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Gene Mapping and Marker-assisted Selection in Channel Catfish (*Ictalurus Punctatus*)

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Abstract: Channel catfish and blue catfish are closely related species, from which fertile progenies can be made. The interspecific hybrid system, therefore, offers an ideal system for genetic linkage analysis because the system not only offers maximum contrast for phenotypic evaluation, but also provide maximum likelihood for genetic marker polymorphism. We have used several DNA fingerprinting technologies including microsatellites, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), EST polymorphic markers, and single nucleotide polymorphism (SNuP) to construct a genetic linkage map of catfish. The long term objectives of the research are to understand genomic structure, organization, composition, and evolution, to identify markers that are linked to phenotypic traits for application in marker-assisted selection, to identify genes involved in various physiological processes and understand regulation of gene expression, and to isolate and clone important genes involved in disease resistance, growth, feed conversion efficiency, body conformation and processing yields, tolerance to low dissolved oxygen, tolerance to low water quality, harvestability, and other economic traits.

We have evaluated several types of polymorphic markers and developed over 70 allozyme markers, 600 RAPD markers, 3000 AFLP markers, and 250 microsatellite markers. Application of such polymorphic markers using selective genotyping allowed identification of markers that are linked to disease resistance, growth rate, and feed conversion efficiency. These markers should be useful for marker-assisted selection in breeding programs. Genetic linkage mapping using reference families and radiation hybrid panels are underway. Large-scale characterization of ESTs are being carried out and genes involved in resistance reactions are being identified by using gene chips and gene filters. Details for marker development, phenotype-genotype linkage, identification of QTL-linked markers, and progress in functional genomics will be presented.

Key words: *Ictalurus punctatus*; Genomics; Genetic linkage; Quantitative trait loci; Mapping

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斑点鲶鱼的基因图谱及遗传标记选育研究

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摘 要：本文主要总结斑点鲶鱼基因组学研究的最新动态。斑点鲶鱼和蓝色鲶鱼是杂交可育的两个近缘种，这一杂交体系不仅为数量性状的表型带来最大的变异，同时也为基因型的多样性提供了最大的可能性。利用AFLP、RAPD、微卫星顺序标记、EST以及DNA单体多样性标记，以建立遗传连锁图。旨在克隆抗病基因、生长基因、抗逆基因、以及控制食物转化率、产肉率、收获率数量性状的基因。另外还对适合于鲶鱼基因组研究的DNA指纹技术进行了评估。并通过极端表型、基因型的分析鉴定出了与生长速度、食物转化率及抗病性有连锁的遗传标记。使用基因型分析、辐射细胞溶合及BAC基因库，以建立斑点鲶鱼的遗传连锁图及物理图，并用基因薄膜技术、基因芯片技术，以进一步掌握在特定生理条件下的基因调控。

关键词：斑点鲶鱼；基因组学；基因连锁；基因图谱；数量遗传定位

Auburn University has four major research projects in aquaculture genetics: (1) selective breeding, (2) genomic mapping and marker-assisted selection (MAS), (3) gene cloning, and (4) transgenic fish production, all with the long term goal to produce fish brood stocks with improved economic traits. Here we describe some recent progress on gene mapping and marker-assisted selection.

1 Current Status of Catfish Aqulture

Catfish is the most important cultured fish in the US and accounts for over 50% of all US aquaculture production. The catfish industry is valued at over 2 billion dollars

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and production in 1999 should exceed 750 million pounds. It is the only agricultural sector with a steady annual growth rate of over 8%. In Mississippi, Alabama, Arkansas, Louisiana, Georgia, and several other southern states, catfish is one of the top agricultural commodities. Although many species are generally referred to as catfish, the major species for the US catfish industry is channel catfish, which accounts for over 95% of catfish production. Tilapia culture is relatively small in the US, although ranked as the second or third largest cultured finfish.

Despite the development of the aquaculture and catfish industry in the US, a large US trade deficit, 3～6 billion dollars, exists annually for seafood products. This deficit is still increasing. Aquaculture appears to be more and more important, especially considering the collapsing natural fisheries[1]. According to the USDA estimates, the US demand for seafood is increasing steadily and wild fisheries will be able to supply only 25%～30% of the additional demand . Future projections predict a steadily widening gap between the world's demand for fish and the ability of the oceans to meet it[1]. Development of a profitable, productive, environmentally sound, and sustainable aquaculture industry, therefore, provides an alternative to the already over-exploited, collapsing natural fisheries.

