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# The history of the aquaculture genetics program at Auburn University

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ABSTRACT

The Auburn University Aquaculture Genetics Program has an 80-year history with focused efforts on genetics and breeding, transgenic technology and genomics being initiated 43, 27 and 18 years ago. This resulted in the first successful selection experiments for channel catfish, *Ictalurus punctatus*, the basis of the monosex tilapia industry, the first production of transgenic fish in the US, major advances in catfish genomics and the first production of a xenogenic catfish. The most significant impact has been the establishment of the technology to allow a hybrid catfish industry, which produced 150 million hybrid catfish fry in 2012.
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## 1. Early history of aquaculture

Aquaculture genetics had its origin with the beginning of aquaculture in China and the Roman Empire more than 2000 years ago (Dunham, 2011). Unbeknownst to them, early fish culturists changed gene frequencies, altered performance of the wild-caught fish, and genetically enhanced the fish for fish-farming application by closing the life cycles and domesticating species such as the common carp, *Cyprinus carpio* (Dunham, 2011). The early Chinese, Europeans and other aquaculturist observed mutations and phenotypic variation for color, body conformation, scale pattern and finnage, and by selecting for these specific phenotypes as well as body size, the field of aquaculture genetics and selective breeding for fish and shellfish was born. However, directed breeding and genetics programs were probably not intense and strongly focused until the Japanese bred koi in the 1800 s and the Chinese developed ornamental goldfish.

Modern fish genetics programs 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 programs until the 1960s because of the infancy and small scale of aquaculture, and a lack of knowledge of fish genetics (Dunham, 2011).

## 2. Aquaculture genetics at Auburn University

The aquaculture genetics program at Auburn University has a history spanning 80 years. R. Oneal Smitherman (Fig. 1) began a focused aquaculture genetics and breeding program in 1969 with initial efforts on catfish genetic enhancement. Because of the foresight of Smitherman and the continuing efforts of Rex Dunham, a continuous goal oriented aquaculture genetics research and teaching program has been in operation during the past 43 years. This program is unique and inherently valuable as the only other institution with a similar longevity, fortitude and commitment is the Norwegian salmon breeding program (Eknath et al., 1991; Gjedrem et al., 2012).

In actuality, without realizing it, Homer Swingle began the Auburn University Aquaculture Genetics Program (AUAGP) in 1933. A species is a group of animals or plants possessing in common one or more distinctive characteristics and which are fully fertile when inter-mated. They are kept genetically distinct through various forms of reproductive isolation from other species (Dunham, 2011). Thus, species exist because of genetic differences. Therefore, when Dr. Swingle was comparing related species for farm pond management and related catfish species for aquaculture application, and choosing the best species for culture, he was actually making a genetic comparison.

Ellis Prather conducted the first selection experiment for the AUAGP in 1941 (unpublished). He selected largemouth bass, *Micropterus salmoides*, for feed conversion efficiency when fed forage fish in tanks. Prather's multiple generation selection experiment apparently improved feed conversion efficiency of largemouth bass when fed forage in tanks more than 10-fold. Unfortunately, similar to other early researchers such as Lauren Donaldson, who unlike Prather received great notoriety for the product of his selection, the Donaldson trout (Donaldson and Olson, 1957), Prather kept no genetic control. The importance of this initial selection effort at Auburn will never be known because the experimental design does not discern between improvement due to genetics and that due to environment (in this case improved culture).

Israeli scientists played a key role in the establishment of the AUAGP. The Auburn University–Israel connection was probably founded at the 1966 FAO World Symposium where Rom Moav (Fig. 2), a pioneer

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**Fig. 1.** R. Oneal Smitherman (pond bank) with Ali Khater, Rex Dunham and Abdel Rahman El Gamal seining selectively bred catfish.

**Fig. 2.** Rom Moav with his beloved common carp.

common carp geneticist, met Homer Swingle, E.W. Shell and R.O. Smitherman who presented their early works on tilapias (G. Hulata, personal communication at the International Symposium on Genetics in Aquaculture XI). In 1968, Moav came to Auburn University (AU) as a visiting scientist in the Department of Poultry, and shared his knowledge in genetic improvement with both the poultry and aquaculture groups.

