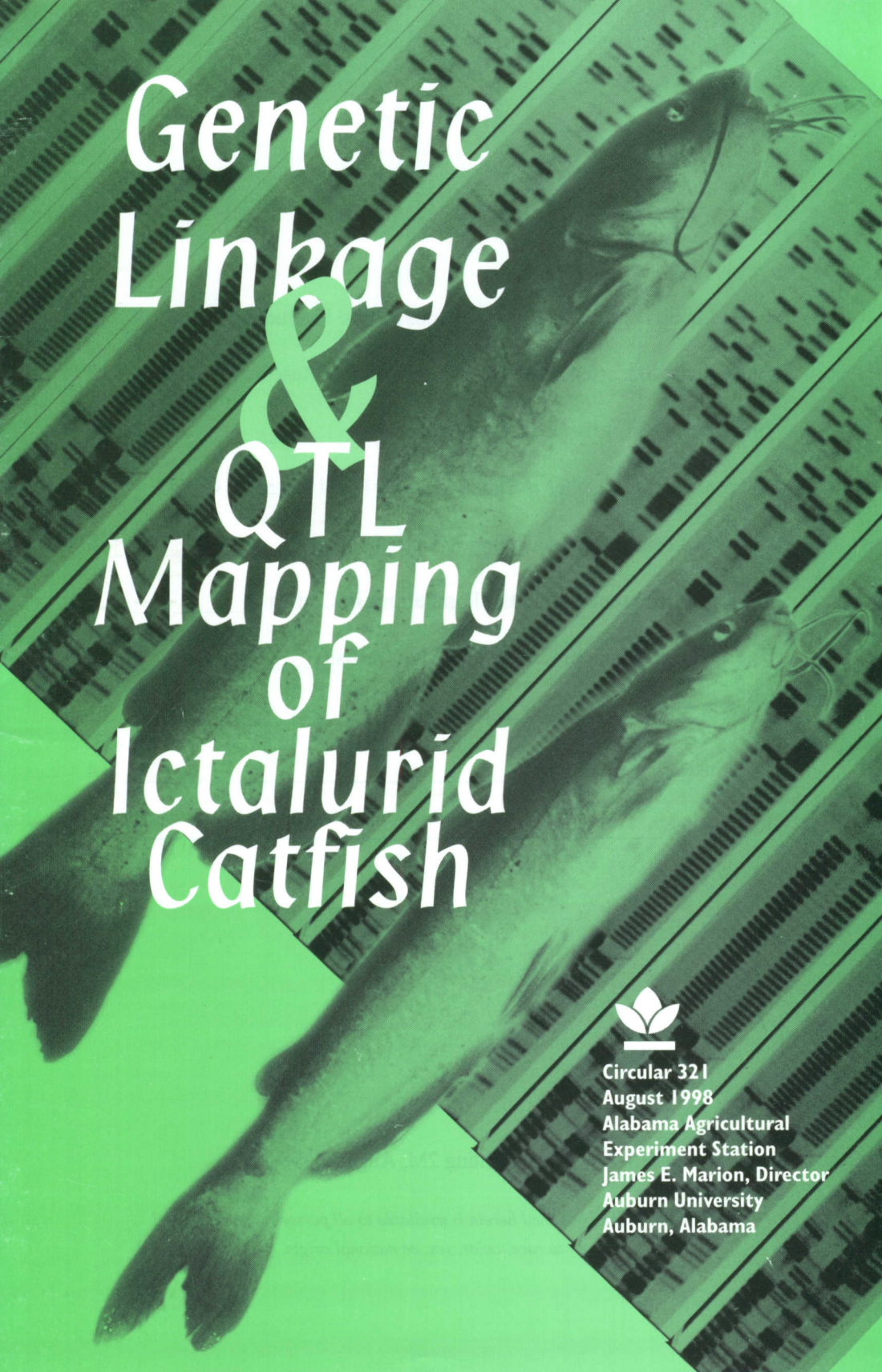
Genetic Linkage & QTL Mapping of Ictalurid Catfish



Circular 321
August 1998
Alabama Agricultural
Experiment Station
James E. Marion, Director
Auburn University
Auburn, Alabama

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First Printing 2M, August 1998

GENETIC LINKAGE AND QTL MAPPING OF ICTALURID CATFISH

ZHANJIANG LIU AND REX A. DUNHAM

CATFISH ACCOUNT for more than 50% of all aquacultural production in the United States. Finding ways to increase catfish production and efficiency through genetic mapping is the subject of current research at Auburn University. Researchers hope to improve catfish brood stock by locating the gene sites that are responsible for economically important traits such as disease resistance, growth, feed conversion, resistance to low oxygen, carcass yield, and harvestability. These gene sites are referred to as quantitative trait loci or QTLs.

In this review, the researchers will discuss the need for increased and efficient aquaculture production, the importance of catfish production and genetics research, the importance of catfish QTL mapping, the theoretical approaches to gene mapping of catfish, the choice of molecular markers suitable for genetic linkage and QTL mapping, the choice of a mapping population in catfish, the QTLs of channel catfish and blue catfish, and the current status of catfish genetic mapping.