In spite of its importance, the catfish industry is still a young industry and suffers from various production problems. A recent survey indicated that disease problems are ranked as the top concern of the catfish industry. Other concerns include traits in growth, feed conversion efficiency, carcass and fillet yields, tolerance to low dissolved oxygen, tolerance to poor water quality, reproductive success, and harvestability. Brood stocks with enhanced culture traits are urgently needed for a sustainable catfish aquaculture.

Our research goal is to provide necessary scientific and technological information for improving the catfish brood stocks by MAS, by introgression of important QTLs from channel catfish and blue catfish, and by genetic engineering using beneficial genes. To reach this goal, our initial steps are to construct genetic linkage maps using various polymorphic markers, and establish linkages of QTLs with markers.

In spite of dramatic development in many livestock animals, genomic mapping of aquaculture species is still at its infancy[20]. However, gene mapping in catfish is timely since well-developed, efficient marker systems are now available. Genetic improvement of catfish is a proven method of addressing production problems such as diseases. Our previous research in the areas of traditional selective breeding and molecular genetics has resulted in genetically improved catfish and four releases of genetically improved catfish to the industry[6].

2 Research Progress in Genomic Mapping2.

1 Economic traits, genetic variation, and heritability

Domestic channel and blue catfish exhibit significant phenotypic and genetic variation for economic traits such as disease resistance, growth rate, feed conversion efficiency, environmental stress tolerance, carcass yield, scinability, and reproduction[5,23]. 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, intraspecific crossbreeding, interspecific hybridization and genetic engineering. Disease resistance has been improved primarily through interspecific hybridization, intraspecific crossbreeding and strain selection. Tolerance to low oxygen was improved primarily by interspecific hybridization. Seinability can be improved by interspecific hybridization and strain selection, and carcass yield by strain selection, hybridization and indirect selection. Heritabilities and genetic correlations have been calculated[2]. The rationale for creating genetic maps of catfish is to increase the efficiency of selection[20]. Breeders wish to find molecular markers correlated with genetic loci controlling economic traits and use these markers to select superior brood stocks. Because traits such as growth rate are relatively easy to measure with traditional selection, a genetic map will be more useful to select fish for traits for which measurement is difficult or expensive, is lethal to brood stocks, and for introgression of alleles into channel catfish from other species with which hybrid production is feasible such as blue catfish.

2.2 Polymorphic marker development and evaluation

In the last three years, we evaluated various polymorphic markers for their usefulness in catfish gene mapping, in addition to studies on genomic organization and structure. These markers include allozymes, restriction fragment length polymorphism (RFLP), expressed sequence tags (EST), random amplified polymorphic DNA (RAPD), microsatellites, amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNuPs). Allozyme markers are type I markers and should be highly useful as anchorage points for comparative mapping. However, total numbers of polymorphic loci are small and polymorphic rates are low at each locus. RFLP markers are co-dominant markers. However, previous knowledge is required (such as probes for Southern analysis or sequences for PCR facilitated RFLP analysis). Only a few RFLP markers are available for use in catfish mapping. We do not anticipate major increases in numbers of RFLPs because developing RFLP markers is slow and costly. Our results using EST from a pituitary cDNA library indicated low polymorphism between channel and blue catfish (about 10%). After sequencing and PCR analysis of 100 cDNA clones, we obtained 11 EST markers[8]. We have evaluated RAPD markers[21～22] for their application in catfish gene mapping. Polymorphic rates are low among strains of channel catfish, but high between channel and blue catfish.

Therefore RAPD should be only useful for mapping using the interspecific hybrid system. Inheritance of RAPD markers was normal following Mendelian expectations. To date we have tested 142 primers and identified 682 polymorphic markers using the channel catfish x blue catfish hybrids. We obtained reproducible results with RAPD markers of sizes 200-1,500 bp. These markers should be useful for gene mapping analysis in the future.

Microsatellites are abundant, highly polymorphic, easy for genotyping with PCR, and are reliable because of their co-dominance. We constructed six microsatellite-enriched, small-insert libraries with channel catfish genomic DNA using the procedure of Orstrander et al[15]. Two of the six libraries were screened using radioactively labeled (CA)15 and (GA)15 oligonucleotide primers. We purified 1,530 microsatellite-containing clones (900 CA and 630 GA). Plasmid DNA containing microsatellites was prepared from 890 clones [500 (CA), 390 (GA)]. To date, we have sequenced 590 clones, of which 403 clones generated enough flanking sequences to design PCR primers[12,18]. In more than a hundred clones, microsatellite sequences were immediately at the cloning site making them useless. We tested the 403 pairs of primers for their performance in amplifying channel catfish genomic DNA. Two hundred fifty-seven of the 403 pairs successfully amplified genomic DNA and generated PCR products of expected sizes. High levels of heterozygosity were observed.

