of the restriction enzyme led to the generation of a ladder-like pattern representing monomers, dimers, trimers, etc. (Fig. 3). These elements appeared to be specific only for channel catfish and blue catfish but were not in the genomes of several other ictalurid catfishes (Liu et al. 1998c). Fluorescent in situ hybridization (FISH) experiments suggest that these elements are located in the centromeric regions of the catfish chromosomes (Quiniou et al. 2005).

The second major class of repetitive elements identified from catfish was the Tc1-like transposons. Several families of Tc1-like transposon elements have been identified by PCR using a single primer designed from the inverted repeats. Three of these families have been characterized. The largest Tc1-like element, referred to as *Tip1*, is 1.6 kb in size, representing the full-length Tc1-like elements. They are highly similar to those identified from zebrafish and other teleosts (Radice et al. 1994; Izsvak et al. 1995; Liu and Li 2003). The second family of Tc1-like elements identified from catfish, referred to as *Tip2*, was 1.0 kb in size. They represent the deleted forms of Tc1-like elements. Sequence comparison of the *Tip2* with known Tc1-like elements from various organisms indicated that they are more similar to Tc1-like elements from invertebrates than to those from vertebrates, especially when the functional domains were considered. The third family of Tc1-like elements is non-autonomous Tc1-like elements, referred to as *Tipnon* (Liu et al. 1999e). They include inverted repeats that share a sequence identity with the Tc1-like elements but do not have any sequences homologous to the transposase gene. They are very small with a size of about 530 bp. However, *Tipnon* is highly abundant with about 32,000 copies

accounting for about 1.6% of the catfish genome. Most of the Tc1 elements appear to exist as short remnants of the transposon. Further analysis through large-scale EST sequencing suggested that a significant fraction (0.6%) of the entire transcriptome contains Tc1-related sequences in both the natural and antisense orientations (Nandi et al. 2007).

In addition to the *Xba* elements and the Tc1-like elements, the *Mermaid* and *Merman* short interspersed elements (SINE) were also identified and characterized in catfish. About 9,000 copies of *Mermaid* and 1,200 copies of *Merman* exist in the channel catfish genome. They were so named because of their coexistence (Kim et al. 2000).

3.5 BAC Libraries and Physical Mapping

Development of genomic resources and technology in catfish has been a major focus in the last few years. To date, one genomic λ-DNA library has been made (Kim et al. 2000; Kocabas et al. 2002a). Two large insert BAC libraries have been made (Quiniou et al. 2003; Wang et al. 2007). The two libraries were constructed using different restriction enzymes EcoR1 and Hind III, and should be useful to complement each other for gap filling.

BAC contig-based physical maps have been constructed in catfish (Xu et al. 2007; Quiniou et al. 2007). One physical map was constructed using BAC library CHORI 212, and the other using CCBL1 BAC library. With the CHORI 212 BAC library, fingerprints of 40,000 BAC clones (6.5× genome coverage) were processed, generating 34,580 BAC clones (5.6× genome coverage) for the fingerprinted contig (FPC) assembly of the BAC contigs. A total of 3,307 contigs were assembled. Each contig contains an average of 9.25 clones with an average size of 292 kb. The combined contig size for all contigs was 0.965 Gb, approximately the genome size of the channel catfish. The reliability of the contig assembly was assessed by both hybridization of gene probes to BAC clones contained in the fingerprinted assembly and validation of randomly selected contigs using overgo probes designed from BAC end sequences (Xu et al. 2007). With the CCBL1 BAC library, 46,548 BAC clones were fingerprinted and assembled into 1,782 contigs and covered an estimated physical length of 0.93 Gb (Quiniou et al. 2007). The use of gynogenetic catfish DNA for the construction of the CCBL1 BAC library allowed cloning of nearly completely homozygous DNA into the CCBL1 library, providing advantages for physical mapping. These physical maps should greatly enhance genome research in the catfish, particularly aiding in the identification of genomic regions containing genes underlying important performance traits. These genome resources lay solid ground for whole genome sequencing in catfish.

The successful sequencing of the human and mouse genomes stirred up a wave of excitement in genome biology. As a result, and partly also due to advances in sequencing technology, sequencing the entire genomes of vertebrate species is no longer an overwhelming challenge. Currently, whole-genome sequencing has been completed or is being completed for a number of vertebrate animals, including cattle, pigs, chickens, and several species of fish, such as the zebrafish, *Danio rerio*, *Takifugu rubripes*, *Tetraodon nigroviridis*, and medaka. In addition, the NIH announced the whole genome sequencing for tilapia – the first aquaculture species. The availability of these genomic sequences will make it possible to trace genomic differences related to functional differences and evolution and fundamentally understand the genetic elements determining expression and function.

For the most efficient whole-genome sequencing, the ideal situation is to avoid repeated sequencing of overlapping regions, but also to cover all gaps such that the sequence of the entire genome can be assembled. Three paradigms have been used for selecting minimally overlapping clones for sequencing. The first is a map-based approach in which fingerprints of clone pairs that appear to have minimum levels of overlapping are picked. This approach was used by the *C. elegans* project and on human chromosomes 1, 6, 20, 22, and X. This approach works well, except that the average overlaps were found to be significant (47.5 kb). If the fingerprinting is conducted by four-color fluorescence-based labeling, this situation probably could be improved, but such a prediction is yet to be proved.