NEED FOR INCREASED AND EFFICIENT AQUACULTURE PRODUCTION

Despite the development of the aquaculture and catfish industries, the United States is suffering from a large international trade deficit of $4.5 billion per year for aquaculture products. This is the third largest contribution to the trade imbalance after petroleum and automobiles. According to USDA estimates, the U. S. demand for seafood will increase from 700 million to one billion kilograms per year, of which wild fisheries will be able to supply only 25 to 30% of the additional demand *(59, 58)*. The worldwide demand for fish products is also increasing. Consequently, the trade deficit in seafood products is expected to increase.

Liu is an assistant professor and Dunham is a professor in the Auburn University Department of Fisheries and Allied Aquacultures.

One way of increasing seafood supply is to increase the marine fishing industry, but the "world fish stocks are in crisis. Almost two-thirds of marine stocks in the Pacific and Atlantic Oceans are being fully exploited or have already been overfished. Future projections predict a steadily widening gap between the world's demand for fish and the ability of the oceans to meet it" *(67)*. Overfishing is a global threat, warns the Food and Agricultural Organization of the United Nations. While fish production will remain fairly constant, the world population will approach seven billion by the year 2010, with a 19 million ton shortfall in fish production. The solution to this problem lies in development of a sustainable aquaculture *(67)*.

IMPORTANCE OF CATFISH PRODUCTION AND GENETICS RESEARCH

Catfish production in 1996 was 295 million kilograms, generating over two billion dollars of value-added revenue. Concentrated mainly in the southern United States, the catfish industry is very important for the rural economy in Mississippi, Alabama, Louisiana, Arkansas, Texas, Georgia, Florida, Missouri, Oklahoma, Idaho, and part of California.

Several production problems face the catfish industry. These production problems include disease resistance, growth, feed conversion, resistance to poor water quality, harvesting, carcass yield, and reproduction. Catfish production needs to be increased and made more efficient to improve farm profitability, increase exports and decrease the trade deficit, reduce pressure on natural fisheries and enhance the environment, and help make catfish aquaculture more sustainable. Brood stocks with superior traits for disease resistance, growth, feed conversion, resistance to low oxygen, carcass yield, and harvestability are required to increase catfish production and make aquaculture sustainable.

Genetic improvement of catfish is a proven method of addressing these problems *(14)*. In fact, catfish genetics and breeding programs at Auburn University have resulted in several releases of genetically improved catfish to the industry *(21, 22, 24, 18, 15)*. The catfish industry has reached a new milestone with the establishment of the first two breeding companies within the past three years, as well as the increased use of genetically improved catfish and breeding principles by catfish farmers during the past 13 years.

IMPORTANCE OF CATFISH QTL MAPPING

While classical selection programs are successful, more effective selection programs need to be developed for traits that cannot be easily measured. Disease, for instance, is the most important production problem in the catfish industry, but genetic determination of disease resistance is difficult. Marker-assisted selection (MAS) and biotechnology offer great potential as alternatives to solve these problems, but genetic linkage information for economically important genes is needed first. For the long-term development and sustainability of aquaculture, it is important to construct a genetic linkage map for application in selection programs through MAS, or for isolation of economically important genes to be used to improve catfish brood stocks through biotechnology.

A growing body of evidence indicates a high degree of genetic conservation in fish genomes (complete sets of genetic material and their organization), and genetic conservation in fish genomes is the basis for synteny or comparative anchorage mapping. Perhaps many gene locations are conserved among different species of fish, including zebrafish for which a detailed genetic linkage map is available *(48, 33)*. If this assumption is correct, it would make sense to construct an anchorage comparative map. However, except for its scientific value in helping increase understanding of genomic organization and evolution, the value of the catfish map would be significantly discounted if QTLs were ignored. It is the mapping of QTLs that ultimately makes cloning of economically important genes and MAS possible. For instance, disease resistance to enteric septicemia (ESC) is the top priority in catfish industry, but is irrelevant in zebrafish.

The quantitative (or economically important) traits of catfish include disease resistance, feed conversion efficiency, tolerance of low oxygen, harvestability, carcass and fillet yield, and growth rate. Two major bacterial pathogens, *Edwardsiella ictaluri* and *Flavobacterium columnarae*, cause in excess of $50 million annual losses. Feed conversion efficiency is important since more than 60% of variable costs are in feeding. Lethal and sublethal effects of low oxygen increase production costs by $50 to 100 million, annually. Harvesting inefficiency has not only a direct cost, but also an indirect cost of higher feed conversion and greater mortality because of large, harvestable fish left in the pond. A 1% increase in carcass or fillet yield would generate an additional annual $15 to 20 million of product and a 5% improvement of $75 to 100 million is not unrealistic. Obviously, increased growth is an important trait, since it should increase production and profitability.

Genetic linkage and QTL mapping are important both for practical purposes and for basic genetic understanding of the organism. They provide basic information about genomic organization, structure, and evolution. They reveal mechanisms of inheritance of phenotypes (the appearance or other measurable characters of an organism) relevant to molecular markers. Correlation of recombination genome size with physical sizes of the genome of the organism makes it possible to estimate physical distances between markers. Microsynteny (the analogy of a chromosome segment from one organism to that from another organism) and comparative genetic mapping among the closely related species of catfish or between catfish and other fish or non-fish species will allow comparison of their genomic conservation and divergence, thus giving information on genomic evolution. Of course, the most important result of linkage and QTL mapping is that they will generate a map that assigns various markers to linkage groups and reveals the physical distances among markers and distances of markers to important QTLs.