As discussed below, the channel catfish x blue catfish hybrid system offers great advantages for gene mapping of catfish. However, one requirement for using microsatellite markers with the interspecific system is that the microsatellite flanking sequences must be conserved between the two species. We addressed this issue by determination of amplifiability of blue catfish DNA using PCR primers designed from channel catfish microsatellite sequences. Over 90% loci were amplified from both species using channel catfish primers. In fact most of the microsatellite flanking sequences are conserved across the genus borders of Ictalurid catfish[12]. This indicates that more than 230 microsatellites will be useful for mapping using the catfish interspecific system. The conservation of microsatellite loci between channel and blue catfish is important for practical applications. This will allow a comprehensive linkage map to be constructed using the interspecific hybrid system and various types of markers; allozymes, RFLP, EST, RAPD, AFLP, microsatellites, and SNuPs.

In addition to type II microsatellite markers, we also developed type I microsatellite markers. Type I markers represent genes of known functions thus are more useful for comparative gene mapping. A pituitary cDNA library was enriched for microsatellites-containing clones. We obtained 50 clones of cDNAs containing microsatellite CA or GA repeats, which belong to 15 unique cDNAs after sequencing. Most clones harbor microsatellite sequences at their 3'non-translated regions (NTR) or 5'-NTR, but two clones harbor CCA and CA repeats in their coding regions[12].

AFLP markers[19] combine the strengths of RFLP and RAPD markers and overcome their problems. It is a PCR-based approach and requires no probe or previous sequence information as required by RFLP. It is highly reliable because of high stringent PCR in contrast to RAPD's problem of low reproducibility. The weakness is that they are dominant markers thus on average half of which are useful for a given backcross reference family. We evaluated usefulness of AFLP markers for gene mapping in catfish. AFLP polymorphic markers are highly abundant between channel catfish and blue catfish. They are inherited in the interspecific hybrids as dominant markers and segregated normally according to Mendelian ratios. We have tested 64 primer combinations and produced over 3,000 AFLP markers suitable for genetic mapping of catfish.

We recently started to evaluate SNuP markers. To date, we have sequenced 100 each of cDNAs from the channel and blue catfish muscle cDNA libraries. Preliminary results indicate that SNuPs (type 1 in this case since they are cDNAs) are abundant using the catfish interspecific hybrid system.

In summary, we have evaluated feasibility of using seven types of polymorphic markers in gene mapping of catfish. We concluded that microsatellite and AFLP are the two most useful types of markers for catfish mapping. Mapping the available microsatellite and AFLP markers should generate a map with less than 2 cM resolution.

2.3 Resource/reference families

Although channel catfish is the major cultured catfish, channel catfish x blue catfish hybrid system offers great advantages. The F1 hybrid is fertile and in fact we have produced F_2, F_3, and various backcrosses[2,11]. They are a major resource for QTL mapping and for MAS. We have successfully produced backcross progeny designed for this QTL mapping project. Sixteen backcross families (8 from channel and 8 from blue backcrossed with the heterozygous F_1) have been produced. These families are reared in 0.1-acre ponds ready for QTL evaluation. The interspecific hybrid system for gene mapping of catfish is advantageous because; (1) high rates of polymorphic markers are assured to exist between blue and channel catfish; (2) several important economic trait loci (ETLs) are possessed by blue catfish, mainly the disease resistance gene(s) to enteric septicemia of catfish (ESC), carcass yield genes, and the genes controlling better seinability (Dunham et al., 1993a). Mapping these important genes is of great importance by itself; (3) mapping ETLs in blue catfish is important to selective breeding programs using backcrossing to introgress beneficial genes from blue catfish into channel catfish; and (4) Drastic phenotypic variation of the hybrid system offers tools to be exploited for easy QTL evaluation and segregation.

2.4 Genotyping

Genotyping is in progress now using AFLP and mi-

crosatellite markers. It is reasonable to assume that in about a year we should have produced a linkage map for catfish.

3 Reseapch Progress in QTL Mapping and Marker-assisted Selection

We have started two types of experiments toward marker-assisted selection. The first is within our efforts for genetic linkage mapping. Phenotypes were evaluated for all the reference families to be used for mapping with the following traits: growth, body conformation (including body length, body width, body depth, head length, head width, head depth, caudal width, and caudal depth), disease resistance to ESC, and disease resistance to columnaris. The selective genotyping approach is used to have a strong phenotype selection pressure on the potential genotype differences. The top and bottom 12.5% of the individuals in each trait will be genotyped. It is reasonable to expect that markers that are linked to the QTLs controlling these traits will be differential between the best performers and the worst performers.