The second approach is based on sequence-tagged connectors using BAC-end sequences (Venter et al. 1996). This approach alleviated the problem of large overlapping regions, but the risk of false positives is high. The third approach is a hybrid of the first two approaches and has been used in various sequencing

projects such as *Arabidopsis* and *Drosophila*. By examining both the overlapping fragments and the overlapping sequences, this approach has been both efficient and reliable. However, many genome resources must be developed to prepare a species for effective whole-genome sequencing. Among many resources, large-insert BAC libraries are crucially important because of their capacity to serve as the basis for physical mapping as well as for the standard clone sets for high-throughput genomic sequencing (Tomkins et al. 2001; Osoegawa et al. 2000, 2003). The BAC-end sequencing (BES), or sequence-tagged connector (STC), approach has proven to be an effective strategy for large-scale sequencing (Venter et al. 1996). This approach involves sequencing the ends of BAC inserts to scatter sequence tags randomly across the genome (Mahairas et al. 1999). Once any BAC or other large segment of DNA is sequenced to completion by conventional shotgun approaches, the STCs can be used to identify a minimum tiling path of BAC clones overlapping the nucleation sequence for sequence extension (Siegel et al. 1999; Chen et al. 2004), thereby greatly reducing redundant sequencing. BES is also useful for confirming genome assembly and for obtaining a non-biased sample of the genome for the purpose of analysis for gene contents, status of repetitive elements, and simple sequence repeats (Larkin et al. 2003; Winter et al. 2003). Thus, production of a BAC-based physical map and BAC-end sequences in channel catfish should lay the scientific foundation for sequencing the whole genome of this species. Although other methods have been used to generate contigs for targeted comparative sequencing, large-scale BAC-end sequencing is currently the most efficient strategy for building whole-genome comparatively anchored physical maps in map-poor species (Larkin et al. 2003) such as catfish.

Recently, we have sequenced 25,000 BAC ends, generating almost 50,000 BESs. This project produced more than 27 million base pairs of genomic sequences, representing approximately 3% of the catfish genome, allowing, for the first time in catfish, a look at gene content, repeat structure and arrangements, and conserved syntenies. The BAC-end sequences are equivalent to one BES every 20 kb, providing a valuable resource toward whole-genome sequencing of catfish. Furthermore, many genes and microsatellites were assigned to BACs, allowing them to be used for integration of the physical map with linkage maps. We also confirmed conserved syntenies using overgo hybridization, demonstrating the value of the draft zebrafish genome sequence to catfish research. Once BAC-based contigs are produced, this BES resource will allow integration of the maps, development of regional markers for fine QTL mapping, and large-scale comparative genome analysis. Such resources, therefore, are important for genetic analysis of performance traits important to aquaculture, whether or not the catfish genome will be sequenced in the near future.

3.6 EST Analysis and Transcriptome Analysis

Twenty-one cDNA libraries have been made from channel catfish, including 15 cDNA libraries made from various channel catfish tissues and six cDNA libraries made from cultured cell lines. Tissues used for construction of the cDNA libraries include head kidney (anterior kidney), spleen, skin, liver, brain, stomach, intestine, ovary, gill, muscle, testis, pituitary, olfactory tissue, and trunk kidney (posterior kidney). Fourteen of the 15 cDNA libraries were made in the pSport-1 vector (Life Technologies, MD), and one was made in the lambda Unizap cloning vector (Stratagene, CA). In consideration of their uses for the identification of SNPs, tissues from 15 fishes were used; as genomic resources to include potentially most, if not all, transcripts for the study of disease-related genes, tissues were collected from both healthy and infected fishes at various times after infection (Li et al. 2007). The six cDNA libraries from the cultured cell lines of channel catfish were from the catfish autonomous (immortal) B cell line, the catfish autonomous (immortal) T cell line, one-week-old catfish mixed leukocyte culture, the catfish autonomous (immortal) macrophage cell line, and the catfish nonautonomous (mortal) cytotoxic T cell lines (http://morag.umsmed.edu/libraries/index.html).

Six cDNA libraries were also constructed from blue catfish using tissues of head kidney, spleen, liver, gill, skin, and heart. These cDNA libraries were used to conduct comparative analysis of ESTs between the channel catfish and blue catfish for the identification

of eSNPs. EST analysis has proven to be one of the most efficient ways for gene identification, gene expression profiling, and cataloguing. It also produces resources for the development of cDNA microarrays. To date, about 43,000 ESTs from channel catfish and 10,000 from blue catfish have been produced and deposited in GenBank. The Institute of Genome Research (TIGR) has constructed a gene index that includes more than 23,000 unique sequences (http://www.tigr.org). This number will increase, since several more thousands of ESTs sequenced recently have not been deposited in GenBank yet.

The majority of cDNA libraries have only been sequenced at a low depth (Fig. 4). In order to acquire a greater coverage of the catfish transcriptome, more ESTs must be sequenced. In our previous sequencing experience, the gene discovery rate was quite high. Our initial sequencing efforts were limited to non-normalized libraries, including libraries made from brain (Ju et al. 2000), head kidney (Cao et al. 2001), skin (Karsi et al. 2002a), and spleen (Kocabas et al. 2002b). On top of these efforts, a recent EST sequencing project generated 30,000 additional ESTs (Li et al. 2007). In this project, overgo probe hybridization subtraction was used to enhance the gene discovery rate. Overall, the sequencing redundancy rate was below 30%, an excellent rate. In a recent effort attempting to characterize the entire catfish transcriptome, the author of this chapter took a lead along with 80 scientists and obtained approval for a large EST sequencing project by the Joint Genome Institute of the Department of Energy. In the project, 300,000 catfish EST clones will be sequenced from both ends to generate 600,000 ESTs. I believe that through this project, we should be able to capture the largest proportion of the catfish transcriptome, and this project will soon be completed.