Practically, there are two reasons for genetic linkage and QTL mapping: first, the direct goal of linkage and QTL mapping is to isolate genes of high interest, such as major genes controlling important economic traits; a second goal of linkage and QTL mapping is to conduct selection programs with marker-assisted selection. Genetic linkage and QTL mapping allow specific genes to be mapped in proximity to molecular markers in a range of less than a million base pairs for gene cloning.

While gene cloning is an important goal of gene mapping, MAS has been proven to speed genetic improvement of important economic traits, such as milk production

in dairy cattle *(6, 29),* soluble solid content of tomatoes *(53),* and yield and ear number of maize *(52).* MAS is even more important for traits that are difficult to measure on a routine basis, such as disease resistance and feed conversion in catfish. Determination of disease resistance requires naive fish and determination of feed conversion efficiency requires family information. Challenging catfish with deadly pathogens is impractical in a production scheme. Therefore, MAS is necessary for catfish selective breeding programs because of the economic importance of disease resistance and feed conversion.

THEORETICAL APPROACHES TO LINKAGE AND QTL MAPPING OF CATFISH

The genomic size of catfish has been estimated at 1 X 10^9 base pairs (bp) per haploid genome (1.97 picogram/cell) *(56, 55).* The genome size in terms of recombination is not known. Assuming the recombination genome size to be 2,500 cM (centiMorgan), 500 evenly distributed genetic markers would be required for a 5-cM resolution map (equivalent to a map with any locus being within 2.5 cM to the nearest marker on average). Poompuang and Hallerman *(47)* indicate that a map for fish with 100 random markers has a high probability to allow thorough marker-QTL linkage analysis. There would be a greater chance for mapping QTLs with 500 markers. With the assumption of a 2,500 cM recombination genome size, each catfish cM is equivalent to about 400,000 bp. Thus a map with 10 cM resolution is equivalent to one marker every four million base pairs which puts any gene less than two million base pairs to the nearest marker.

Theoretically, there are two approaches to map any gene of interest to a highly detailed map. The first approach randomly maps as many markers as possible on the chromosomes without discrimination. This approach is costly. For instance, to obtain a resolution of 1 cM (mapping the gene in proximity of 400,000 base pairs as assumed above), 2,500 markers are required. Development of 2,500 good markers and mapping them on the chromosomes requires great effort and expense, although this approach is straightforward.

The second approach is to construct a detailed map (sometimes referred to as a saturated map). There are two steps to this approach. The first step is to generate an intermediately detailed map with a resolution of 5 to 10 cM, which requires 250 to 500 markers. This map uses markers to establish linkage between the genes (QTLs) of interest. The second step is to generate a high resolution map with as many markers as possible in the targeted region. A high density of markers in a targeted region allows fine mapping of the QTLs of interest. The linkage maps can be constructed with a resolution of 1 cM or less. This can be achieved by marker development for a specific chromosome or a segment of a chromosome.

To construct a "regional map" containing the QTLs of interest, physical mapping information is important. Actual gene cloning starts from the closely linked marker and is achieved by "chromosome walking" or "chromosome jumping" to the region containing the gene of interest. Large insert gene libraries such as yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) are used to obtain sequence contigs to arrange the chromosome region in a "linear chain." (Contigs

are linear arrays of large DNA segments overlapping each other, reflecting their positional relationships.) Sequence analysis by direct sequencing or exon-trapping is then carried out to clone the gene. (An exon is a polynucleotide sequence that forms messenger RNA when combined with other such sequences.) The cloned genes can then be used for gene transfer.

How many markers are required? Practically, a map with 1 cM resolution requires 2,500 markers for complete genome coverage. One may not need to cover the whole genome if the second approach focusing on QTLs is taken, because the most important QTLs may reside on only a subset of the chromosomes. Major genes for disease resistance to ESC, for example, may reside on one chromosome. The key is to identify the chromosome carrying the QTLs of interest. This is the initial mapping phase. If such chromosomes can be distinguished from others and can be isolated, chromosome-specific markers can be developed.

Physical size of an average channel catfish chromosome should be 3.4×10^7 bp. Again, assuming the recombination frequency is equal along arms of all chromosomes, this chromosome would be equivalent to 1/29 of the 2,500 cM, or 86 cM. To gain a resolution of 1 cM on this chromosome, only 86 chromosome-specific markers are needed. This seems to be absolutely accessible. However, the prerequisite is that chromosome specific libraries can be established. If this chromosome cannot be identified and, therefore, chromosome-specific libraries are difficult to establish, specific markers based on physical mapping can be developed when the major QTL genes are mapped to a specific chromosome.

If one expressed sequence tag (EST) marker can be mapped to the chromosome, microsatellite markers can be developed from BAC or YAC contigs starting from this mapped EST markers. (Microsatellites consist of simple, tandemly repeated mono- to penta-nucleotide sequence DNA segments flanked by unique sequences.) Assuming each YAC or BAC clone can cover 100 kb, more than 340 contig clones are required to cover this whole chromosome. This seems to be a dramatic undertaking. However, if a unique sequence is mapped within 5 cM to the gene of interest, the physical distance averages about two million base pairs. About 20 to 40 BAC contig clones would cover the region. When the 20 contig clones are identified, "region-specific" markers can be developed.

