

## ENVIRONMENTAL EFFECTS AND GENOTYPIC VARIABILITY OF FISHES

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**Keywords:** polymorphism, genotype, environmental

**Abstracts:** With increased demand for aquacultured foods has come a need for more efficient production systems. Major improvements have been achieved through enhanced husbandry procedures, improved nutrition, enhanced disease diagnosis and therapies and the application of genetics to production traits. Although several aquaculture species have been greatly improved through the application of genetics, much greater improvements can be accomplished (Dunham *et al.*, 2001). Genetics can greatly contribute to production efficiency, enhancing production and increasing sustainability. Resource utilization can be greatly improved and impediments to sustainability, such as slow growth of fish, inefficient feed conversion, heavy mortality from disease and the associated use of chemicals, loss of fish from low oxygen levels, inefficient harvest, poor reproduction, inefficient use of land space and processing loss, can all be diminished by utilizing genetically improved fish. Genetic enhancement of farmed fish has advanced to the point that it is now having an impact on aquaculture worldwide, but potential maximum improvement in overall performance is not close to being achieved

### INTRODUCTION

Aquaculture genetics actually had its origin with the beginning of aquaculture in China and the Roman Empire more than 2000 years ago. Without realizing it, the first fish culturists changed gene frequencies and altered performance of the wild-caught fish, actually genetically enhancing the fish for fish-farming application by closing the life cycles and domesticating species such as the common carp, *Cyprinus carpio*. When the Chinese, Europeans and others observed mutations and phenotypic variation for colour, body conformation and fins studies, and then selected for these phenotypes as well as for body size, selective breeding, the predecessor of molecular genetics and biotechnology, of fish and shellfish was born. Additionally, fish culturists and scientists who compared and evaluated closely related species for their suitability for aquaculture application over the past two millennia were also unknowingly conducting some of the first fish genetics research. Closely related species are reproductively isolated and have species status because of their genetic distance from one another; therefore, the comparison of different species is a genetic comparison (Dunham *et al.*, 2001). However, directed breeding and genetics programmes were probably not intense and strongly focused until the Japanese bred koi in the 1800s and the Chinese developed fancy goldfish. Of course, fish biotechnology and molecular genetics research and development share the same beginnings as biotechnology and molecular genetics as applied to other organisms when in 1665 Robert Hooke described cellular entities and developed the cell theory. Shortly thereafter, in 1667, Anton van Leeuwenhoek discovered that semen contained spermatozoa and theorized that they could fertilize eggs, although this was not substantiated for another 200 years. A series of discoveries during the 338 years that followed have led to the current state of biotechnology and molecular genetics. The foundation for electrophoretic analysis was laid in 1816 when R. Parnet reported the effect of electric fields on charged particles, including proteins (Richardson *et al.*, 1986). Brown recognized the nucleus as a regular, constant cellular element within cells in 1831, and in the 1840s Carl Nageli had observed that the nucleus divided first in dividing cells but did not understand the significance of this observation.

Of course, one of the most important keys for the emergence of the field of genetics occurred in 1866 when Gregor Mendel discovered the existence of genes and their transmission from generation to generation. Shortly thereafter, in 1869, Friedrich Miescher discovered deoxyribonucleic acid (DNA), although the full implications of this discovery were obviously not completely understood.

Fish genetics programmes first emerged in the 1900s after the basic principles of genetics and quantitative genetics had been established. However, there was not a substantial effort in fish genetics research and the application of genetic enhancement programmes until the 1960s because of the infancy and small scale of aquaculture, a lack of knowledge of fish genetics and a lack of appreciation of genetic principles by natural resource managers regarding genetic enhancement, population genetics and conservation genetics.

In 1985, Jeffreys developed DNA fingerprinting technology (Jeffreys *et al.*, 1985), revolutionizing not only population genetic analysis and gene-mapping technology, but also forensic and criminal science. The current state of modern molecular genetics and genomics research would not have been possible without the revolutionary invention of the polymerase chain reaction (PCR) by Kary Mullis in 1985.