One of the major uses of ESTs is to provide technological resources for the development of microarrays. The first catfish microarray was a 660 gene channel catfish (*Ictalurus punctatus*) cDNA array printed on nylon and used to identify channel catfish brain genes responsive to cold acclimation at four time points (0, 2, 24, and 48 h) after a shift from 24 °C to 12 °C (Ju et al. 2002). Cultured catfish in the US must be able to tolerate wide ranges in water temperatures throughout the year. The findings of this study (e.g., transient induction of chaperone and signal transduction pathway genes) provided insight into the genes and molecular pathways altered in the catfish brain during exposure to a type of environmental stress that is relevant to the aquaculture industry (decreased ambient temperature) (Ju et al. 2002). A comprehensive understanding of the genes involved in cold acclimation may reveal suitable targets (e.g., expression biomarkers or SNPs) for marker-assisted selection (MAS) of cold-resistant catfish broodstock. A 19 K oligo microarray platform was developed to identify catfish spleen genes responsive to LPS (Li and Waldbieser, 2006). This study confirmed that many immune-relevant genes (e.g,. TLR5, interferon regulatory factor 1, a chemokine receptor) responded in LPS-stimulated catfish spleen.

Infectious diseases are among the most serious threats to the global aquaculture industry. Therefore, one of the main applications of fish genomic resources and techniques is to identify genes that may be suitable targets for MAS of disease-resistant broodstock. Channel catfish is the most economically important aquaculture finfish species in the US, and the immune system and responses of this species are well-characterized. While channel catfish are relatively susceptible to *E. ictaluri* infection, blue catfish are relatively resistant. A 28 K catfish in situ oligo microarray platform was recently developed, with features representing all discrete transcripts currently known in *I. punctatus* (21 K genes) and *I. furcatus* (7 K genes). This is an outstanding genomics platform for comparative transcriptomic studies aimed at identifying candidate genes or heritable expression biomarkers of natural resistance to *E. ictaluri*. Two studies using the 28 K catfish microarray provide a comprehensive look at the hepatic gene expression responses of channel catfish and blue catfish to challenge with *E. ictaluri* (Peatman et al. 2007, 2008). A comparison of the results of these two microarray studies identifies a suite of 58 genes that responded to the pathogen in blue catfish but not in channel catfish. This differentially responsive suite included immune-relevant genes such as CC chemokine SCYA106, MHC class I alpha chain, and matrix metalloproteinase 13 (MMP13) (Peatman et al. 2007b). These studies identified numerous genes that potentially play roles in the differential sensitivity of channel and blue catfish to *E. ictaluri*, forming a solid foundation for future functional characterization, genetic mapping, and QTL analysis of immunity-related genes from catfish (Peatman et al. 2008).

EST analysis allows a brief assessment of tissue expression of genes. It also allows identification of alter-

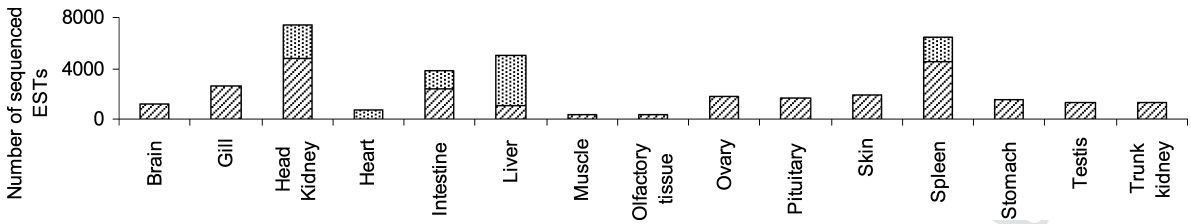

Fig. 4 Catfish libraries and EST sequences produced to date. *Sketched areas* indicate channel catfish libraries, and *dotted areas* indicate blue catfish libraries

native splicing and alternative polyadenylation. Most importantly, it is probably the most efficient way to identify polymorphic markers such as EST-associated microsatellites. In a recent analysis of 43,000 ESTs, we have identified more than 4,000 EST-associated microsatellites. Though additional work is still required for these microsatellites to be used as markers, it is the first step to the mapping of a large number of type-I markers.

ESTs also provide the material basis for the study of gene duplication. Gene duplication is a widespread phenomenon in teleost fishes. The most popular theory currently states that teleost fish went through a stage of whole-genome duplication, making them tetraploid organisms, followed by rapid random gene losses in various lineages. Such high levels of gene duplications present many difficulties for aquaculture genomicists. The availability of ESTs from closely related species of channel catfish and blue catfish provide phylogenetic approaches to differentiate if the genes are orthologous or not. For instance, if a channel catfish EST is more similar to a blue catfish EST than another channel catfish EST, it would suggest the presence of two copies of the related genes in channel catfish.

3.7 Systematic Characterization of Genes and/or Full-Length cDNAs

Systematic analysis of complete cDNAs and/or genes using a genomic approach is part of our genome program. While understanding the genome on a large scale is important, detailed analysis of genes and their expression is mandatory for the understanding of gene structure, gene evolution, gene families, orthologs versus paralogs, and gene expression in relation to functions. Additional information concerning transcript processing can also be obtained regarding alternative splicing and alternative polyadenylation. All such information should facilitate comparative functional genomics.