In 1969, Rom Moav (of the Hebrew University in Jerusalem, originally trained in tobacco breeding and statistics), followed by Giora Wohlfarth (Fig. 3) (from the Dor Fish Culture Research Station), who had established one of the first aquaculture genetics programs, visited Auburn University and assisted Dr. R. O. Smitherman in establishing a catfish genetics research program and a graduate level course in fish genetics and breeding. Smitherman traveled to Israel, and Moav or Wohlfarth traveled to Auburn on multiple occasions to learn, exchange ideas and to establish fish genetics and breeding as a focused discipline at Auburn. The AU–Israel connection was further strengthened in 1973 when Moav and Shell were awarded a grant from the United States–Israel Binational Science Foundation (BSF) to further develop "Breeding Schemes for the Genetic Improvement of Edible Fish" (G. Hulata, personal communication at the International Symposium on Genetics in Aquaculture XI). Towards the end of this grant, Wohlfarth spent a sabbatical year at AU and taught the first fish genetics and breeding course at the Department of Fisheries and Allied Aquacultures. This collaboration continued during 1980–1982 through a BARD project "Selective Breeding of Farmed Fish" awarded to Smitherman, Wohlfarth, William Shelton, Dunham, Gideon Hulata and Shmuel Rothbard. Dunham used part of this funding to establish the Department of Fisheries and Allied Aquacultures' first biochemical genetics laboratory, although some earlier isozyme data for catfish had been generated via collaboration with Craig Weatherby in the Department of Zoology. The initial objective of the biochemical genetics laboratory was to find protein markers associated with increased performance in catfish as well as study population genetics in fish.

Several technologies were exchanged between the two groups, sometimes in amusing fashion. Since the Israelis were using mirror carp, they had developed heat branding techniques to mark their

