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Integration of Molecular Genetic Information into the NCCCWA Selective Breeding Program for Rainbow Trout

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Abstract

The USDA/ARS National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, is working to integrate molecular genetic technologies into a selective breeding program aimed at the genetic improvement of rainbow trout for aquaculture production efficiency. Our multidisciplinary approach aims to incorporate genome information with respect to disease resistance and stress, growth, and reproductive physiology into our selective breeding program. Selective breeding is based on the hypothesis that sufficient genetic variation exists in broodstock to realize genetic improvement through contributions of superior performing germplasm to future generations. Our associated goal is to develop and transfer improved germplasm and technologies to the aquaculture industry. Our approach to implementing molecular information into our breeding program is outlined under three objectives: (a) development of a genetic map to reveal chromosomal locations affecting traits associated with aquaculture production, (b) use of a candidate gene approach to identify and characterize genes affecting important aquaculture production traits through functional genomic technologies, and (c) development of bioinformatic strategies to implement genetic mapping and functional genomic information into a selective breeding program.

Genetic Markers and Mapping

To date, much of our efforts have focused on developing the diverse arsenal of species-specific genome tools and reagents that will be required to exploit molecular genetic data in selective breeding. To this end, a suite of DNA genetic markers called microsatellites has been developed to enhance our breeding program through a number of applications.

Microsatellites are DNA repeats of 1-6 nucleotides that occur naturally in the genome. The number of times a repeat occurs in tandem (alleles) can differ between individuals; fish that share common alleles are more closely related to each other than fish with different sets of alleles (Weber and May, 1989).

The most basic application of microsatel-

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lites is to determine parentage, allowing the evaluation of families in a common garden. For instance, fry that are too young to mark by other technologies can be combined to conduct an experiment; parents of superior performers can be identified after performance traits are measured (Palti et al., 2006). A second application is to characterize the genetic structure of a population to avoid inbreeding throughout generations of selection (Silverstein et al., 2004). Although one result of selection is increased inbreeding, the use of genetic markers can estimate the degree of inbreeding due to selection and enable monitoring to maintain an acceptable level of genetic diversity within broodstock across generations.

The most complex use of markers is the genetic mapping of genes affecting production traits. Genetic maps are constructed by observing the inheritance of genetic markers across generations of families. Traits of interest (e.g., disease resistance) can be mapped to a specific chromosomal region (or locus) by analyzing co-segregation of marker alleles with the phenotype in the same families that were used to construct genetic maps. This allows for the identification of a chromosome region (and perhaps a gene and its allelic variants) that affect a trait. Identification of such genes allows for selection based on marker genotypes, referred to as marker or gene-assisted selection, where phenotypic performance has been shown to be correlated with markers or gene allelic variants. Genetic mapping approaches target (a) traits that are expensive or difficult to measure or require sacrificing fish, (b) traits that are to be introgressed from an outside population into highly performing broodstock, (c) selection programs where multiple traits can not be evaluated in the same fish, or (d) traits estimated to have low heritability.

Genetic markers and maps have been developed for rainbow trout and other salmonids at Washington State University, the University of Guelph, INRA, and NCCCWA (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003a; Gilbey et al., 2004; Moen et al., 2004; Woram et al., 2004). These maps have been used to identify loci influencing natural killer cell-like activity (Zimmerman et al., 2004), temperature tolerance (Jackson et al., 1998), spawning date (Sakamoto et al., 1999), body weight (O'Malley et al., 2003), resistance to infectious pancreatic necrosis virus (Ozaki et al., 2001), resistance to *Ceratomyxa shasta* (Nichols et al., 2003), embryonic development rate (Robison et al., 2001), and albinism (Nakamura et al., 2001). We elected to target three traits due to their relevance to rainbow trout aquaculture production efficiency: stress tolerance (crowding stress measured by cortisol response), resistance to the bacterial pathogen *Flavobacterium psychrophilum*, and feed efficiency.

Genetic mapping aims to identify DNA sequence variation associated with a gene that is responsible for variation in a trait. Once a set of markers have been associated with a trait of interest, the next step is to identify the gene(s) responsible for the change in the trait. For this, molecular biologists and geneticists conduct a series of fine mapping experiments. This includes genotyping more markers, development of more markers targeting that specific region of the genome, investigating additional crosses or populations with linkage disequilibrium mapping, and identification of candidate genes from gene function or sequence information. Often candidate genes are selected based on comparative information from another species, whether it is functional or mapping information. In salmonids, we have the benefit of closely related species having comparative maps (Danzmann et al., 2005). We recently began to develop comparative mapping information between trout and model research species (Rexroad et al., 2005).

Fine mapping is the process of "zooming in" on a smaller and more specific chromosomal region and narrowing down the list of genes in the locus affecting the trait. An example of the need for fine mapping is the chromosomal region that controls natural killer cell-like activity in rainbow trout (Zimmerman et al., 2004). This locus is responsible for 63.4% of the genetic variation and spans a genetic distance of 25.7 centiMorgans. This

corresponds to roughly 21 million base pairs of DNA and includes roughly 200 genes.

Fine mapping is greatly facilitated by bacterial artificial chromosome (BAC) libraries. The rainbow trout genome has been estimated to be 2.4 x 10^9 base pairs. To facilitate genome research, molecular biologists work with small fragments of the genome retained in bacterial cells. These BAC libraries are manageable and conducive towards DNA sequencing and other laboratory manipulation. Libraries for rainbow trout have been constructed at the Tokyo University of Marine Science and Technology (2; Katagiri et al., 2001), NCCCWA (2; Palti et al., 2004), and Washington State University (Phillips et al., 2003). The BAC libraries can be used to construct a physical map of the genome based on overlapping restriction enzyme digest fragments, a method also referred to as DNA fingerprinting (e.g., Ng et al., 2005). Once a set of clones representing a chromosome region of interest has been obtained, they can be sequenced to identify candidate genes. These genes and the surrounding regions are then investigated to identify differences in DNA sequences between individuals that result in different phenotypes.

Candidate Gene Approach

The candidate gene approach uses information about known genes to investigate potential effects on traits of interest. For instance, the Major Histocompatibility Complex in humans and many other vertebrates has been shown to mediate immune response to pathogens, therefore it is reasonable to expect that the corresponding Major Histocompatibility Regions in trout would serve the same function. The candidate gene approach is greatly facilitated by a large volume of gene sequence data from which to identify candidates. This is obtained by partially sequencing genes from different tissues, periods in the life cycle, or experimental treatments. The goal of obtaining these representative sequences is to identify as many unique genes as possible and associate them with a function.

GenBank, a genome database hosted by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), currently has 238,460 gene sequences from rainbow trout, about 50% of which were submitted by NCCCWA. Use of this large amount of data is facilitated by bioinformatic analyses such as those conducted by The Institute for Genome Research (TIGR). The TIGR gene indices are a user friendly and open access database to retrieve information (Rexroad et al., 2003). This includes potential or predicted protein sequences, potential mutations, functional annotation, and comparative information obtained by comparing with sequences from other species. The large volumes of gene sequence information have enabled the use of microarray technology for salmonids (Rise et al., 2004; Krasnov et al., 2005; Tilton et al., 2005; Von Schalburg et al., 2005). This technology allows investigators to examine the expression of thousands of genes simultaneously, whereas traditional protocols address each gene individually.

Conclusions

Significant progress has been made through international collaborations in developing tools for rainbow trout genome research including genetic maps, expressed sequence tags, and bacterial artificial chromosome libraries (Thorgaard et al., 2002). The addition of these resources enhances our research programs and facilitates the development of new selective breeding strategies which employ molecular genetic data.

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