A set of transcripts involved in a specific metabolic pathway or a specific process can be obtained systematically during large-scale EST analysis without screening for specific cDNAs one by one. After the initial identification of ESTs representing genes of interest, complete cDNA sequences can be easily obtained and their expression analyzed. Using such an approach, we have characterized a complete set of all 32 small ribosomal protein cDNAs and a complete set of all 47 large ribosomal protein cDNAs from channel catfish (Karsi et al. 2002b; Patterson et al. 2002). Other genes we have analyzed include myostatin (Kocabas et al. 2002a), gonadotropin (GnRH) alpha subunit (Liu et al. 1997), GnRH beta subunits 1 and 2 (Liu et al. 2001b), alpha actin gene (Kim et al. 2000), creatine kinase (Liu et al. 2001c), a large number of CC (He et al. 2004; Bao et al. 2006; Peatman et al. 2005, 2006) and CXC chemokine genes (Baoprasertkul et al. 2004, 2005; Chen et al. 2005), hepcidin (Bao et al. 2005), LEAP-2 antimicrobial peptide (Bao et al. 2006), bacterial permeability increasing protein (Xu et al. 2005), three NK-lysin genes (Wang et al. 2005, 2006), a number of cytokine genes, a number of complement genes, and a large number of cytochrome P450 genes (Z Liu unpublished).

The significance of characterizing complete coding sequences has been realized. NIH started the mammalian Full-Length cDNA Initiative (http://grants.nih.gov/grants/guide/rfa-files/RFA-CA-99-005.html) in 1999 for the purpose of functional and comparative genomics. Full-length cDNA databases of human (http://www.ornl.gov/meetings/wccs/helix.htm) and mouse (http://www.jsbi.org/journal/GIW99/

GIW99P34.pdf) cDNAs have also been established in Japan. While our efforts and resources for the analysis of full-length cDNAs are limited, our high-quality cDNA libraries should be a valuable resource for such purposes.

3.8 Future Perspectives

Efforts in catfish genome research should be enhanced. Specifically, I believe the following areas need to be addressed in the very near future. First, genetic linkage mapping should be continued. Denser genetic maps must be constructed for practical usefulness in breeding programs. QTL mapping efforts should be increased. With the initial identification of performance traits-linked markers, genome regional markers should be developed for fine-mapping the putative QTLs. Candidate gene identification using microarray and other approaches should be conducted in order to pin down the potential genes involved in important QTLs, especially for disease resistance. Integration of the linkage and physical maps is very important for the identification of genes underlining production and performance traits. More importantly, regional markers can then be developed from adjacent BAC clones for fine-mapping of QTLs and for the eventual cloning of economically important genes. In the long term, the BAC contigs should be useful as the guide for entire or partial genome sequencing in catfish. Mapping of common markers on the physical map and the linkage maps will also allow integration of various maps that will greatly increase the resolution of catfish maps. Comparative mapping efforts should be increased. Much information can be obtained by the comparative mapping.

Benefits of great investment into basic studies using model species can only be realized by comparative genomics. This includes the "transfer" of information from map-rich species to catfish and also comparative studies among several aquaculture fish species such as tilapia and salmonids. A large number of type-I markers are being mapped in catfish. Upon completion, comparison of the map location of these type-I markers can be directly compared to those of the human, cattle, swine, and zebrafish. Coordination and mapping of the same set of type I markers in other aquaculture fish species will also allow development of comparative maps in several other aquaculture species. In addition, hybridization of a common set of type-I marker probes to catfish, tilapia, and salmonids has been planned (TD Kocher, pers. comm.). A direct comparison of their location on the physical map is also possible using BAC contigs. Gene expression in relation to function should be studied in a comparative way through evolution. While many genetic mechanisms may have been evolutionarily conserved, specific mechanisms discovered from catfish should fill the gap it represents as an important aquaculture species among the lower teleost fish.

Whole-genome sequencing must be considered for catfish in the near future. This is mandated by several realities. Several funding agencies are now starting to limit their funding to species with entire genome sequences. This would mean a double penalty on aquaculture species if we do not push for genome sequencing. First, because of the lack of genome sequences, we cannot do in silico cloning, in silico Southern blot, and many other functions available to species with genome sequences; and second, the lack of genome sequences certainly does not disqualify anyone for applying for grants to do functional genome research. Also, the genome research of catfish has come to a stage where further progress is limited without the genomic sequences, and advances in sequencing technology make it feasible now to carry out the entire genome sequencing of catfish. At present, it would take about $10 million to sequence and assemble the catfish genome. Whole-genome sequencing was not previously even a possibility at this price.

In spite of funding limitations, catfish genome research has made major advances in recent years. We expect that as the genome research progresses, genome information will be applied to selective breeding programs, allowing enhanced broodstock development. Along with research from many other fields, genome research will prove to be the fundamental basis for a sustainable aquaculture industry.

Acknowledgement. Our catfish genome work has been supported by the USDA-NRICGP Animal Genome and Genetic Mechanisms Program and the Genome Reagents and Tools Program (Award #2006-35616-16685). The author is especially indebted to his past and current dedicated staff and hard-working

students: Huseyin Kucuktas, Ping Li, Attila Karsi, Zhenlin Ju, Soohag Kim, Arif Kocabas, Andrea Patterson, Jinian Feng, Micah Simmons, Dongfeng Cao, Kathryn Mickett, Liqiao Chen, Eric Peatman, Puttharat Baoprasertkul, Jerry Serapion, Peng Xu, Shaolin Wang, Benjaporn Somridhivej, Qun Wang, Yaping Wang, Guiling Wang, Baolong Bao, Jason Abernathy, Samiran Nandi, and Wonko Lee. The author is also grateful to his former department head, Dr. John Jensen, for his spiritual support, and is thankful to Dr. Michael Moriarty, the vice president for research, and Dr. Richard Guthrie, the dean of the College of Agriculture, for their support of the Catfish Genome Program. Some of the figures were reproduced from our published articles.

