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What Can History Tell Us about the Future of Aquaculture Genetics

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Abstract

Historically, genetics has not been a major part of aquatic sciences education or practices. However, it is interesting to note that one of the first animals used for genetic research after rediscovery of Mendel's results was a Poeciliid, i.e., the guppy. Furthermore, the use of genetic principles and practices for improvement of aquatic species raised under controlled or semi-controlled conditions has not become a standard component of aquaculture production, contrary to the situation with other agricultural plants and animals. A number of explanations for the lack of utilization of otherwise accepted genetic approaches will be explored. In addition to the seeming reticence to employ the standard genetic approaches in aquaculture, realization of the purported promise of some of the more recently developed molecular genetic tools has been slower than anticipated. In addition to the difficulties experienced with deployment of this technology, the costs and the need for larger multidisciplinary teams to develop the tools for reliable analyses have raised challenges not previously experienced in the field. These factors have led to increased efforts to utilize cooperative approaches to major genetic problems that need to be solved. It would appear that future employment of modern genetic analytical tools to improve aquaculture production will be enhanced and, in fact, further assured via cooperative research ventures.

Introduction

Among the results we look forward to from this workshop are (1) definition of the most beneficial directions to proceed with future aquaculture genetics research and (2) identification of research opportunities that will facilitate cooperative approaches for making rapid

advances to enhance and sustain aquaculture production. It is anticipated that the collective imaginings of the expertise gathered for this event will provide the resource to envision the combinations of genetic methods, tactics, investigators, etc., that will fully utilize avail-

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able scientific "power" for advancing the industry to its full potential. However, before the future research picture can be envisioned with respect to what is useful and beneficial we must define our starting parameters and do some assessment of the constraints and advantages these may impart. Consequently, the following presents a short review of the status of aquaculture genetics research and an assessment of the application and utility of the results to date.

Before starting on this endeavor, it would be beneficial to define the breadth of what will be included in considering aquaculture genetics research. Aquaculture is a relatively new and developing enterprise, particularly from a commercial production perspective. Consequently, there is a broader array of genetic factors to consider than simply making a comparison with traditional agricultural production genetics. Frequently, reviews of aquaculture genetics include only those areas of genetics that deal with the improvement of organisms for growing under artificial conditions (i.e., quantitative genetics, selection, breeding, etc.). These areas have had a demonstrably important impact on traditional agricultural plants and animals and have been shown to be effective in modifying species raised in aquaculture. However, in addition to those aquatic species raised primarily for food production, there are important commercial interests in other types of production (e.g., aquarium fish, sport fishing enhancement, endangered species, and pharmaceuticals) into which may be interjected somewhat different genetic factors and approaches. Furthermore, since domestication of most species used for aquaculture production is a relatively recent occurrence, natural populations for these species are still extant and should be treated as a crucial genetic resource for the future. The genetic foci for tackling these latter issues will require different methods than those traditionally utilized for agricultural production.

The problem these considerations raise for reviewing the status of aquaculture genetics is primarily one of developing an organization to encompass the relevant information into a meaningful and concise presentation. In

attempting to accomplish this task I re-read a paper by Dr. Jim Lester in which he presented his ideas on the role of genetics in responsible aquaculture in a symposium on "Responsible Marine Aquaculture" (Lester, 2002). In this paper he divides the role of genetics into two major categories, monitoring and modification. Genetic monitoring encompasses those techniques that provide information on the diversity and similarity of stocks and that can be used to identify biological units such as species, populations, hybrids or families. Genetic modification utilizes knowledge of the phenotypes, genotypes and sometimes pedigrees of breeders to effectively design and evaluate genetically-based improvement programs. While these two categories cover a great deal of the range of genetic analyses in aquaculture, I would like to add another, mining. Genetic mining comprises those techniques geneticists use to search for information on the control, function and gene composition of biological systems. These results may be useful for future genetic modification or may provide new "tools" for genetic monitoring of biological groupings of organisms. This third category of genetic investigation is primarily an outcome of increasing analytical capabilities with DNA, RNA, proteins and other biochemical constituents of cells. These three categories of genetic inquiry cover a major portion of the analyses currently utilized in aquaculture genetics, so let's take a look at the status of each with respect to its influence and potential for exploitation.

