5 Genetics and Breeding

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5.1 INTRODUCTION

Research on channel catfish genetics and breeding began in the late 1960s and early 1970s (Dunham and Smitherman 1987); however, applications of genetic improvement in channel catfish culture have lagged behind genetic improvements made in other farm animal industries. These improvements are most apparent in the poultry, beef and dairy cattle, and swine industries where genetic research, particularly in the areas of quantitative inheritance, has made major contributions to industry advancement and profitability. In dairy cattle, milk yield per cow more than doubled from 1930 to 1976. Significant advances in pork production have also been made through genetic improvement. Since 1945 the time required to produce a marketable pig (100 kg; 220 pounds) has been reduced from 200 to 160 days. In the poultry industry, producers have reduced the time required to produce a marketable broiler (1.7 kg; 3.75 pounds) from 14 to 7 weeks and doubled the feed efficiency (Warwick and Legates 1979; Gall et al. 1988).

The successful breeding programs and increased production realized in these industries have been the result of a long effort in basic and applied research coupled with information transferred

TABLE 5.1. Potential for increased efficiency of producing foods of animal origin in 2000 and 2030 (Smith 1991).

		Percent Improvement	
Animal or product	Unit	2000	2030
Beef	Live weight marketed per breeding female	25	60
Pork	Live weight marketed per breeding female	35	60
Dairy	Milk marketed per breeding female	30	65
Sheep	Live weight marketed per breeding female	35	70
Broiler chicken	Live weight marketed per breeding female	30	35
Turkeys	Live weight marketed per breeding female	40	40
Laying hens	Number of eggs	20	25
Catfish	Age to market weight (0.45 kg, 1 pound)	50	200

to industry. Animal products from these industries in the United States supply 53% of national food consumed and 69% of the protein. The potential for continued improvement and increased efficiency still exists in these animals, and will result from further improvements in germplasm, reproductive efficiency, nutrition, and production systems. Projections for future increases in production efficiency are summarized in Table 5.1.

The potential increases in production efficiency from catfish breeding are projected to be larger than in other animals. Improving production efficiency in catfish culture by utilizing improved germplasm is possible, but will occur only through long-term genetic research. Breeding programs must be integrated with improvements in culture technology along with identification of constraints limiting the potential of channel catfish breeding and selection programs.

The goal of a breeding and selection program in an agricultural system should be to alter the animal's characteristics to make it more profitable to culture and more efficient to produce. The task facing the research geneticist is to determine the amount and type of genetic control over the animal's performance to implement a system of breeding and selection to improve production efficiency. An undisputed need exists for improving channel catfish production in aquaculture through planned breeding programs. Future breeding programs will be required to address areas of qualitative and quantitative genetics, reproductive efficiency, molecular and cellular genetics, and to incorporate new biotechnologies.

The goal of this chapter is to overview the major topics in catfish genetics to provide a review of what is currently known and to indicate where further information is necessary. We also hope to illustrate the linkage of research and production necessary to establish a system of genetic improvement for future development of the catfish industry.

5.2 GENETIC ISSUES IN REPRODUCTION AND SPAWNING

Control of reproduction or spawning is a necessary element of an effective genetic improvement program. The lack of sustained genetic selection in channel catfish has effectively maintained a high level of phenotypic variation in commercial and research populations. Commercial catfish farms typically maintain large populations (thousands) of broodfish and have access to wild broodstocks if necessary to supplement the genetic variation. Large numbers of offspring (10,000-20,000 full sibs) in each spawn or family would permit high selection intensity if commercial

producers could effectively select individual catfish or broodfish for specific traits. However, genetic selection in commercial culture is generally not feasible because most producers simply culture fingerlings or foodfish for sale and do not have any information on individual or family performance. Because commercial producers typically do not follow any program of selective breeding, improved lines of catfish in the future are likely to only be developed by research laboratories or in cooperation with commercial producers.