References

Argue B, Liu ZJ, Dunham RA (2003) Dress-out and fillet yields of channel catfish, *Ictalurus punctatus*, blue catfish, *I. furcatus*, and their F_1, F_2 and backcross hybrids. Aquaculture 228:81–90

Bao B, Peatman E, Li P, He C, Liu ZJ (2005) Catfish hepcidin gene is expressed in a wide range of tissues and exhibits tissue-specific upregulation after bacterial infection. Dev Comp Immunol 29:939–950

Bao B, Peatman E, Xu P, Li P, Zeng H, He C, Liu ZJ (2006a) The catfish liver-expressed antimicrobial peptide 2 (LEAP-2) gene is expressed in a wide range of tissues and developmentally regulated. Mol Immunol 43:367–377

Bao B, Peatman E, Xu P, Baoprasertkul P, Wang G, Liu ZJ (2006b) Characterization of 23 CC chemokine genes and analysis of their expression in channel catfish (*Ictalurus punctatus*). Dev Comp Immunol 30:783–796

Baoprasertkul P, Peatman E, Chen L, He C, Kucuktas H, Li P, Simmons M, Liu ZJ (2004) Sequence analysis and expression of a CXC chemokine in resistant and susceptible catfish after infection of *Edwardsiella ictaluri*. Dev Comp Immunol 28:769–780

Baoprasertkul P, He C, Peatman E, Zhang S, Li P, Liu ZJ (2005) Constitutive expression of three novel catfish CXC chemokines: homeostatic chemokines in teleost fish. Mol Immunol 42:1355–1366

Bondari K (1983) Response to bidirectional selection for body weight in channel catfish. Aquaculture 33:73–81

Bondari K (1984) Reproductive performance, growth, and survival of selected and wild × selected channel catfish. Theor Appl Genet 68:391–395

Cadieu GM (1993) Heritability of tolerance to low dissolved oxygen, high ammonia and high nitrite for channel catfish, *Ictalurus punctatus*. MS thesis, Auburn University

Cao D, Kocabas A, Ju Z, Karsi A, Li P, Patterson A, Liu ZJ (2001) Transcriptome of channel catfish (*Ictalurus punctatus*): initial analysis of genes and expression profiles from the head kidney. Anim Genet 32:169–188

Carmichael GJ, Schmidt ME, Morizot DC (1992) Electrophoretic identification of genetic markers in channel catfish and blue catfish by use of low-risk tissues. Trans Am Fish Soc 121:26–35

Chappell JA (1979) An elevation of twelve genetic groups of catfish for suitability in commercial production. Dissertation, Auburn University

Chen L, He C, Baoprasertkul P, Xu P, Li P, Serapion J, Waldbieser G, Wolters W, Liu Z (2005) Analysis of a catfish gene resembling interleukin-8: cDNA cloning, gene structure, and expression after infection with *Edwardsiella ictaluri*. Dev Comp Immunol 29:135–142

Chen R, Sodergren E, Weinstock GM, Gibbs RA (2004) Dynamic building of a BAC clone tiling path for the Rat Genome Sequencing Project. Genome Res 14:679–684

Dunham RA (1996) Contribution of genetically improved aquatic organisms to global food security. International conference on sustainable contribution of fisheries to food security, Government of Japan and FAO, Rome

Dunham RA, Smitherman RO (1983a) Response to selection and realized heritability for body weight in three strains of channel catfish, *Ictalurus punctatus*, grown in earthen ponds. Aquaculture 33:88–96

Dunham RA, Smitherman RO (1983b) Crossbreeding channel catfish for improvement of body weight in earthen ponds. Growth 47:97–103

Dunham RA, Smitherman RO (1984) Ancestry and breeding of catfish in the United States. Circular 273, Alabama Agricultural Experiment Station, Auburn University

Dunham RA, Smitherman RO (1987) Genetics and breeding of catfish. Regional Research Bulletin 325, Southern Cooperative Series, Alabama Agricultural Experiment Station, Auburn University

Dunham RA, Smitherman RO, Brooks MJ, Benchakan M, Chapell JA (1982) Paternal predominance in channel–blue hybrid catfish. Aquaculture 29:389–396

Dunham RA, Smitherman RO, Webber C (1983) Relative tolerance of channel × blue hybrid and channel catfish to low oxygen concentrations. Progressive Fish-Culturist 45:55–56

Dunham RA, Benchakan M, Smitherman RO, Chappell JA (1984) Correlations among morphometric traits of 11-month-old blue, channel, white, and hybrid catfishes and the relationship to dressing percentage at 18-months of age. J World Mariculture Soc 14:668–675

Dunham RA, Joyce JA, Bondari K, Malvestuto SP (1985) Evaluation of body conformation, composition and density as traits for indirect selection for dress-out percentage of channel catfish. Progressive Fish-Culturist 47:169–175

Dunham RA, Smitherman RO, Goodman RK (1987a) Comparison of mass selection, crossbreeding and hybridization

for improving body weight in channel catfish. Progressive Fish-Culturist 49:293–296

Dunham RA, Eash J, Askins J, Townes TM (1987b) Transfer of metallothionein-human growth hormone fusion gene into channel catfish. Trans Am Fish Soc 116:87–91