In the last few years, technological advances in DNA marker technologies and DNA microarray and gene chip technologies have further accelerated the pace of aquaculture and aquaculture genomics. Genomic research has produced vast amounts of information towards an understanding of the genomic structures, organization, evolution and genes involved in the determination of important economic traits of aquatic organisms. Positional cloning of genes from aquatic species is no longer a dream. Zhanjiang Liu, in the 1990s–2000s, has isolated and sequenced more than 12,000 genes

from aquaculturally important species, such as channel catfish, and an almost complete genomic sequence for *Sphaeroides maculatus* has just been published (Aparicio *et al.*, 2002).

Currently, efforts are well established in the areas of traditional selective breeding, biotechnology and molecular genetics of aquatic organisms. Cultured fish are being improved for a multitude of traits, including growth rate, feed conversion efficiency, disease resistance, tolerance of low water quality, cold tolerance, body shape, dress-out percentage, carcass quality, fish quality, fertility and reproduction and harvest ability. For many years there has been a cry in the wilderness that aquaculture is impeded by the lack of genetically improved fish and the utilization of essentially wild fish. This is still true for some species and for new aquaculture species; however, for a few well-established aquatic species, large genetic gain has been realized, and there is evidence of up to tenfold improvement of some traits compared with that of poor-performing, unimproved wild strains by combining various combinations of traditional selective breeding and biotechnology.

The development and utilization of genetically improved fish are widespread across the world in the 21st century. A variety of genetic techniques are being implemented commercially, including domestication, selection, intraspecific crossbreeding, interspecific hybridization, sex reversal and breeding and polyploidy, to improve aquacultured fish and shellfish. Genetically improved fish and shellfish from several different phylogenetic families are utilized. Genetic principles and biotechnology are also being utilized by fisheries managers and by researchers to enhance natural fisheries, to protect native populations and to genetically conserve natural resources. Genetically modified aquatic organisms are already having an impact on global food security in both developed and developing countries. However, in general, much more progress can and needs to be made. The combination of a variety of genetic improvement programmes – traditional, biotechnological and genetic engineering – is likely to result in the best genotypes for aquaculture and fisheries management.

### **THE CLONING PROCESS AND POPULATIONAL GENETICS**

Clonal populations of fish can be generated in a non-specific manner, utilizing gynogenesis and sex reversal. Mitotic gynogens are produced by blocking the first cell division, and these individuals are 100% homozygous. However, they are not clones of their mother as their genotype is different from their dam because of independent assortment and recombination during meiosis and because of the absolute homozygosity induced from the gynogenesis. Additionally, each mitogynote is different from its siblings, and each is variably homozygous for a variety of allelic combinations. Further, the breeder has no control over which genotype survives and is generated by the gynogenesis. Clonal populations can be produced from these mitogynotes by performing a second generation of mitotic gynogenesis on the first generation mitotic gynogen. Presumably, more than one offspring would be produced. Then a portion of the fry would be sexreversed, both sexes would exist, although genetically identical, and large numbers of identical fry would be produced via natural mating. Future male brood stock would again be perpetuated via sex reversal. Theoretically, clonal populations could be generated in a similar way from androgens. The primary difficulty in this breeding scheme is that the mitogynotes and androgens are maximally inbred and 100% homozygous, often resulting in reproductive problems. Cloned populations have been produced in zebra fish, ayu, common carp and rainbow trout (Komen *et al.*, 1993). Theoretically, clones – individuals with identical genotypes for their entire genome – should have identical, non-variable performance. However, individuals with extreme homozygosity appear to lose significant ability to respond to the environment in a consistent stable manner, and microenvironmental differences affect performance among individuals (Komen *et al.*, 1993). Thus, as the component of genetic variation decreases, actually becoming zero, the component of environmental variation increases and at a more rapid rate than the genetic component decreases, resulting in populations with extreme phenotypic variation. Homozygous gynogens of ayu (*Plecoglossus altivelis altivelis*, Temminck & Schlegel, 1846) show increased variation for size and for meristic traits. Rainbow trout clones exhibit greater amounts of bilateral asymmetry – unequal counts for meristic traits on the right and left sides of the bodies – compared with controls, isogenic crossbreeds and normal crossbreeds. Bilateral asymmetry has been previously documented in inbred rainbow trout and is