Obviously, efforts must be made in both linkage/QTL mapping and physical mapping. Data from linkage mapping will be very useful for physical mapping and vice versa. Catfish gene mapping efforts, therefore, should be addressed in three continuous phases: the initial mapping phase, the physical mapping and fine linkage and QTL mapping phase, and the gene identification and cloning phase.

Catfish, and fish in general, offer several advantages for QTL mapping. Their high reproductive capacity (each catfish can produce over 10,000 eggs) means that a large number of individuals is available for QTL measurement.

Large numbers of individuals make catfish an excellent system for performing selective genotyping *(10, 12)*. Extensive phenotype selection for QTL mapping and selection can be applied to obtain phenotypic extremes, thus significantly enhancing success rate for detecting QTLs. Higher success rates can be achieved if selective genotyping is coupled to phenotype pooling (selective DNA pooling) *(11)* where appropriate.

CHOICE OF MARKERS FOR MAPPING IN CATFISH

The choice of markers for gene mapping is a reflection of what markers are available, reliable, easy to use, less expensive, and more rapid. The choice of markers also depends on the expertise of the laboratory. Choice of markers has been largely a result of technological development. As more sophisticated markers become available, the use of older marker technology declines. The various marker systems offer both advantages and disadvantages. Their usefulness in catfish gene mapping depends on their current availability and possibility of their development in catfish in the near future.

Isozyme markers in catfish were first examined in the 1980s. (Isozymes are multiple forms of an enzyme, and allozymes–see below–are isozymes as a result of allelic variation at a single locus.) Hallerman *et al. (32)* found that allozyme frequencies at nine of 13 polymorphic loci changed in response to selection for growth rate, indicating these isozyme loci influenced growth directly or were linked to other loci related to growth. Unfortunately, these relationships were not further explored. (Polymorphic is the occurrence of multiple forms at a marker locus. The existence of polymorphic markers is known as polymorphism.) Three electrophoretic studies *(23, 32, 9)* have documented extensive genetic variability within and between blue catfish and channel catfish at 70 isozyme loci. Isozymes are inexpensive and technically accessible markers. However, they are too limited to give large numbers of markers to generate a high density map or to have a broader genome coverage.

Restricted fragment length polymorphism (RFLP) markers are extremely useful markers. Their main strength is that they are co-dominant markers and a large difference in length can be detected due to gain or loss of restriction sites; therefore, RFLP markers are easy to score. In addition, if complementary DNA (cDNA) probes are used, comparative anchorage maps can be constructed in a closely related species. However, RFLP analysis requires development of probe libraries and involves Southern blot hybridization–procedures that are time consuming and labor intensive. RFLP analysis also often involves use of radioisotopes. Furthermore, because RFLP polymorphism depends on sequence differences due to insertion, deletion, rearrangements, and sequence differences within the restriction enzyme recognition sites, the capability to detect polymorphism is low in consideration of the tedious blotting procedures. This approach was popular in the 1980s because more advanced marker systems were not available. Because of its time-consuming process, RFLP is slow and costly. Currently, RFLP markers in catfish are rare. Development of large numbers of RFLP markers in catfish to construct a high density genetic map would be costly and time-consuming.

Preliminary results using expressed sequence tags (EST) from a pituitary cDNA library indicate that less than 20% of independent clones exhibited polymorphism between channel catfish and blue catfish. Thus EST markers are inefficient in identifying polymorphism. Limited numbers of EST markers are available in catfish. Large scale development of EST markers involves higher cost than microsatellite markers. However, further development of more EST markers may be useful for

comparative mapping because EST markers represent genes and, therefore, a higher level of evolutionary conservation is expected.

Microsatellites consist of simple, tandemly repeated mono- to penta-nucleotide sequence DNA segments flanked by unique sequences. In mammalian systems, microsatellite sequences generally are abundant and evenly distributed. Variability at these sites is mostly due to a difference in the number of repeat units in the DNA segments. In catfish, the most abundant microsatellite is the (CA)n/(GT)n repeats followed by (GA)n/(CT)n. Tri-nucleotide repeats are intermediately abundant. The main strengths of microsatellites as markers are (1) they are highly polymorphic, thus allowing rapid and economical development of large numbers of polymorphic markers; (2) they are co-dominant markers and therefore highly reliable; and (3) they are generally short and thus can be facilitated in genotyping by polymerase chain reaction (PCR). The main disadvantage of microsatellites as markers is that the initial characterization of microsatellite clones involves a lot of sequencing and PCR optimization.

Twenty-two microsatellite markers have been developed in the Catfish Genetics Unit, USDA ARS *(61),* and they are highly polymorphic, with more than 10 alleles at some loci. (Alleles are any of the alternative forms of a gene that may occur at a given locus.) Waldbieser and Bosworth *(61)* indicated that the heterozygosity of microsatellites is the same for wild and domestic channel catfish. This is an unexpected result since domestic fish populations are usually more homozygous than wild populations. However, the equivalent heterozygosity of microsatellites is an artifact because of the large number of alleles per locus. Actually, wild channel catfish have a slightly higher number of alleles per locus compared to domestic fish as expected, but heterozygosities were near maximum values for both types of catfish because of the large number of alleles.

More than 800 microsatellite clones have been isolated and 250 clones have been characterized. Sequencing of the remaining clones is expected to be finished in 1998. The data indicate that about half of the clones results in enough non-repetitive sequences, allowing design of PCR primers; the other half of the clones either harbors microsatellite sequences at the proximal end of the small insert or has adjacent sequences that are also highly repetitive. This indicates that 400 good microsatellite markers will be available when characterization of the 800 clones is finished. Large numbers of microsatellite clones can be isolated readily since several microsatellite-enriched small insert libraries have been constructed *(45).* If indeed several thousand microsatellite markers are required, they can be available within a few years.