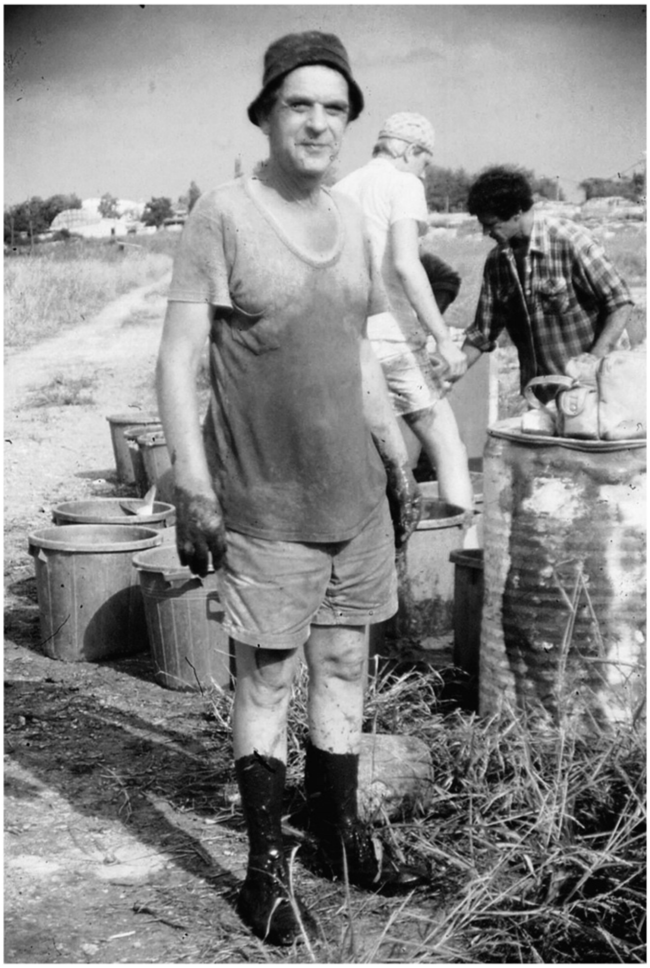

**Fig. 3.** Mud-dirty Giora Wohlfarth during draining of experimental pond.

genetic groups. Since ictalurid catfish are scaleless, the Israeli heat branding technique was found to be the best marking technology for catfish genetic groups at that time. The technology used a car battery to provide the energy to generate the heat. A simple branding gun was clipped to the brander and paper clips to provide the resistance and the actual metal branding surface. The Israelis were able to brand fish in the field this way without having electricity in the field. Smitherman's initial attempts to use the technique were a frustrating failure as the paper clips continually burned through and broke. Moav and Wohlfarth had neglected to inform Smitherman that they used a nail as an electrode in the center of the car battery to reduce it from a 12 V source to a 6 V source to generate a reduced and appropriate level of heat.

Smitherman conducted the first strain evaluations of channel catfish about 1969–1972 with the collaboration of O.L. Green and Garland Pardue of the U.S. Fish and Wildlife Service and others. A graduate student, Randell Goodman, compared the morphology of different channel catfish strains and produced the first AUAGP Master of Science thesis under the supervision of Smitherman and Shell. Smitherman then left on a two-year assignment to Panama. It is important to have dedicated caretakers watching over a breeding program that appreciate its importance. While Smitherman was temporarily stationed overseas, the initial brood stock collection of several strains was destroyed (actually eaten). The other researchers at Auburn did not have the same appreciation for genetics as Smitherman, necessitating the re-establishment of the catfish genetics program upon his return. The initial genetic enhancement programs examined were strain selection, intraspecific crossbreeding, mass selection, and interspecific hybridization (Dunham and Smitherman, 1983a,b; Green et al., 1979; Yant et al., 1975).

In the early to mid 1970s, major efforts were also made in tilapia genetics lead by Smitherman and Dr. William Shelton. They produced the first work and manuscripts on sex reversal to produce all-male populations and coupled that with genetic breeding to develop genetically monosex populations. Other researchers took these basic findings and continued to develop the all-male genetic technology for tilapia that is used by most of the world today. The lead doctoral student on this first work was Rafael Guerrero who returned to his home country, the Philippines, continued this work, and has won several international awards for his role in developing this high impact technology.

In the mid-1960s, John Giudice of the US Fish and Wildlife Service had observed that the hybrid between channel catfish females and blue catfish, *I. furcatus*, males had exceptional heterotic growth (Giudice, 1966). However, this experiment was conducted at very low stocking densities. Another of Smitherman's students, Dr. Roger Yant, evaluated the performance of this hybrid at commercial densities, and found its growth, feed conversion, survival and processing traits to be superior to that of channel catfish (Yant et al., 1975). Thus, AUFGP embarked on a long-term commitment to research on the channel × blue catfish hybrid that is now impacting and transforming the US catfish industry.

About 1975, Wohlfarth returned to Auburn as it was in a major growth phase, and once again assisted in the research and planning of Auburn's catfish genetics program. Efforts by a doctoral student, Jesse Chappell, were key to Auburn's continued growth in catfish genetics. From 1975 to 1979, Chappell, Mark Brooks, Manote Benchakan, Andrew McGinity, J.C. Lee, Douglas Tave, Paul Youngblood and Dunham laid the ground work for the AU selective breeding program in catfish and tilapia. Dunham then demonstrated that selection for body weight could improve body weight in three strains of channel catfish (Dunham and Smitherman, 1983a,b).

David Teichert-Coddington found that selection for body weight in Ivory Coast strain of tilapia was not successful, probably because of a lack of additive genetic variation in tilapia from narrow origins (Teichert-Coddington, 1988). Also, in the early 1980s, a tri-lateral relationship was established between AU, Israel and Egypt to work on tilapia genetic enhancement. Although selection failed, Ali Khater and later Tave working with Smitherman found large strain differences in Nile tilapia.

By the early 1980s significant genetic improvement of catfish had been accomplished, and farmers were anxious for access to the improved research lines. Auburn University conducted the first release of three selected lines in 1984–1985. Farmers were offered improved young brood stock or fingerlings above market price. Fish were released to 69 farms in six states and availability met demand. In the late 1980s early 1990s, Auburn University made two additional releases, one was an additional selected channel catfish, and the other a line of channel catfish and blue catfish that had high pen spawning rates to produce the channel female × blue male hybrid.