Dunham RA, Brummett RE, Ella MO, Smitherman RO (1990) Genotype–environment interactions for growth of blue, channel and hybrid catfish in ponds and cages at varying densities. Aquaculture 85:143–151

Dunham RA, Ramboux AC, Duncan PL, Hayat M, Chen TT, Lin CM, Kight K, Gonzalez-Villasenor I, Powers DA (1992) Transfer, expression and inheritance of salmonid growth hormone genes in channel catfish, *Ictalurus punctatus*, and effects on performance traits. Mol Mar Biol Biotechnol 1:380–389

Dunham RA, Brady Y, Vinitnantharat S (1993a) Response to challenge with *Edwardsiella ictaluri* by channel catfish, *Ictalurus punctatus*, selected for resistance to *E. ictaluri*. J Appl Aquaculture 3:211–222

Dunham RA, Hyde C, Masser M, Smitherman RO, Perez R, Plumb J, Ramboux AC (1993b) Comparison of culture traits of channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus*. J Appl Aquaculture 3:257–267

Dunham RA, Lambert DM, Argue BJ, Ligeon C, Yant R, Liu ZJ (2000) Comparison of manual stripping and pen spawning for production of female channel catfish × blue catfish hybrids and aquarium spawning of channel catfish. North Am J Aquaculture 62:260–265

Engler FW, Hatfield J, Nelson W, Soderlund CA (2003) Locating sequence on FPC maps and selecting a minimal tiling path. Genome Res 13:2152–2163

Hallerman EM, Dunham RA, Smitherman RO (1986) Selection or drift isozyme allele frequency changes among channel catfish selected for rapid growth. Trans Am Fish Soc 115:60–68

He C, Peatman E, Baoprasertkul P, Kucuktas H, Liu Z (2004) Multiple CC chemokines in channel catfish and blue catfish as revealed by analysis of expressed sequence tags. Immunogenetics 56:379–387

Izsvak Z, Ivics Z, Hackett PB (1995) Characterization of a Tc1-like transposable element in zebrafish (*Danio rerio*). Mol Gen Genet 247:312–322

Ju Z, Karsi A, Kocabas A, Patterson A, Li P, Cao D, Dunham R, Liu ZJ (2000) Transcriptome analysis of channel catfish (*Ictalurus punctatus*): genes and expression profile from the brain. Gene 261:373–382

Ju Z, Dunham R, Liu ZJ (2002) Differential gene expression in the brain of channel catfish (*Ictalurus punctatus*) in response to cold acclimation. Mol Genet Genomics 268:87–95

Karsi A, Cao D, Li P, Patterson A, Kocabas A, Feng J, Ju Z, Mickett K, Liu ZJ (2002a) Transcriptome analysis of channel catfish (*Ictalurus punctatus*): Initial analysis of gene expression and microsatellite-containing cDNAs in the skin. Gene 285:157–168

Karsi A, Patterson A, Feng J, Liu ZJ (2002b) Translational machinery of channel catfish: I. A transcriptomic approach to the analysis of 32 40S ribosomal protein genes and their expression. Gene 291:177–186

Kim S, Karsi A, Dunham R, Liu ZJ (2000) The skeletal muscle α-actin gene of channel catfish (*Ictalurus punctatus*) and its association with piscine-specific SINE elements. Gene 252:173–181

Kocabas AM, Li P, Cao D, Karsi A, He C, Patterson A, Ju Z, Dunham RA, Liu Z (2002) Expression profile of the channel catfish spleen: analysis of genes involved in immune functions. Mar Biotechnol 4:526–536

Kocabas A, Kucuktas H, Dunham RA, Liu ZJ (2002a) Molecular characterization and differential expression of the myostatin gene in channel catfish (*Ictalurus punctatus*) Biochim Biophys Acta 1575:99–107

Kocabas A, Li P, Cao D, Karsi A, He C, Patterson A, Ju Z, Dunham R, Liu ZJ (2002b) Expression profile of the channel catfish spleen: analysis of genes involved in immune functions. Mar Biotechnol 4:526–536

Kocabas A, Dunham R, Liu ZJ (2004) Alterations in gene expression in the brain of white catfish (*Ameirus catus*) in response to cold acclimation. Mar Biotechnol 6:S431–438

Larkin DM, Everts-van der Wind A, Rebeiz M, Schweitzer PA, Bachman S, Green C, Wright CL, Campos EJ, Benson LD, Edwards J, Liu L, Osoegawa K, Womack JE, de Jong PJ, Lewin HA (2003) A cattle–human comparative map built with cattle BAC-ends and human genome sequence. Genome Res 13:1966–1972

LeGrande W, Dunham RA, Smitherman RO (1984) Comparative karyology of three species of north American catfishes (*siluriformes: Ictaluridae:Ictalurus*) and four of their hybrid combinations. Copeia 1984:873–878

Li P, Peatman E, Wang S, Feng J, He C, Baoprasertkul P, Xu P, Kucuktas H, Nandi S, Somridhivej B, Serapion J, Simmons M, Turan C, Liu L, Muir W, Dunham R, Brady Y, Grizzle J, Liu ZJ (2007) Towards the catfish transcriptome: generation and analysis of 31,215 catfish ESTs. BMC Genomics 8:177

Li RW, Waldbieser GC (2006) Production and utilization of a high-density oligonucleotide microarray in channel catfish, *Ictalurus punctatus*. BMC Genomics 7:134

Liu Q, Goudi CA, Simco BA, Davis KB, Morizot DC (1992) Gene–centromere mapping of six enzyme loci in gynogenetic channel catfish. J Hered 83:245–248