Two PCR-based marker systems–random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers–have been developed recently. RAPD markers have been demonstrated to be an extremely efficient way to detect DNA polymorphisms and generate large numbers of molecular markers for genetic mapping and genomic fingerprinting applications *(57).* RAPD has been successfully used to genetically mark a variety of eukaryotic organisms, including humans, domestic animals, fungi, plants, and fish *(63, 62, 28).* RAPD markers have allowed significant advances in the ability to generate linkage maps quickly *(31).* RAPD is powerful in detecting polymorphisms because oligonucle-

otide primers scan the whole genome for its perfect and sub-perfect binding sites in a low-stringency PCR reaction. Because of its power for identifying polymorphic markers without any previous sequence information, RAPD permits the construction of a highly saturated genetic map in a short time span *(62, 63)*. The most outstanding examples of this rapid analysis are the linkage maps in *Arabidopsis (50)* and Loblolly pine *(44),* which were constructed in a few months as compared with construction of linkage maps with conventional molecular markers, such as RFLP, which takes several years.

With catfish genomic DNA as templates, each RAPD primer can generally amplify five to 16 bands, many of which are polymorphic in closely related species such as channel catfish and blue catfish *(39)*. In fact, results using 142 primers indicate that RAPD markers are abundant for channel catfish and blue catfish. Over 400 good RAPD markers have been identified, 200 each for channel and blue catfish. More RAPD markers can be identified readily by using more RAPD primers. Genetic analysis with RAPD markers is relatively easy, fast, efficient, and economical. RAPD markers are usually scored as dominant alleles, since the amplified DNA product is present in one parent but absent from the other. This is a potential disadvantage for RAPD analysis when coupled to lower reproducibility due to low stringent PCR. Research experience indicates that if only prominent and highly reproducible bands are used as markers, this problem can be circumvented. Bands with low yield, smearing, or less reproducibility will reduce reliability and, therefore, should not be used as markers. As mapping of QTLs to a limited region becomes imminent, more markers are needed. RAPD markers, therefore, may offer a convenient way for generating large numbers of markers rapidly. RAPD markers are not useful for intraspecific mapping populations because polymorphisms are low among strains of channel catfish or blue catfish *(39)*.

AFLP markers *(60)* are another type of PCR-based marker. Compared to RAPD markers, reliability of AFLP is high because PCR conditions are stringent. In this procedure, genomic DNA is digested with *Eco*R I and *Mse* I. *Eco*R I and *Mse* I adaptors are ligated to the restriction fragments at either ends. A pair of primers is used to pre-amplify the *Eco*R I/*Mse* I fragment populations. The amplified library of *Eco*R I/*Mse* I fragments is further selectively amplified by PCR primers with two or three extra bases, with each extra base on each primer reducing the fragments to be amplified to one-quarter of the original fragments (A, C, G, or T at a given position; only one of the four chances matches with the extra base added in the primer). *Eco*R I PCR primer is labeled, and the detected fragments represent *Eco*R I/*Mse* I fragments, for the most part. The products are analyzed by electrophoresis on a sequencing gel. Each primer pair combination has generated 60 to 150 bands in catfish *(40)*.

High levels of polymorphism were detected between channel catfish and blue catfish, but very low polymorphism was detected within channel catfish or blue catfish. Thus, similar to RAPD markers, AFLP markers are highly useful for interspecific hybrid mapping populations, but they are less useful for intraspecific mapping populations. AFLP markers are also dominant markers. Their inheritance is similar to that of RAPD markers *(39, 40),* with every marker transmitted into F1 except that at rare loci, markers were segregating in the F1 population because the P1 were

heterozygous at these loci. Segregation in F2 or backcross follows ratios of Mendelian genetics *(40)*.

With the use of 10 primer pair combinations, more than 250 AFLP markers have been identified. Experience indicates that–similar to the RAPD markers–only strong and reproducible bands, and bands that are not too close to the adjacent bands, should be used as markers. For instance, two polymorphic bands may be highly reproducible, but if they are too close to each other, it would be difficult to score in the segregating population. Large number of AFLP markers can be identified rapidly. Similar to RAPD markers, AFLP markers can be highly useful for economically and rapidly mapping large numbers of markers.

CHOICE OF REFERENCE MAPPING POPULATIONS IN CATFISH

There are two options for production of mapping populations for construction of genetic linkage and QTL mapping in catfish: the interspecific hybrid system and the intraspecific mating plan.

The first choice is to use the channel catfish x blue catfish hybrid system (the interspecific hybrid system). The two catfish species have nearly identical karyotypes: each has 58 chromosomes (Table 1). The fertile F1 from the channel catfish and blue catfish mating can serve as an excellent model system for gene mapping. F2 populations have been produced. Backcross individuals can be readily obtained by backcrossing the F1 with channel catfish or blue catfish. The interspecific F2 or backcross progeny can be used as mapping populations.