In 1985–86, Dunham and Smitherman developed a proposal to build a separate catfish/fish genetics facility. This was needed to relieve pressure on existing facilities at the lower station, to upgrade facilities for genetic research, to conduct genetics research in a more environmentally secure and confined environment and to isolated genetics research from other aquaculture research to decrease the chances of genetic contamination. This proposal was submitted to USDA for funding as a center for Catfish Genetics Research. USDA rejected this proposal. Within a year, USDA solicited advice from Dunham and Smitherman to build a Catfish Genetics Research Unit at Stoneville, Mississippi. Many components of the USDA research program and scope of the program were identical to the Auburn proposal. USDA-ARS established the Catfish Genetic Research Unit in Stoneville, Mississippi first lead by Gary Carmichael followed by William Wolters and now lead by Geoff Waldbieser and Brian Bosworth (Li et al., 2001; Tomasso and Carmichael, 1991; Waldbieser et al., 2001; Wolters et al., 1996). Dunham and Smitherman re-drafted the proposal and submitted it to Mellon Foundation. Shell and Smitherman were instrumental in Mellon Foundation funding of this facility, which was constructed during 1987–1992.

Long-term dedication of administrators and scientists is necessary for the success of genetic enhancement programs. A dean at the University of Georgia closed the catfish breeding program at Tifton, Georgia and bulldozed the facilities transforming it into a cow pasture about 1984. The germplasm and some research projects were absorbed by Auburn University (Bondari and Dunham, 1987).

In 1984–85, AU initiated research in genetic engineering. With a minimal budget, Dunham initiated the AU transgenic fish research and molecular genetics program. With the low level of funding, collaboration was essential for growth of the transgenic program. Dunham collaborated with the AU veterinary school (Dwight Wolfe) and the University of Alabama at Birmingham (Tim Townes) initially. AU (Dunham) produced a transgenic channel catfish in 1986 (Dunham et al., 1987, 2002), the first transgenic fish in the US and fourth worldwide. This evolved into collaboration with Tom Chen and Dr. Dennis Powers at Johns Hopkins University. The US–Israeli collaboration continued in molecular genetics and transgenics as Dunham, Powers, Chen, Boaz Moav (Tel Aviv University), Benjamin Cavari (IOLR) and Wayne Knibb (IOLR) obtained BARD support to research transgenic fish. Also, during this time AU developed outdoor confinement facilities for transgenic fish research, obtained approval from the USDA Office of Agricultural Biotechnology to conduct outdoor research with transgenic fish and conducted the first government approved outdoor research with a transgenic animal in the world.

Environmental concerns about the application of biotechnology and genetic engineering emerged in the 1980s. Application of gene-transfer technology will not happen until genetic engineering is proved to be a safe technology. In the mid-1990s, Dunham conducted the first environmental-risk research with transgenic fish, channel catfish, demonstrating that in natural conditions the transgenic catfish were slightly less fit than non-transgenic cohorts.

Molecular genetics and genomics will hopefully be the next genetic enhancement on the horizon. Zhanjiang (John) Liu joined the Department of Fisheries and Allied Aquacultures in 1994 to conduct molecular genetics and genomics research to lead to eventual application for genetic improvement. Under his direction, research on DNA marker development, gene mapping and functional genomics accelerated at a rapid pace. Technological advances in DNA marker technologies and DNA microarray, gene chip and sequencing 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. Liu and his students including Eric Peatman who joined the faculty in 2009 lead efforts to isolate and sequence more than 25,000 genes in catfish, and produced the largest numbers of expressed sequence tags (ESTs) among aquaculture species.