Genetic Modification

Genetic modification is the array of techniques that has the most direct influence on aquaculture production, especially since it accompanies domestication and human mediated change in cultured organisms. The classic approach to genetic modification is selective breeding, but there are a number of other methods that have also been employed in aquaculture studies; these include cross-breeding and hybridization, chromosome set manipulation, and transgenesis. The status of each of the procedures and its impact on aquaculture are considered below.

Selective breeding. This approach to genetic modification probably has the longest history of any of the methods mentioned. One of the first selective breeding programs with an aquatic species reported in the scientific literature was by Embury and Hayford (1925) in which they reported increased resistance to the disease furunculosis in brook trout (*Salvelinus fontinalis*) by selectively breeding fish that survived endemic exposure to the disease. Another early selection program is one initiated in 1932 to produce the “Donaldson” strain of rainbow trout (*Oncorhynchus mykiss*) for which significant increases in growth and fecundity were reported (Donaldson and Olson, 1955). Interestingly, in spite of the early reports of success with selective breeding, very little research in this area was apparently conducted on aquacultured species prior to 1970. This is also about the time that interest in aquaculture was emerging as a viable commercial endeavor.

Subsequently a number of selective breeding programs were developed on a wide array of aquaculture species in a variety of countries (Moav and Wohlfarth, 1976; Smitherman and Dunham, 1985; Gjerde, 1986; Hershberger et al., 1990; Eknath et al., 1993). The status of these programs has been periodically reviewed (e.g., Gjedrem, 1997; Hulata, 2001). Based on the results obtained with traditional agriculture plants and animals and the initial results from selective breeding programs with aquaculture species, such an approach should prove to be very effective in producing genetic modifications that are beneficial for the industry.

Results to date with aquaculture species suggest that 10% change per generation in some selected traits can be anticipated with most commercial conditions. Even with this relatively high level of trait improvement and the fact that 10 of the 19 principal aquaculture species, as defined by FAO, are involved in selective breeding programs, very few of these programs have been assumed by the industry; most are supported by state, national or international research organizations. The most mature industry-operated selective

breeding program is that for improvement of Atlantic salmon (*Salmo salar*) for the net pen industry in Norway (Gjedrem et al., 1987). This is contrary to the situation with most other traditional agricultural species and may be a reflection of the early stages of industry development.

With the advances made in the techniques for molecular analysis of DNA, a number of new analytical approaches to assist genetic selection have been developed to potentially make selection more precise and more rapid (Poempuang and Hallerman, 1997). For example, the ability to identify and locate quantitative trait loci (QTL) within the genome of aquaculture species will allow a more directed approach to trait improvement. If we add to this the capability to use genetic variation that is closely associated with the QTL in either marker assisted selection (MAS) or gene assisted selection (GAS), the precision and speed with which gains can be realized should be greatly improved. Although we will undoubtedly hear a lot about advances in this area in the next several days, it appears that practical application of these technologies in the industry is 10-15 years into the future.

Crossbreeding/hybridization. The use of crossbreeding, or crosses among organisms of the same species for genetic modification is grounded in the concept that heterotic expression in traits can be obtained when individuals from genetically different groups are crossed. Results with aquaculture species have been very variable when this approach has been utilized. Common carp (*Cyprinus carpio*) crossbreds are the basis for the carp aquaculture industries in Israel, Vietnam, China and Hungary (Wohlfarth, 1993; Bakos and Gorda, 1995; Hulata, 1995). However, in many other aquaculture species crossbreds yielded very variable proportions of heterotic expression. The explanation for these results may reside in the amount of inbreeding introduced into the species either naturally or artificially. There has not been much directed development of inbred lines with aquatic species.