associated with a reduction in biochemical genetic variation. Isogenic crossbreeds – crossbreeds resulting from mating two clonal lines – had a similar bilateral asymmetry to that of outbred crossbreeds (Komen *et al.*, 1993). Almost identical results have been obtained for clonal rainbow trout. Clonal lines of rainbow trout were produced by androgenesis, followed by another generation of androgenesis or gynogenesis, followed by retention of the second polar body (Young *et al.*, 1995).

Clonal populations were more asymmetrical and showed higher variance in total meristic counts than either the isogenic crossbreeds or outbred crossbreeds, indicating reduced fitness in the clonal populations and their ability to react to microenvironmental variation. There was no difference in asymmetry between outbred crossbreeds and isogenic crossbreeds. Fluctuating asymmetry of spotting pattern was also investigated as a potential measure of developmental stability. Asymmetry of spotting pattern was not different in homozygous clones compared with isogenic crossbreeds, but the spotting pattern had a low coefficient of variation within lines and may be useful for identifying isogenic lines and monitoring genetic divergence between lines. The increased developmental stability and decreased variance of meristic traits in the isogenic crossbred rainbow trout indicate that they were less influenced by environmental conditions and may make better research organisms than the homozygous clones. Vandeputte *et al.* (2002) used a homozygous clone outcrossed to normal common carp as a powerful internal scaled control for the evaluation of mirror carp strains.

#### **GENETIC VARIATION AND POPULATION BIODIVERSITY**

Theoretically, genetic variation is beneficial and important. Genetic variation is important for the long-term survival of a species. Genetic variation can ensure the fitness of a species or population by giving the species or population the ability to adapt to changing environments. Obviously, a lack of genetic variation or too much homozygosity can be detrimental to an individual's or a population's survival traits and fitness. The cheetah is a prime example of the potential detrimental effects of excess homozygosity. This highly homozygous species has severe reproductive problems. Homozygosity has also been correlated with bilateral asymmetry (fluctuating asymmetry) – unbalanced meristic counts on the right and left halves of the body – in fishes. Additionally, highly or totally homozygous individuals and populations actually exhibit greater phenotypic variation than outbred controls because they are more greatly affected by environmental or microenvironmental change and have reduced homeostatic ability compared with more heterozygous individuals and populations. Inbreeding in small, natural populations increases extinction rate (Doyle, 2003). Inbreeding depression resulting from increased homozygosity is well documented in fish (Dunham *et al.*, 2001). Field crops have been endangered when they did not have the genetic variation to respond to new pathogens or plagues. Clearly, the existence of genetic variation is important to the long term survival and fitness of a species. Many natural populations respond to different forms of selection, such as directional, bidirectional, cyclical and stabilizing selection, which help to ensure the maintenance of the genetic variability and/or fitness of populations. Levels of homozygosity and inbreeding can be important not only in domestic or aquaculture populations, but in wild populations as well. Inbreeding does adversely affect reproductive success in wild deer (Slate *et al.*, 2000).

The majority of inbreeding experiments on fish (Dunham *et al.*, 2001) and other organisms have been done in aquaculture and laboratory-type environments. Some have hypothesized that inbreeding depression would be more severe and affect fitness more adversely in the harsher natural environment compared with the laboratory environment or aquaculture environment where animals are well taken care of. Depending upon population structure, inbreeding can be

prevented in natural populations via migration. However, migration rates may need to be larger than previously expected to prevent inbreeding.

Genetic variation is usually considered desirable; cases may exist where a lack of genetic variation may enhance an organism's short-term fitness when a population is highly adapted for a particular environment. Theoretically, the introduction of inferior genotypes could reduce a population's fitness, and some conservation geneticists have coined the term outbreeding depression for this population phenomenon, although it is not well documented. Outbreeding depression is usually related to temporary relaxation of selection pressure. For some critical developmental events and biochemical pathways, canalization and epistasis negate potentially detrimental genetic variation. Geographical location, environment and climate have major effects on population genetic variation (May and Krueger, 1990). Generally, the greater the geographical separation of breeding areas, the more genetically differentiated will be the populations of fish that use them (Wright, 1943).

