APPLICATIONS OF MOLECULAR GENETICS TO FISH BREEDING

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Molecular genetic studies relevant to fish and shellfish breeding have advanced significantly in recent years. This has been true both because of the applied interest in aquaculture for a number of these species and the use of a few aquatic species to address fundamental questions in genome structure, development and other basic research areas. The principal areas of molecular genetic research include genetic mapping, sequencing, QTL analysis, and studies of transgenics.

Genetic mapping has progressed in several important aquaculture species, including tilapia, catfish, trout, oysters and shrimp. Most of these species are not closely related to each other, requiring independent marker development and inhibiting simple extrapolations regarding marker distributions on chromosomes. The tilapia are a complex of related species which are important in tropical aquaculture around the world. Efforts in tilapia have been directed toward development of a microsatellite-based map which can be used for QTL analysis of complex traits (Kocher et al., 1998). The channel catfish is the leading aquaculture species in the USA. A map of microsatellite markers has been developed for this species (Waldbieser et al., 2001) but has not yet been applied for QTL analysis. Two rainbow trout maps have been developed (Young et al., 1998; Sakamoto et al., 2000), the first primarily AFLP-based and the second microsatellite-based, and these maps are in the process of being merged by placement of common markers on both. Other salmonid species for which maps are under development include the brown trout, Arctic char and Atlantic salmon. Among invertebrate aquaculture species, mapping efforts have primarily been directed at shrimp of the genus Penaeus (Wilson et al., 2002) and the oyster Crassostrea gigas, in which a very high genetic load results in unusual segregation ratios, complicating mapping (Launey and Hedgecock, 2001).

Sequence data is also beginning to be collected for many species. To date, among aquaculture species, this has primarily involved EST analysis of cDNA libraries (e.g., Nam et al., 2000). Larger-scale genomic efforts are likely to proceed in the near future, as BAC libraries have been prepared for several species and ordered arrays of these libraries are being developed. Major efforts of this type are now underway for Atlantic salmon in Norway and Canada.

Genomic and mapping studies of model species such as zebrafish, fugu and medaka are progressing even more rapidly. The fugu genome will soon be fully sequenced and is of particular interest because it is one of the smallest genomes among the vertebrates (Venkatesh et al., 2000). The zebrafish has been widely adopted as a representative lower vertebrate model for biomedical research and has both a well-developed microsatellite map (Knapik et al., 1996) and the prospect of a complete genome sequence in the near future. The medaka is also an important representative lower vertebrate model species with a well-developed genetic map.
(Naruse et al., 2000). The extent to which data from model species can be extrapolated to cultured species is a major question. The zebrafish genetic map is being related to that of mammals (Postlethwait et al., 1998) but the extent of genome reorganization among fishes is still unclear. If gene orders are substantially conserved among fish species, the more intensive mapping and sequencing efforts being applied to model species may be quite valuable for making extrapolations to commercially important but less intensively studied fish species. The principal invertebrate aquaculture species (oysters and shrimp) are not closely related to intensively studied invertebrate model species such as Drosophila and the nematode Caenorhabditis elegans, and this will make extrapolations about genome structure much more difficult for those animals.

Genetic analysis of complex traits requires appropriate crosses and reference families and has generally not progressed as rapidly as mapping per se. Lines with distinctive phenotypic differences have rarely been developed and characterized in aquaculture species and when available they have not often been used for QTL analyses. The most progress in this area has come in trout. QTL studies in rainbow trout include investigations of spawning time (Sakamoto et al., 1999), thermal tolerance (Perry et al., 2001), development rate (Robison et al., 2001) and resistance to the virus IPNV (infectious pancreatic necrosis virus) (Ozaki et al., 2001). A study in tilapia has associated growth rate with the prolactin locus (Streelman and Kocher, in press). These studies are likely to progress significantly as marker coverage improves and as mating designs allow finer localization of loci and association with candidate genes.

Advantageous chromosome engineering methods which aquatic species can tolerate have not been fully exploited. Aquatic species are more like plants than like mammals in their ability to tolerate genetic manipulations such as production of doubled haploids through androgenesis and gynogenosis. Such manipulations allow rapid production of reference lines which can be valuable for research purposes and allow mating designs such as segregation in doubled haploids which maximize experimental power (Robison et al., 2001). Studies with clonal line crosses also allow less costly (but admittedly more difficult to extrapolate) marker systems such as AFLPs to be utilized in mapping and QTL analyses. The rainbow trout clonal model (Young et al., 1996; Robison et al., 1999) has been the principal example to date of exploiting such manipulations for mapping and QTL analysis, although clonal lines are available in other aquatic species including zebrafish (Streisinger et al., 1981), tilapia (Sarder et al., 1999), medaka (Naruse et al., 1985) and carp (Bongers et al., 1997). Fertility problems of homozygous females are likely one of the major impediments to wider application of these approaches.

Transgenic approaches have been highly successful on a technical level with fishes. The ease of introducing genes into the eggs of externally fertilizing aquatic species and the ease and low cost of rearing these animals has facilitated dramatic progress over the last two decades. Fish with large improvements in growth rate have been produced and propagated (e.g., Devlin et al., 2001; Rahman et al., 2001). Enhancements in disease resistance are likely to be another focus area in the future.

Although the technology has progressed very well, utilization of transgenics has been limited due to concerns regarding the potential impacts of escape of transgenic fishes on natural
populations and the environment (Hallerman and Kapuscinski, 1995; Hallerman et al., 1999). These concerns have limited the application of this technology in many countries for regulatory reasons, although some countries may not enforce such limitations. Addressing the concerns is likely to involve better confinement as well as the application of methods to sterilize transgenic individuals to prevent their possible reproduction.

Overall, progress in the application of molecular genetics to aquatic species has been substantial and in some cases (such as transgenic technologies) has equaled or advanced beyond the state-of-the-art in mammals. By exploiting the advantages associated with external fertilization, tolerance to genetic manipulation and the availability of well-studied model species, even more rapid advances are likely in the near future.

REFERENCES