Liu ZJ (2003) A review of catfish genomics: progress and perspectives. Comp Funct Genomics 4:259–265

Liu ZJ, Li P, Argue B, Dunham R (1997) Gonadotropin α subunit glycoprotein from channel catfish (*Ictalurus punctatus*) and its expression during hormone-induced ovulation. Mol Mar Biol Biotechnol 6:221–231

Liu ZJ, Li P, Argue B, Dunham R (1998a) Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I furcatus*) and their F_1, F_2 and backcross hybrids. Anim Genet 29:58–62

Liu ZJ, Nichols A, Li P, Dunham R (1998b) Inheritance and usefulness of AFLP markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I furcatus*) and their F_1, F_2 and backcross hybrids. Mol Gen Genet 258:260–268

Liu ZJ, Li P, Dunham R (1998c) Characterization of an A/T-rich family of sequences from the channel catfish (*Ictalurus punctatus*). Mol Mar Biol Biotechnol 7:232–239

Liu ZJ, Tan G, Kucuktas H, Li P, Karsi A, Yant DR, Dunham RA (1999) High levels of conservation at microsatellite loci among Ictalurid catfishes. J Hered 90:307–312

Liu ZJ, Li P, Argue BJ, Dunham RA (1999a) Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture 174:59–68

Liu ZJ, Li P, Kucuktas H, Nichols A, Tan G, Zheng X, Argue BJ, Yant R, Dunham RA (1999b) Development of AFLP markers for genetic linkage mapping analysis using channel catfish and blue catfish interspecific hybrids. Trans Am Fish Soc 128:317–327

Liu ZJ, Tan G, Li P, Dunham RA (1999c) Transcribed dinucleotide microsatellites and their associated genes from channel catfish, *Ictalurus punctatus*. Biochem Biophys Res Commun 259:190–194

Liu ZJ, Karsi A, Dunham RA (1999d) Development of polymorphic EST markers suitable for genetic linkage mapping of catfish. Mar Biotechnol 1:437–447

Liu ZJ, Li P, Kucuktas H, Dunham RA (1999e) Characterization of nonautonomous Tc1-like transposable elements of channel catfish (*Ictalurus punctatus*). Fish Physiol Biochem 21:65–72

Liu ZJ, Li P, Kocabas A, Ju Z, Karsi A, Cao D, Patterson A (2001a) Microsatellite-containing genes from the channel catfish brain: evidence of trinucleotide repeat expansion in the coding region of nucleotide excision repair gene RAD23B. Biochem Biophys Res Commun 289:317–324

Liu ZJ, Kim S, Karsi A (2001b) Channel catfish follicle stimulating hormone and luteinizing hormone: cDNA cloning and their expression during ovulation. Mar Biotechnol 3:590–599

Liu ZJ, Kim S, Kucuktas H, Karsi A (2001c) Multiple isoforms and an unusual cathodic isoform of creatine kinase from channel catfish (*Ictalurus punctatus*). Gene 275:207–215

Liu ZJ, Karsi A, Li P, Cao D, Dunham R (2003) An AFLP-based genetic linkage map of channel catfish (*Ictalurus punctatus*) constructed by using an interspecific hybrid resource family. Genetics 165:687–694

Mickett K, Morton C, Feng J, Li P, Simmons M, Dunham RA, Cao D, Liu ZJ (2003) Assessing genetic diversity of domestic populations of channel catfish (*Ictalurus punctatus*) in Alabama using AFLP markers. Aquaculture 228:91–105

Morizot D, Schmidt M, Carmichael G (1994) Joint segregation of allozymes in catfish genetic crosses: designation of *Ictalurus punctatus* linkage group I. Trans Am Fish Soc 123:22–27

NAHMS (1997) USDA animal and plant health inspection service. Reference of 1996 U.S. catfish health and production practices

Nandi S, Peatman E, Xu P, Wang S, Li P, Liu ZJ (2007) Repeat structure of the catfish genome: A genomic and transcriptomic assessment of Tc1-like transposon elements in channel catfish (*Ictalurus punctatus*). Genetica 131:81–90

Osoegawa K, Tateno M, Woon PY, Frengen E, Mammoser AG, Catanese JJ, Hayashizaki Y, de Jong PJ (2000) Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. Genome Res 10:116–128

Padi JN (1995) Response and correlated responses to four generations of selection for increased body weight in the Kansas strain channel catfish, *Ictalurus punctatus*, grown in earthen ponds. MS thesis, Auburn University

Patino E (1986) Heritabilities for body weight, feed consumption and feed conversion and the correlations among these traits in channel catfish, *Ictalurus punctatus*. MS thesis, Auburn University

Patterson A, Karsi A, Feng J, Liu ZJ (2003) Translational machinery of channel catfish: II. Complementary DNA and expression of the complete set of 47 60S ribosomal proteins. Gene 305:151–160

Peatman E, Baoprasertkul P, He C, Li P, Kucuktas H, Liu Z (2005) *In silico* identification and expression analysis of 12 novel CC chemokines in catfish. Immunogenetics 57:409–419

Peatman E, Baoprasertkul P, Terhune J, Xu P, Nandi S, Kucuktas H, Li P, Wang S, Somridhivej B, Dunham R, Liu ZJ (2007) Expression analysis of the acute phase response in channel catfish (*Ictalurus punctatus*) after infection with a Gram negative bacterium. Dev Comp Immunol 31:1183–1196