Hybridization, or crosses between different species of organisms, has been attempted rather broadly among aquaculture finfish

species to address a variety of needs. While most hybrids produced have not proven beneficial for aquaculture, a few valuable hybrids have been commercially exploited. The hybrid between the female white bass (*Morone chrysops*) and the male striped bass (*M. saxatilis*) and its reciprocal is commercially cultured, mainly in the U.S. (Van Olst and Carlberg, 1990) because it has better overall culture characteristics than either parent species (Harrell, 1998).

Combinations of other species have been exploited for sex control. For example, crosses of the grass carp (*Ctenopharyngodon idella*) with the bighead carp (*Aristichthys nobilis*) yield triploids (Marian and Kraznai, 1978) which are sterile. This hybrid attracted the attention of the U.S. fisheries biologists because it was herbivorous and could be used to control aquatic vegetation in important waterways and would not be able to naturalize due to its sterility. Hybridization among tilapia species was considered as a promising approach to produce all-male progeny and control unlimited reproduction that led to overpopulation of culture ponds. Crosses between Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*O. aureus*) yield predominantly male offspring; crosses of other species were also found to produce mostly males (Wohlfarth and Hulata, 1983). The most widespread use of this technology is in Israel, although it suffers from management problems dealing with the maintenance of pure species (Wohlfarth, 1994).

The need for aquaculturists to maintain at least two pure species for the utilization of hybridization, or at least two inbred lines for crossbreeding presents a big obstacle in the use of this technique for genetic modification. Furthermore, the results from hybridization or crossbreeding are not possible to predict and therefore require a considerable investment into a "trial and error" approach to the definition of crosses that yield positive aquaculture attributes.

Chromosome set manipulation. This type of genetic modification is induced by changing the number or composition of chromosome "sets" (haploid number) that an organism con-

tains (Ihssen et al., 1990). Normally most organisms contain two sets of chromosomes (diploid), one set from each parent. This can be altered by the use of physical or chemical treatments that interrupt the normal meiotic or mitotic division cycle in very young embryos (i.e., prior to the first cell division). The aquatic organisms resulting from these procedures can vary from completely homozygous diploid individuals to individuals that have four or more sets of chromosomes. The biological consequences from such changes can also vary from gigantism to complete sterility. Thus, the changes we see from these genetic modification procedures are major and can be beneficial to scientific research as well as to aquaculture production.

The most broadly commercially utilized type of chromosome set manipulation is that producing triploid animals, or those with three sets of chromosomes (3N). The presence of three chromosome sets leads to the uneven distribution of chromosomes during division of the cells and, consequently, induces sterility in such animals (Thorgaard, 1983). This can have particularly large impacts on the production of animals where reproduction adversely affects survival or the quality of the flesh. Beyond the elimination of the negative effects of reproduction, energy normally shunted to this biological process is available for growth and leads to increased size. Commercial production of 3N oysters and all-female 3N rainbow trout is a growing part of these two aquaculture industries (Allen et al., 1984; Benfey, 1996).

The other type of chromosome manipulation that has been beneficial, although mostly from a research perspective, is the production of mitotic gynogens and androgens. Mitotic gynogens are diploid, contain only female chromosomes and are completely homozygous; androgens are also diploid and completely homozygous, but have only male chromosomes. By performing two consecutive treatments to make either gynogens or androgens, aquatic animals can be produced that are genetically identical, or the equivalent to clonal individuals. Such animals have been extremely useful for genetic identification of

QTL differences among populations and for mapping of these loci (Young et al., 1996). It might also be speculated that, with the high levels of homozygosity in these genetically modified organisms the raw material may be available for the use of crossbreeding to realize some benefits from heterosis.

Overall, the efficacy of chromosome set manipulation as a potential method to affect major genetic modifications in aquatic organisms has been demonstrated and the benefits of some of these realized. Additional experimentation is needed to more precisely define the treatment procedures to realize consistent results.