Physiological and ecological differences among Florida largemouth bass, *Micropterus salmoides floridanus*, northern largemouth bass, *M. salmoides salmoides*, and their hybrids have been documented that are probably related to natural selection at different environmental temperatures (Philipp *et al.*, 1985). A number of studies have shown a difference in their response to various temperature regimes (Fields *et al.*, 1987; Carmichael *et al.*, 1988). Other studies have shown differences in timing of spawning, growth rate, reproductive success and survival of the two subspecies that are probably related to temperature and selection (Isley *et al.*, 1987; Maceina *et al.*, 1988; Philipp and Whitt, 1991).

Such selective pressures related to temperatures are well documented in other species. Using one hundred of *Poecilia reticulata* Peters individuals, homozygote for the coldwater resistance gene, were further tested for resistance to heat. The results indicated a significant higher tolerance to heat of these ones compared to control lot (data not shown). These data supported the hypothesis of Karayücel *et al.* (2006) and indicated the common identity of coldwater resistance gene and heat tolerance gene. This X-linked gene (for resistance) seems to encode a protein with multiple roles, a molecular chaperone, a protein formally included in HSPs group. The conclusion was that at least some of the HSPs were involved in both coldwater resistance and heat tolerance, modulating animal metabolism under different environmental conditions (Petrescu-Mag *et al.*, 2008a).

Fish in the natural environment have little contact with humans, are at relatively low densities, have relatively low exposure to diseases, live in relatively good water quality, are almost never subjected to low oxygen levels, must catch and feed on prey items and must avoid predators. In contrast, the associated selective pressures in the aquaculture or hatchery environment are very different. In this environment, fish are in frequent contact with humans, are at tremendous densities, have frequent exposure to pathogens, are often exposed to poor water quality and low oxygen levels, are provided with artificial diets and are relatively protected from predators. When fish are removed from the natural environment and placed in the culture environment, random genetic drift and domestication effects (new and greatly different selective forces act upon fish in the domestic environment compared with the natural environment) alter gene frequencies and reduce genetic variation as measured by isozyme analysis and DNA markers. Domestication reduces genetic variability in fish (Allendorf and Utter, 1979; Allendorf and Phelps, 1980; Ryman and Stahl, 1980; Stahl, 1983; Dunham and Smitherman, 1984; Hallerman *et al.*, 1986; Koljonen, 1989) through both selective processes and random genetic

drift. The majority of this research was demonstrated with salmonids. The same trend has been observed in limited studies of channel catfish.

Several factors may contribute to the domesticated fishes' potential decreased fitness and competitiveness in these examples. Possibly the loss of certain variation may make these fish less adaptable in the natural environment. Wild trout tend to be stronger than domesticated trout and exhibit superior swimming stamina (Dickson and Kramer, 1971; Woodward and Strange, 1987). Wild trout have a greater ability to raise blood parameters in response to stress than domesticated strains (Woodward and Strange, 1987). Lepage *et al.* (2000) obtained similar results with sea (brown) trout. The metabolic stress responses of wild and domesticated fish originating from the same river were measured by placing the fish in a new environment, alone as well as in combination with predators. This stress induced elevated plasma concentrations of glucose and cortisol and brain levels of cortisol, dopamine, serotonin and metabolites of dopamine and serotonin. The stress responses in the domestic brown trout were weaker than those of wild cohorts, and alterations in brain monoamine neurotransmission were part of this effect.

Other studies revealed the fact that percentage of black individuals of *Poecilia sphenops* seems to decrease significantly in aquaria. The color pattern evolves step by step from the ornamental full-black to the ancestral wild phenotype. In the wild, the situation is different: although the black phenotype is one generally limited to some domesticated strains, this was found highly and naturally conserved in the feral populations from both Pețea Lake and Băile Felix spring (Petrescu-Mag *et al.*, 2008b).