The interspecific hybrid system offers several advantages. First, more polymorphisms exist between the channel catfish and blue catfish than among strains of channel catfish when RAPD markers *(39)*, AFLP markers *(40)*, or EST markers *(64)* are used. Levels of polymorphism for microsatellite markers are also higher between the channel catfish and blue catfish than between strains within channel catfish or blue catfish. In fact, previous research data indicated that very low levels of polymorphisms exist among intraspecific strains in channel catfish or blue catfish using either RAPD or AFLP markers, and essentially no polymorphism was discovered with EST markers *(39, 40, 64)*, thus making it difficult to obtain large numbers of molecular markers if an intraspecific mating plan is used. Limited types and numbers of polymorphic markers within channel catfish hinder construction of a high density map. The interspecific hybrids will also allow construction of linkage maps for blue catfish with slightly higher cost and slightly greater efforts. Technically, there are no drawbacks with backcrosses as mapping populations.

The interspecific hybrid system has another advantage. Interspecific polymorphism is much greater than intraspecific polymorphism in catfish, allowing a more complete and saturated map to be generated more rapidly, efficiently, and less expensively. Not only does the higher level of marker polymorphism lend the interspecific hybrid backcrossing system to linkage mapping, but extreme differences between the quantitative traits and presumably the QTL alleles of these two species should ease and accelerate the QTL mapping because of the obvious polymorphism for the QTLs.

TABLE 1. SUMMARY OF KARYOTYPE DATA FOR 30 SPECIES OF ICTALURID CATFISH

Species	No.[1]	2N[2]	FN[3]	LC[4]	LM[5]	Formula	HoM%[6]	M%[7]	HrM%[8]
Ictalurus punctatus	4	58	92	—	—	32msm[9], 26stt[10]	25.8	74.2	0.0
Ictalurus furcatus	6	58	84	—	—	—	—	—	—
Ameiurus natalis	2	62	84	2	2	22msm, 40stt	30.5	67.8	1.7
Ameiurus melas	3	60	76	—	—	16msm, 44stt	38.9	58.3	2.8
Ameiurus brunneus	—	62	96-106	—	—	—	—	—	—
Ameiurus nebulosus	9	60	76	—	—	16msm, 44stt	31.5	64.5	4.0
Ameiurus platycephalus	—	54	92	—	—	—	—	—	—
Ameiurus serracanthus	1	52	90	8	6	38msm, 14stt	33.4	53.3	13.3
Ameiurus catus	3	48	64-68	—	—	—	—	—	—
Pylodictus olivaris	3	56	82	4	2	26msm, 30stt	31.1	67.2	1.5
Noturus gilberti	2	54	82	4	2	28msm, 26stt	34.7	65.3	0.0
Noturus insignis	6	54	74	4	—	20msm, 34stt	27.4	71.0	1.6
Noturus exilis	2	54	68	6	—	14msm, 40stt	43.0	57.0	0.0
Noturus nocturnus	10	48	72	10	8	24msm, 24stt	24.1	75.1	0.8
Noturus leptacanthus	10	46	72	16	12	26msm, 20stt	24.5	75.5	0.0
Noturus funebris	2	44	68	14	12	24msm, 20stt	48.7	51.3	0.0

Source: Adapted from LeGrande *(36)*.

[1]No.= number of specimens.
[2]2N = diploid number.
[3]FN = fundamental number.
[4]LC = number of large chromosomes.
[5]LM = number of large msm's.
[6]HoM% = percent of hypomodal counts.
[7]M% = percent of modal counts.
[8]HrM% = percent of hypermodal counts.
[9]msm = metacentric and submetacentric chromosomes.
[10]stt = telocentric and subtelocentric chromosomes.

TABLE 1, CONTINUED. SUMMARY OF KARYOTYPE DATA FOR 30 SPECIES OF ICTALURID CATFISH

Species	No.[1]	2N[2]	FN[3]	LC[4]	LM[5]	Formula	HoM%[6]	M%[7]	HrM%[8]
Noturus phaeus	3	42	68	14	12	26msm[9], 16stt[10]	23.3	73.4	3.3
Noturus gyrinus	11	42	72	14	10	30msm, 12stt	26.6	71.2	2.2
Noturus lachneri	9	42	72	12	10	30msm, 12stt	34.8	63.0	2.2
Noturus flavus (Cooper Cr.)	2	50	70	6	—	20msm, 30stt	44.8	52.6	2.6
Noturus flavus	8	48	70	8	2	22msm, 26stt	27.8	71.2	1.0
Noturus flavipinnis	2	52	82	10	4	30msm, 22stt	38.7	59.6	1.7
Noturus miurus	11	50	74	12	8	24msm, 26stt	39.6	58.6	1.8
Noturus albater	13	66-72	82	4	—	—	—	—	—
Noturus elegans	3	46	82	8	8	36msm, 10stt	46.7	53.3	0.0
Noturus h. hildebrandi	15	46	80	12	10	34msm, 12stt	35.7	61.1	3.2
Noturus hildebrandi lautus	6	46	80	12	10	34msm, 12stt	33.8	64.2	2.0
Noturus flavater	1	44	64	14	10	20msm, 24stt	33.3	66.7	0.0
Noturus eleutherus	7	42	66	16	10	24msm, 18stt	32.7	63.6	3.7
Noturus stigmosus	1	42	62	12	8	20msm, 22stt	26.7	73.3	0.0
Noturus munitus	8	42	62	16	10	20msm, 22stt	42.4	57.6	0.0
Noturus taylori	9	40	63-64	16	12	24msm, 16stt	40.1	59.4	1.4

Source: Adapted from LeGrande *(36)*.