Transgenesis. As the molecular technology improved to allow the assembly of functional DNA units containing genes that had the potential to improve performance traits (e.g., growth or disease resistance) research was conducted to investigate the consequences of injecting such assemblages into production organisms. Aquaculture species were a major part of these investigations beginning in the 1980s (Maclean, 1998). Most of the investigations with aquaculture species were devoted to growth enhancement by injection of foreign growth hormone constructs. As an aside, many aquaculture species were attractive candidates for such studies because their external reproduction and relatively large ova facilitated the injection of DNA constructs during the initial developmental stages. While a number of constructs containing genes such as antifreeze genes and antimicrobial peptides have been attempted with aquaculture species, the transgenic that is closest to commercial application for food production is one involving improved growth in Atlantic salmon (Entis, 1997). Interestingly, success in this case was achieved with a construct comprised of all fish genes (ocean pout promoter and chinook salmon growth hormone gene).

Some major production benefits have been realized with the use of transgenic plants in agriculture, although in some places (e.g., Europe) consumer response to these materials has not been positive. It appears that transgenic agriculture animals have not

demonstrated attributes that are as beneficial as those shown in plants and the sensitivity of the farmers to the lack of positive consumer perception have limited their use in production. Furthermore, the random nature of the incorporation of the DNA construct into the genome has resulted in some undesirable side-effects (e.g., skull malformations, arthritic conditions). In addition, with aquaculture there is a major concern about escapees and their impact on natural populations. Overall, while transgenesis holds the potential to provide some major benefits to aquaculture production it is currently of limited commercial interest and additional experimental work is needed to improve the precision of the technology.

Genetic Monitoring

The relevant analyses utilized for this category of genetic evaluations are based on obtaining information on distinct gene differences that can be counted and quantified to identify relationships among biological groupings of organisms. Genetic monitoring analyses are based on the use of statistical models that require distinct, countable gene variants. Consequently, broad utilization of these models required the expansion of our capabilities to identify gene products that could be individually identified, counted and quantified. While a number of what have been termed “qualitative gene differences” were identified and analyzed (e.g., blood types and color variants) in early studies, the most significant step forward was the utilization of electrophoretic separation of macromolecules combined with histochemical staining techniques (Utter, 1991). This combination of biochemically-based analytical approaches led to a veritable “Pandora’s box” of genetic variability that had not been previously identifiable: the development of molecular DNA technology has expanded the power of these analyses tremendously (Wright and Bentzen, 1995).

With the current array of genetic variants that can be analyzed relatively easily from populations, both natural and artificial, it is possible to obtain a lot of useful genetic information. Studies will yield data to identify populations and other reproductive subgroups,

evaluate genetic diversity within a group, measure changes in populations, and allow genetic marking of different groups. Utilization of the results from genetic monitoring is somewhat of a separate topic on which major emphasis is placed for investigating and instituting the management and conservation of naturally produced aquatic species resources. However, there are important intersections between these areas and aquaculture genetics that should be emphasized and not be ignored.

The importance of most of these intersections is, in my view, couched in the concept that natural populations are the ultimate sources of future genetic variability and, thus, are invaluable resources that need to be conserved. Among agriculture organisms aquatic species have the rather unique characteristic of extant natural populations which should be treated with appropriate care to enable future utilization. Genetic monitoring will provide the evaluation needed to ensure this goal is met.

Genetic Mining

It could be logically argued that genetic mining is an activity that has been part of this area of science since the rediscovery of Mendel's principles and this would be hard to dispute. However, geneticists have not had the opportunity previously to conduct "mining" studies and expect a yield that provided information on such large numbers of genes and in the specificity of activity that can now be realized. These characteristics have been possible via molecular DNA technology development and innovation. Utilization of microarray techniques can yield information on the activation of hundreds, perhaps thousands of individual genes in an organism. Further, depending on the extent of the information available about the array, the genes being activated and their relative activities can be determined. Putting this information together with a physiological or disease challenge can provide many insights into the functional relationships among genes with respect to their response.

Genetic mining in aquaculture species at this level is a relatively new, but rapidly evolving endeavor. Much of the basic DNA infor-

mation required for detailed analysis of the results of such investigations needs further development. However, it has already become apparent that data from these studies will require much more powerful analytical tools for thorough analysis and interpretation. Information developed to date has demonstrated the exciting potential contained within this arena and it will provide new insights about the genetics of the organisms being raised in the aquatic environment.

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