Channel catfish reproduction in commercial culture is practiced only during the spring spawning season, therefore any selective matings in practical breeding programs are currently limited to yearly intervals. Spawning is most commonly done by the open-pond method where male and female broodfish are allowed to mate randomly in large ponds supplied with spawning containers (Tucker and Robinson 1990). Spawning containers are checked periodically during the spawning season and eggs are usually removed and taken to a hatchery for artificial incubation and training of newly hatched fry to accept formulated diets. The spawning season is protracted when this method is used, lasting from early May until late June or early July. Generally 30 to 50% of the female broodfish spawn, but the spawning success of male broodfish is likely to be lower because individual males have been shown to spawn multiple times during the spawning season and could thus have a disproportionate effect on the total number of matings (Waldbieser and Wolters 1999). Ponds used for commercial spawning are typically 2 to 4 ha (5 to 10 acres), and when stocked at approximately 1,100 kg/ha (ca. 1,000 pounds/acre) will produce several hundred spawns. Consolidation of broodfish into fewer ponds at higher stocking densities following the spawning season mixes the population and further promotes random mating.

Pen and aquarium spawning methods are more intensive, but have value in breeding programs because the culturist has greater control over broodstock selection. Pen spawning is similar to pond spawning, but involves construction of spawning pens in outdoor ponds, and is used primarily for selecting and spawning particular pairs of broodfish. Aquarium or tank spawning is more intensive and involves pairing broodfish in indoor tanks or aquarium supplied with flowing water. Both of these methods require accurate sexing and determination of the stage of reproductive development in individual broodfish, and hormone injections to stimulate ovulation are often used.

All three methods have applications in breeding programs, however, pond spawning is most suitable for producing large numbers of families. Pen spawning can be used to produce within-species crosses and to mate selected sires and dams to estimate heritabilities (estimates of inheritance for particular traits) and genetic correlations from sibling analyses. However, families will often not be contemporaneous and differential fish sizes or age may require statistical adjustment. Aquarium spawning is suited for factorial mating designs (which assist statistical analysis) and can provide contemporaneous families. Both pen and aquarium spawning methods have been used in research to provide statistical estimates of heritabilities and genetic correlations because male and female parents can be easily identified. Genetic (DNA) markers have recently been used to identify parentage in open pond spawning and progeny testing for these families will allow estimates of genetic variation (Waldbieser and Wolters 1999). Facilities allowing the design and implementation of such studies are available at a few research institutions in the southeastern United States (Fig. 5.1). At least one commercial facility has been developed for conducting genetic research, however, their breeding program was recently sold to another company and the future research efforts are uncertain (Roger Yant, Harvest Select Farms, Inc, Inverness, Mississippi, personal communication).