The second approach we used is a whole-genome QTL scan from outcrossing populations. Again, if markers are linked to a specific QTL, they should harbor variable alleles between the best performers and the worst performers. To date, we have identified one marker that are linked to QTLs controlling growth rate. The largest fish and the smallest fish harbored alternative alleles. The marker is not too far from the QTL because the recombination fraction was less than 10%. We have also identified three markers that are linked to QTLs controlling feed conversion efficiency. The relationships among these markers are not known at present because the genomic map is not yet available and linkage groups of the three markers are not known neither. One marker has been identified to be linked to ESC resistance. These identified markers, upon confirmation, will be highly useful for marker-assisted selection programs. Equally important, they will be useful for checking the success of traditional selective breeding programs. Selection for growth, for instance, should produce populations that are highly enriched with alleles linked with fast growing QTLs. This principle should be applicable to all performance traits.

4 Ongoing Research and Future Perspectives

4.1 Radiation hybrid mapping

Although the idea of irradiation and fusion gene transfer was published over 20 years ago[7], the technology was underutilized unh by combining it with efficient genotyping using PCR. Because of its extreme power for gene mapping, radiation hybrid (RH) panels are recognized as a milestone in the human genomic research and is now regarded as the ultimate tool for correction of marker orders for linkage mapping in mammals[24]. Extremely fine chromosome maps have been constructed in humans[13,17], mouse[14], and bovine[24]. Recently, RH panels have been reported for rat, baboon, dog[16], pig[25], chicken and zebrafish[3,9]. No RH panels are available for aquaculture species yet. In catfish, RH panels are desperately needed as we are nearing an initial genetic linkage map. Undoubtedly, development of RH in catfish will accelerate its genomic mapping, thereby accelerating the progress in genetic improvement of catfish brood stocks. We have initiated the development of RH in catfish.

4.2 Physical mapping using large insert libraries

Our second step after construction of a genetic linkage map is to focus on chromosomes that contain important QTLs controlling disease resistance, growth, feed conversion efficiency, and processing yields. Catfish have 29 pairs of homogeneous chromosomes[10] which make it extremely difficult to micro-dissect chromosomes for development of chromosome-specific markers. Therefore, our strategy is to first identify the chromosomes containing important QTLs of interest. Then, large insert libraries such as BAC library will be used to tag the markers that are linked to specific QTLs to specific BAC clones. Once the anchorage points are found, contigs of the BAC clones can be established rapidly surrounding the linked markers. This would allow development of chromosome-specific or regional markers for fine mapping of QTLs from the associated BAC clones. In collaboration with USDA ARS Catfish Genetics Research Center at Stoneville, our work on physical mapping is expected to start in late 1999.

4.3 Large-scale characterization of expressed sequence tags (EST)

We have isolated various organs and tissues for preparation of a universal normalized cDNA library. These tissues include muscle, eyes, brain, pituitary, gill, intestine, stomach, head kidney, trunk kidney, heart, liver, leukocyte, etc. The normalized cDNA library will allow rapid sequencing of large numbers of cDNA tags. To date, we have sequenced over 1,500 cDNA tags and our objectives are to have 30,000 tags. Upon identification and characterization, the EST library will be an indispensable resource for functional genomics.

4.4 Functional genomics and bioinformatics

Physiological genomics or functgional genomics is a newly developed branch of genomics. It applies information from genomics research to study gene expression and gene regulation under various physiological conditions. For instance, gene expression may change after infection of the host with pathogens. Such changes have been always a keen research area. However, in the past, efforts in this area was limited by genetic resources and technologies. In most cases, gene expression changes were studied by subtraction cDNA libraries, by differential display, or by 2-D protein electrophoresis. With the availability of large numbers of ESTs, unique ESTs may be arranged into arrays on gene filters or gene chips. Hybridization with probes isolated under differential conditions may reveal gene expression

changes and gene-gene interactions. Some gene may be up-regulated, while others may be down-regulated. A time course study of gene expression may well provide insight into mechanisms of gene regulation. Functional genomics, therefore, will no doubt advance our understanding of various physiological processes.

As the development of functional genomics, genetic information is explosively increasing. The number of data bases and information within each data bases are dramatically increasing, requiring a highly organized and efficient system for information storage, processing, and retrieval. Thus, bioinformatics, as a new branch of science that combines computers with molecular biology, needs a lot of attention. This requirement is urgent for agricultural sectors where traditional funding levels are low.

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