During the 1990s, progress continued in selective breeding and hybridization. The first long-term cryopreservation of Ictalurid catfish sperm was accomplished by Amrit Bart. Dunham increased research on reproductive technology to artificially produce hybrid catfish. Four generations of selection for increased body weight in channel catfish resulted in a 55% improvement. Selection for resistance to *Edwardsiella ictaluri* and low oxygen levels was unsuccessful. Correlated responses to selection for body weight resulted in improved fecundity and dressout percentage, but decreased tolerance of low dissolved oxygen (Rezk, 1993). In general, genetic engineering, intraspecific crossbreeding and selection increased disease resistance in channel catfish.

As a result of the first 25 years of the focused effort on catfish genetics research, from 1994 to 2005 the first four catfish genetics and breeding companies in the US were spawned based on the AU research. Additional positive impact occurred as 20 years after the first release of improved germplasm, a portion of farms still use these brood stocks with good results (USDA, 2003). Also, in some cases, these fish were bred into existing stocks to top cross and improve the existing stocks. The selective breeding research made a larger impact in the 1990s through the mid 2000s by commercial farms taking the results and applying them to their own brood stock rather than through release of improved germplasm from research institutions (Steeby and Wagner, 2006; USDA, 2003). Improved selected lines were developed. Some farmers have employed their own crossbreeding programs to enhance performance or to avoid inbreeding. By 2005, 70% of the catfish industry was utilizing selected fish that were a result of Auburn research or were developed following the program demonstrated by Auburn. Based on artificial spawning technology developed at Auburn University by students such as Ann Ramboux, Soonhag Kim, Brad Argue, Bart and Dayton Lambert, Gold Kist Inc. began making channel-blue hybrid catfish every year, becoming the first farm to produce significant numbers of hybrids annually.

During the first few years of the new millennium Liu and Dunham have increased emphasis in physical mapping, QTL mapping, sequencing of the catfish genome, microarray analysis, selective breeding for disease resistance, transgenic sterilization and hybrid embryo production. The first DNA markers were identified that were linked to feed conversion efficiency and growth rate in channel catfish. The first genetic linkage map based on channel catfish × blue catfish interspecific hybrid was completed by 2003.

From that point to the present, the genomics research led by Liu and Peatman has reached many milestones. Catfish BAC-end sequence resources were created from 2006 to 2009 with over 63,000 BAC end sequences generated, which resulted in the construction of BAC-based physical map of catfish in 2007. Also in 2007, high density microarray technology was developed. Peatman et al. (2007, 2008) determined the transcriptome of channel catfish and blue catfish when challenged with *E. ictaluri* and columnaris, discovering major changes in gene expressions. By 2009, gene-based genetic linkage map and early comparative genome studies were completed. In 2010, 350,000 channel catfish ESTs and 150,000 blue catfish ESTs were generated using Sanger sequencing and catfish genome sequencing began. Genome-wide discovery of catfish SNPs was completed in 2011. During the past year genetic linkage and physical maps have been integrated, second generation of genetic linkage maps produced, a unique set of 25,144 unique protein-encoding genes identified by transcriptome sequencing, of which over 14,000 full length transcripts were assembled and assembly of the catfish genome is expected to be completed.

During the mid and late 2000s, students such as Anang Kristanto, Gloria Umali-Maceina, Atra Chaimongkol, Joseph Ballenger, Alison Hutson, Andrew Gima and Megan Gima, further refined the luteinizing hormone releasing hormone analog-based artificial fertilization technology to produce hybrid catfish embryos. AU conducted its latest release of blue catfish and channel catfish lines to produce hybrids and improved hybrids. Eagle Aquaculture was formed to specifically market channel-blue hybrid catfish fingerlings and hybrid embryo technology.

Sam Lawrence of Eagle Aquaculture has lead efforts to commercialize interspecific hybrid catfish in the United States making this technology a reality. Based on AU research, a hybrid catfish industry has been established, which produced 200 million hybrid catfish in 2012 using the protocols developed by Auburn.

During the past decade, Dunham and AU have emphasized research on transgenic sterilization (Thresher et al., 2009) and xenogenesis (Perera, 2012). Recent advances in transgenic sterilization (Su, 2012) lead by students such as Baofeng Su, and the production of the first xenogenic catfish lead by Dayan A. Perera and Mei Shang will open the doors to the next wave of impact by AUAGP in the coming years.

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