Generally (in the wild), predators are natural “forces” selective acting over dynamics of the color traits and over poeciliid population behavior. The black individuals of *Poecilia sphenops* should decrease in percentage, due to a presumably female natural preference for grey-olive or yellow (wild type) males and due to their unusual body coloration that could make them an easier prey in the populations with predation risk. Corroborated with the female preference and predation risk, another important “force” that should lead to degeneration of recessive black phenotypes in the molly is the dominance of wild alleles (Petrescu-Mag *et al.*, 2008b).

Dunham in 2004, considered that the domestic fish are more aggressive, which may be related to their increased vulnerability to predation or harvest (Moyle, 1969; Dickson and Kramer, 1971; Fraser, 1974). The nervous behaviour and wariness of wild salmonids relative to domestic salmonids is well documented (Moyle, 1969; Fraser, 1974). Wild trout position themselves deep in hatchery tanks and domesticated trout orient themselves nearer the surface of the water (Moyle, 1969). Johnsson *et al.* (2001) found that 1-year-old wild Atlantic salmon had a stronger heart rate and flight response from a simulated predator than seventh-generation farmed salmon derived from the same founder wild population, but the differences were weaker or reversed in 2-year-old fish. In 2008, Bourne and Sammons have presented a strong evidence corroborating the hypothesis that when behaviors are correlated, individuals express different behavior types under different contexts; because bold pentas explored a T-maze in the shortest time, and initially approached the chamber with a living pike cichlid instead of the conspecific male displaying a willingness to expose themselves to risk of predation. Intermediate pentas spent more time exploring the maze and exhibited no initial interest in the predator chamber or in the conspecific one. Shy individuals took the longest exploring the maze, and initially approached the predator chamber.

Wild fish can also be more reproductively competitive than domestic fish. Wild coho salmon males outcompeted captive reared males and controlled access to spawning females in 11 of 14 paired trials in laboratory stream channels and, in two cases where domestic satellite

males were observed participating in spawning, DNA genotyping indicated that they did not sire any of the progeny (Berejikian *et al.*, 2001). This has additional practical management implications when the objective is to enhance the effective population number of a naturally spawning population with fish reared in captivity (Doyle, 2003).

There are additional explanations for the reduced fitness of domestic genotypes compared with wild genotypes in the natural environment. In a culture environment, if selection is relaxed, some of the genetic gain will be lost when this selection pressure is removed. When aquatic organisms are removed from the natural environment, again a selective pressure is removed and gene frequencies can change. Therefore, reduced natural selection in the benign, domestic environment can theoretically permit unfavourable genes to accumulate in the population by drift and/or mutation pressure even though they are not selected (relaxed selection), which could result in a catastrophic loss of fitness when the organisms are exposed again to the full force of natural selection, such as in fisheries stock enhancement (Doyle, 2003). These effects can occur in captive populations of any size since they are related to selective pressure, not population size. If the size is small for the captive population, inbreeding and the accumulation of deleterious homozygotes can further exacerbate the reduced fitness for natural settings, unless the population is selected for fitness traits (Doyle, 2003).

Genetic variability in a population is important for biodiversity, because, without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction. The principal's sources of variability are considered recombinations and mutational process.

The population genetics of many important species require closer examination. Interactions of wild and domesticated species needs much closer study, including modelling. There should be an intensification of live, frozen and molecular gene-banking efforts. More research is needed in the area of effective sterilization techniques for domesticated and transgenic aquatic organisms. There is a need for greater controls of transboundary movements of aquatic germplasm. Researches on transgenic aquatic organisms should continue because of their potential benefits (especially in developing countries); however, much greater understanding of potential environmental impacts is necessary. Linkages should be formed among civil society, organizations, scientists, industry and governments to address genetic issues and to support the development of practical regulations and sound policy. Dissemination of transgenic aquatic organisms for aquaculture should only be carried out within the framework of adequate regulations and policy (Dunham, 2004).

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