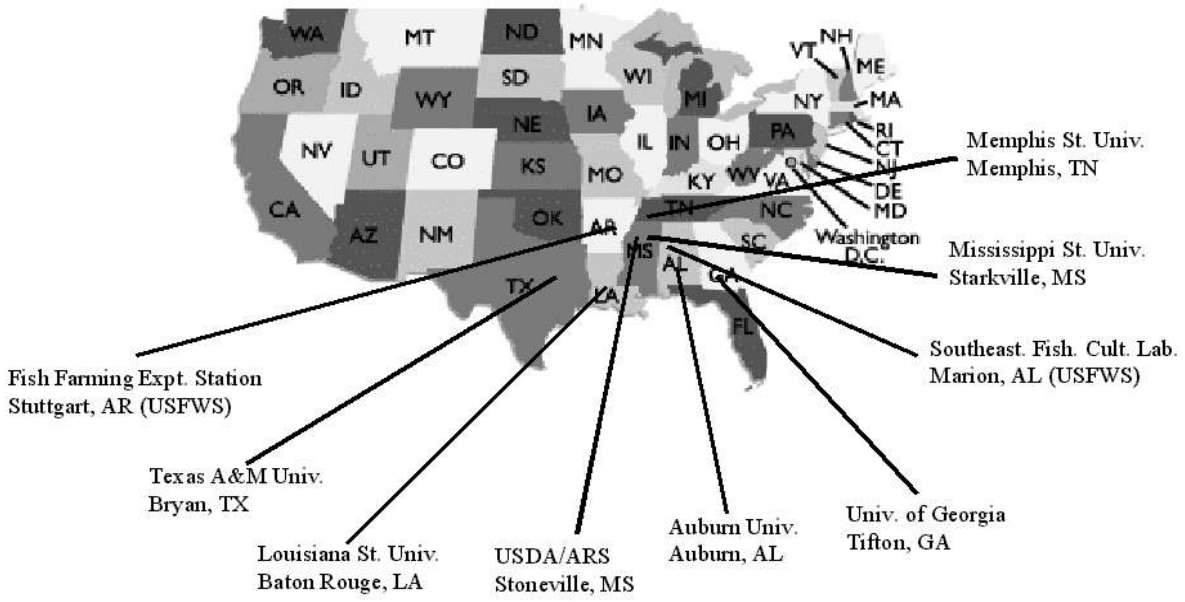

FIGURE 5.1. Federal and university locations conducting research on catfish genetics and breeding in the southeastern United States from the 1960s through 2001.

Gamete manipulation (manual stripping of eggs and sperm) is not easily accomplished in channel catfish as compared with other fishes such as salmonids. Spawning of salmonids is commonly done in research and commercial production by manual stripping of sperm and ovulated eggs followed by artificial fertilization (Leitritz and Lewis 1980). Oviposition in channel catfish occurs over several hours and is a constraint to multiple matings between males and females (Clemens and Sneed 1957; Dupree and Green 1969). Artificial fertilization in channel catfish has generally required male and female broodfish to be stocked into aquaria or tanks to time and observe ovulation, however, recent research has improved the timing of hormone injections and prediction of ovulation (Bates and Tiersch 1998; Dunham et al. 1998). Male gametes cannot be stripped and require dissection and maceration of testes to obtain sperm suspensions. These artificial fertilization procedures have been used successfully to produce catfish hybrids (Dupree and Green 1969; Tiersch and Goudie 1993) and allow the induction of polyploidy (Wolters et al. 1981a).

Attempts to induce spawning outside the normal spawning season with temperature and photoperiod manipulations have had limited success. Culturists and breeders currently can consistently obtain gametes and make matings only during the natural spawning season (May through July). Experimental manipulation of temperature and photoperiod in recirculating systems has facilitated successful reproduction catfish outside the spring spawning season (Kelly and Kohler 1996), but commercial application has not been realized and may not be cost effective. Extension of the spawning season is possible in research settings by heating of small (0.08-ha; 0.2-acre) broodstock ponds with thermostatically controlled geothermal water (Hall et al. 2002). This has enabled natural (Lang et al. 2003a) and artificial spawning and production of hybrid catfish in Louisiana as early as February and March (Lang et al. 2003b). Industry

application has begun with utilization of geothermal wells at a commercial producer of hybrid catfish fry (Harvest Select Farms, Inc., Inverness, Mississippi), and this management scheme is currently available in areas with access to suitable ground water. Early spawning would benefit fingerling producers desiring a longer first summer growing season to raise larger fingerlings. Out-of-season spawning also provides the benefits of having a source of fry for year-round research projects, and synchronized conditioning of broodstock for predictable spawning schedules. At present, commercial producers typically manipulate stocking densities and feeding rates to provide a suitable supply of fingerlings for stocking of grow-out ponds when needed.

Future research on catfish endocrinology and control of reproduction could improve the outlook for year-round spawning and potentially a shortening of the generation interval (currently 3 to 5 years) to allow more rapid genetic progress. However, the generation interval is limited by both age and size at sexual maturity. Little information is currently available on effects of these factors on sexual maturity, however, age of first reproduction in channel catfish strains and natural stocks ranges from 1 year (at 10 cm [4 inches] total length) in unique wild populations found in southeastern Louisiana (Lutz et al. 1987; Bates et al. 2002) to the usual 2 to more than 4 years, at a size of from 20 to 40 cm (ca. 8 to 16 inches) (Dunham et al. 1987; Wolters et al. 2000).