[1]No.= number of specimens.
[2]2N = diploid number.
[3]FN = fundamental number.
[4]LC = number of large chromosomes.
[5]LM = number of large msm's.
[6]HoM% = percent of hypomodal counts.
[7]M% = percent of modal counts.
[8]HrM% = percent of hypermodal counts.
[9]msm = metacentric and submetacentric chromosomes.
[10]stt = telocentric and subtelocentric chromosomes.

Blue catfish have performance and genes for production traits such as body shape, uniformity, resistance to *E. ictaluri*, high seinability, and high carcass yield that are radically different and better than those of channel catfish. Channel catfish have performance and genes for growth, feed conversion efficiency, tolerance of low oxygen, and resistance to *F. columnarae* that are different and better than those of blue catfish. These polymorphic QTLs of both species could be located for future physical mapping and gene isolation by positional cloning, and for transfer into channel catfish or blue catfish.

Finally, using the interspecific hybrid system for mapping is also helpful for rapid application of MAS in backcrossing programs to introgress the genomes of blue and channel catfish for production of new, improved synthetic breeds *(2)*. It is logical to construct the genetic linkage map using the same fish whose QTL performance has already been determined.

The second choice for production of mapping populations is to use the intraspecific mating plan. In this case, the two most distantly related channel catfish strains are mated to produce intraspecific F1. F2 or backcrosses to parent strains can be made. This approach may be disadvantageous since very low or no polymorphisms can be found with several types of markers. Additionally, even with microsatellites, several reference families need to be tested to analyze different microsatellite loci since for the mating parents microsatellites may be polymorphic at some loci, but homozygous at other loci.

In either case, brood stock fish are available for production of mapping populations for linkage analysis and QTL measurements. Sexually mature F1 fish are available for production of F2 or backcrosses for both interspecific and intraspecific mating plans.

THE QTLS OF CHANNEL CATFISH AND BLUE CATFISH

There are at least 30 ictalurid catfish species whose karyotypes are known *(36)*. Seven species of the ictalurid catfish are propagated in government or private hatcheries (Table 1) *(36)*, among which channel catfish and blue catfish are the most important in aquaculture. Breeding programs for channel catfish and blue catfish have been established for more than 25 years. Detailed genetic data on strain evaluation, inbreeding, intraspecific crossbreeding, and interspecific hybrids are available *(21, 22, 27, 2)*.

Channel catfish are the most important catfish in the industry because of their superior performance for growth and overall disease resistance. As summarized in Table 2, channel catfish exhibit superior growth, feed conversion efficiency, resistance to the bacterial disease caused by *Flexibacter columnarae*, and tolerance to low oxygen. Blue catfish exhibit superior performance for processing yields, seinability, and resistance to the most serious bacterial disease–enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri*. The channel x blue hybrids exhibit heterosis (hybrid vigor) for almost all economically important traits. However, mass production of the hybrid has been difficult due to problems involved in artificial spawning.

TABLE 2. SUMMARY OF PERFORMANCE DATA OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*), BLUE CATFISH (*I. FURCATUS*), AND THEIR HYBRID—CHANNEL CATFISH FEMALE X BLUE CATFISH MALE[1]

Catfish	Growth	Resistance to ESC	Resistance to columnaris	Feed conversion	Processing yields	Low O_2 tolerance	Seinability
Channel	2	3	2	2	3	2	3
Blue	3	1	3	3	2	3	1
Hybrids	1	2	1	1	1	1	2

[1]Ranking scales: 1 = excellent or good, 2 = intermediate, 3 = poor.

Strong paternal predominance is present in the interspecific hybrid, with the appearance of the F1 more similar to its paternal parent *(25)*. The maternal channel catfish x paternal blue catfish is the best genotype for superior performance of economically important traits. Inheritance studies indicate that many of the QTLs involve major genes in the phenotype determination although all of these economically important traits are controlled by quantitative loci *(21, 22, 27, 16, 20, 26, 18, 19, 17, 4, 5, 7, 2)*. These basic inheritance studies of the important QTLs are the foundation for genetic linkage mapping and QTL mapping. Once the important major genes for the QTLs are mapped in detail and cloned, catfish brood stocks can be improved through biotechnology.

CURRENT STATUS OF CATFISH GENE MAPPING

In the last decade, dramatic progress has been made in the gene mapping of mammals led by the mapping efforts in humans and mice. Such efforts have led to positional cloning of important human genes responsible for a number of diseases. The first such example was the positional cloning of the cystic fibrosis gene CFTR *(51)*, followed by the cloning of the breast cancer gene BRCA-1 *(41)*. Today, the high blood pressure-related genes, the obesity genes, and many other more important genes are being cloned. Cloning of these genes has revolutionized molecular medicine.

In agricultural science, there is a gene mapping effort underway for almost all agricultural species *(49, 46)* with two important ultimate goals: improving agricultural crops by selective breeding using MAS, and cloning important genes controlling economic traits. Positional cloning of genes from plants has been successfully demonstrated by cloning of the ABI3 gene *(30)* and the omega-3 fatty acid desaturase gene *(3)* from *Arabidopsis* and the recent cloning of disease resistance genes from tomato, tobacco, and sugar beet *(13, 35, 8)*. All progress has been made in a relatively short period because of advances in technology that allow quick identification of large numbers of polymorphic markers. Even 10 years ago, mapping a gene to clonable level in plants or animals was unbelievable.