Peatman E, Terhune J, Baoprasertkul P, Xu P, Nandi S, Wang S, Somridhivej B, Kucuktas H, Li P, Dunham R, Liu ZJ (2008) Microarray analysis of gene expression in the blue catfish liver reveals early activation of the MHC class I pathway after infection with *Edwardsiella ictaluri*. Mol Immunol 45:553–566

Plumb JA, Chappell J (1978) Susceptibility of blue catfish to channel catfish virus. Proc Ann Conf Southeast Assoc Fish Wildlife Agency 32:680–685

Plumb JA, Green OL, Smitherman RO, Pardue GB (1975) Channel catfish virus experiments with different strains of channel catfish. Trans Am Fish Soc 104:140–143

Quiniou SM, Katagiri T, Miller NW, Wilson M, Wolters WR, Waldbieser GC (2003) Construction and characterization of a BAC library from a gynogenetic channel catfish *Ictalurus punctatus*. Genet Sel Evol 35:673–683

Quiniou SM, Wolters WR, Waldbieser GC (2005) Localization of Xba repetitive elements to channel catfish (*Ictalurus punctatus*) centromeres via fluorescence in situ hybridization. Anim Genet 36:353–354

Quiniou SM, Waldbieser GC, Duke MV (2007) A first generation BAC-based physical map of the channel catfish genome. BMC Genomics 8:40

Radice AD, Bugaj B, Fitch DH, Emmons SW (1994) Widespread occurrence of the Tc1 transposon family: Tc1-like transposons from teleost fish. Mol Gen Genet 244:606–612

Ramboux AC (1990) Evaluation of four genetic groups of channel–blue catfish hybrids grown in earthen ponds. Dissertation, Auburn University

Rezk MS (1993) Response and correlated responses to three generations of selection for increased body weight in channel catfish, *Ictalurus punctatus*. Dissertation, Auburn University

Serapion J, Kucuktas H, Feng J, Liu Z (2004a) Bioinformatic mining of type I microsatellites from expressed sequence tags of channel catfish (*Ictalurus punctatus*). Mar Biotechnol 6:364–377

Serapion J, Waldbieser GC, Wolters W, Liu ZJ (2004b) Development of type I markers in channel catfish through intron sequencing. Anim Genet 35:463–466

Siegel AF, Trask B, Roach JC, Mahairas GG, Hood L, van den Engh G (1999) Analysis of sequence-tagged-connector strategies for DNA sequencing. Genome Res 9:297–307

Simmons M, Mickett K, Kucuktas H, Li P, Dunham R, Liu ZJ (2006) Comparison of domestic and wild catfish populations provide no evidence for genetic impact. Aquaculture 252:133–146

Tan G, Karsi A, Li P, Kim S, Zheng X, Kucuktas H, Argue BJ, Dunham RA, Liu ZJ (1999) Polymorphic microsatellite markers in *Ictalurus punctatus* and related catfish species. Mol Ecol 8:1758–1760

Tiersh TR, Goudie CA (1993) Inheritance and variation of genome size in half-sib families of hybrid ictalurid catfishes. J Hered 84:122–125

Tiersh TR, Simco BA, Davis KB, Chandler RW, Wachtel SS, Carmicheal GJ (1990) Stability of genome size among stocks of the channel catfish. Aquaculture 87:15–22

Tomkins JP, Wood TC, Stacey MG, Loh JT, Judd A, Goicoechea JL, Stacey G, Sadowsky MJ, Wing RA (2001) A marker-dense physical map of the *Bradyrhizobium japonicum* genome. Genome Res 11:1434–1440

Waldbieser GC, Bosworth BG (1997) Cloning and characterization of microsatellite loci in channel catfish, *Ictalurus punctatus*. Anim Genet 28:295–298

Waldbieser GC, Bosworth BG, Nonneman DJ, Wolters WR (2001) A microsatellite-based genetic linkage map for channel catfish, *Ictalurus punctatus*. Genetics 158:727–734

Wang Q, Bao B, Wang Y, Peatman E, Liu ZJ (2006) Characterization of a NK-lysin antimicrobial peptide gene from channel catfish. Fish Shellfish Immunol 20:419–426

Wang S, Xu P, Thorsen J, Zhu B, de Jong P, Waldbieser G, Kucuktas H, Liu ZJ (2007) Characterization of a BAC library from channel catfish *Ictalurus punctatus*: indications of high rates of chromosomal reshuffling among teleost genomes. Mar Biotechnol 9:701–711

Winter A, Alzinger A, Fries R (2004) Assessment of the gene content of the chromosomal regions flanking bovine DGAT1. Genomics 83:172–180

Wolters WR, Johnson MR (1994) Enteric septicemia resistance in blue catfish and three channel catfish strains. J Aquat Anim Health 6:329–334

Wolters WR, Wise DJ, Klesius PH (1996) Survival and antibody response of channel catfish, blue catfish, and catfish hybrids after exposure to *E. ictaluri*. J Aquat Anim Health 8:249–254

Xu P, Bao B, Zhang S, Peatman E, He C, Liu ZJ (2005) Characterization and expression analysis of bactericidal permeability-increasing protein (BPI) antimicrobial peptide gene from channel catfish *Ictalurus punctatus*. Dev Comp Immunol 29:865–878

Xu P, Wang S, Liu L, Peatman E, Somridhivej B, Thimmapuram J, Gong G, Liu ZJ (2006) Channel catfish BAC end sequences for marker development and assessment of syntenic conservation with other fish species. Anim Genet 37:321–326

Xu P, Wang S, Liu L, Thorsen J, Kucuktas H, Liu ZJ (2007) A BAC-based physical map of the channel catfish genome. Genomics 90:380–388