Gene mapping in the aquacultural species is still at the initial stages. Morizot *et al. (43)* summarized accomplishments of gene mapping in fish, and recent progress is summarized in the proceedings of the aquaculture genome mapping workshop *(1)*. Several hundred markers have been mapped to the salmon map *(54)*, and more than 60 markers have been mapped for tilapia *(34)*. Even though catfish represent more than 50% of U. S. aquaculture, their genetic linkage map is limited. Only 29 isozyme loci, belonging to 17 different linkage groups, have been mapped *(37, 42)*.

Isozyme variability was first used in mapping research to estimate gene-centromere distances for six loci in gynogenetic channel catfish *(37)* and for additional polymorphic loci in blue-channel triploid hybrids. The first *Ictalurus* linkage group (LG I), comprised of loci coding for glutathione reductase and phosphoglucomutase *(43)*, was identified by segregation studies in intraspecific channel catfish crosses and F1 (channel x blue) x channel catfish backcrosses.

Recently, five more isozyme linkage groups have been established through the analysis of 550 backcross individuals from four highly informative channel x blue backcross hybrids *(42)*. Pairwise recombination estimates have been obtained for 28 isozyme locus products and one genomic DNA RFLP from subsets of the more than 550 individuals from four backcross types. These five additional multipoint linkage groups are comprised of 18 loci. Eleven additional loci segregate independently from LG I-VI and from each other. The 29 loci studied so far, then, could reside on as many as 17 of the 29 chromosomal pairs found in channel catfish and blue catfish *(36)*. Body weight, head length, head width, head depth, body depth, body width, caudal depth, caudal width, and carcass yield have been measured in the same fish that were used to establish linkage groups I-VI and the 17 unassigned polymorphisms for QTL analysis.

Opportunity for gene mapping of catfish is timely since well-developed, efficient marker systems are now available. Additionally, background research concerning genetics of economically important traits has been completed in the AAES fisheries laboratory and elsewhere *(21, 22, 24, 65, 15, 66)*. The catfish genetics information and availability of efficient marker systems make genetic linkage and QTL mapping in catfish immediately feasible.

CONCLUSION

Two factors determine the feasibility of a mapping project: availability of a proper mapping population (segregating population in terms of polymorphic markers) and availability of sufficient numbers of polymorphic markers. Interspecific hybrid brood fish from channel catfish and blue catfish are sexually mature. F2 and backcross progeny *(2, 38, 42)* of channel-blue hybrids have been produced. Several backcross reference families have been produced and are currently being evaluated for QTLs. Similarly, intraspecific reference families are being produced at both Auburn University and USDA ARS Catfish Genetics Unit at Stoneville, Mississippi. More than 400 RAPD markers, more than 3,000 AFLP markers, and several hundred microsatellite markers *(39, 40, 61)* have been produced. QTL performance is routinely measured and data about it collected through several research projects *(2, 66)*.

With the reference mapping populations, the large number of developed molecular markers, and the capabilities for QTL performance determination, catfish genetic linkage and QTL mapping should accelerate. Genetic linkage and physical mapping will ultimately result in cloning of economically important genes.

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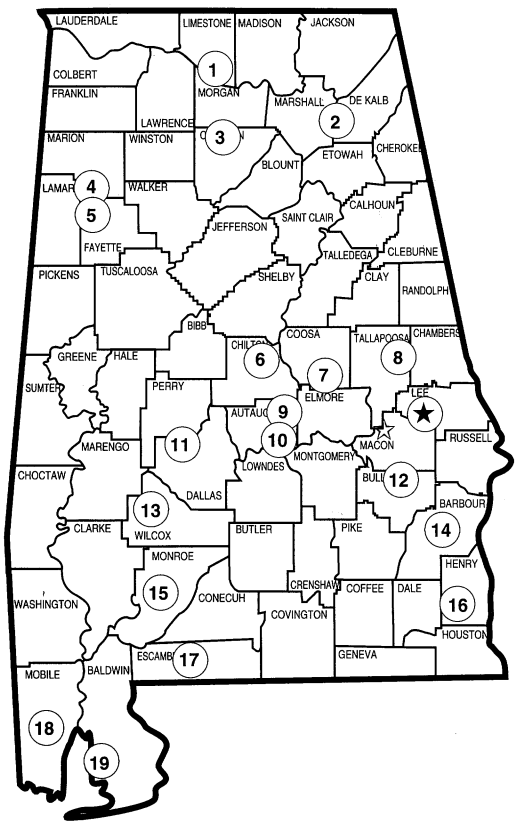

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2. Sand Mountain Substation, Crossville.
3. North Alabama Horticulture Substation, Cullman.
4. Upper Coastal Plain Substation, Winfield.
5. Forestry Unit, Fayette County.
6. Chilton Area Horticulture Substation, Clanton.
7. Forestry Unit, Coosa County.
8. Piedmont Substation, Camp Hill.
9. Forestry Unit, Autauga County.
10. Prattville Experiment Field, Prattville.
11. Black Belt Substation, Marion Junction.
12. The Turnipseed-Ikenberry Place, Union Springs.
13. Lower Coastal Plain Substation, Camden.
14. Forestry Unit, Barbour County.
15. Monroeville Experiment Field, Monroeville.
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