5.3 QUALITATIVE GENETICS

Most traits of economic importance such as growth rate show continuous variation and are controlled by multiple genes (Warwick and Legates 1979; Falconer 1981). Qualitative traits usually modify the appearance of an organism and can be characterized or separated into discrete phenotypic classes such as color, presence or absence of a body modification or unique trait, and are usually controlled by one or a few genes. Variation in enzymes and proteins have been extensively studied in catfish and have demonstrated the ability to differentiate among catfish species and stocks, and to measure changes in gene frequencies caused by selection. Protein electrophoresis often has been described within the area of qualitative genetics because protein variation is usually controlled by one or more specific loci, however, this information will be described within the section on molecular genetics.

5.3.1 Qualitative traits

Many physical deformities such as tailless, side-sprigs, triple-tailed, and stumpbody have been described in channel catfish (Fig. 5.2) and most have been found to be detrimental to overall performance (Bondari 1981; Dunham and Smitherman 1987). The frequency of these traits is variable, and the inheritance has either not been studied or found to not have a genetic basis, and may be environmentally induced. Deformities can lower processing yields if present in a high percentage of the population.

The best known potentially valuable qualitative trait in channel catfish is albinism and it is inherited as a single homozygous recessive trait (Fig. 5.3) (Prather 1961; Bondari 1981). No other consistent skin colors have been reported, although environmental variation caused by turbidity, tank color and external lighting in laboratories has some effect on the intensity of skin pigmentation. Fillets from processed albino catfish are lighter in color, appear to have a fresher quality, and are more appealing to consumers (Tucker and Robinson 1990). Studies comparing

FIGURE 5.2. Aberrant fin morphology on the caudal region of channel catfish yields processed fillets with skeletal abnormalities.

FIGURE 5.3. Normal dark pigmentation and albino pigmentation of channel catfish.

the performance of albino and normally pigmented channel catfish have been contradictory (Dunham and Smitherman 1987). Growth of albinos is similar to normally pigmented fish, however, spawning success is often lower and albinos possibly have more rigid temperature requirements for spawning (Bondari 1981; Goudie et al. 1992). In spite of apparent difficulties in raising albino catfish and a potentially higher incidence of bird depredation in ponds, some commercial producers in the past have attempted to stock larger percentages of albino fish in production ponds. Even though higher consumer appeal exists for albino catfish fillets, they are currently not significantly utilized in commercial culture.

5.3.2 Application of qualitative traits

Additional qualitative traits need to be identified in channel catfish. Except for albinism and protein variation, currently known qualitative traits modifying expression of the catfish phenotype have limited value. Future research linking gene frequencies or markers with performance, particularly disease resistance and polymorphisms of major histocompatibility complex loci, should be high priority and will be closely linked to molecular genetic technologies (Chevassus and Dorson 1990; Stet et al. 1990; Kirpichnikov 1992).

5.4 QUANTITATIVE GENETICS

As stated earlier, quantitative geneticists have been successful in producing new varieties of plants and improving domestic livestock performance utilizing a variety of selective breeding analyses and methods. Quantitative genetics focuses on traits of economic importance such as growth, feed efficiency, disease resistance, and processing characteristics that exhibit continuous variation. These traits are generally measured by variables such as length or weight and do not have descriptive characteristics such as color (albino) or the presence of a characteristic (triple-tail). Complicated statistical analyses are commonly conducted to describe the characteristics of these traits. Qualitative traits such as albinism are known to be controlled by a single gene and it is generally accepted that most quantitative traits are controlled by the interactions of many genes. Utilizing quantitative genetics to improve fish performance and develop fish with specific traits is complicated and beyond the scope of this chapter. Detailed aspects of quantitative genetics applied to fish breeding can be found in various references that describe the genetic mechanisms and statistical analyses used (Tave 1986). The following information will describe some important aspects of quantitative genetics and specific examples of improving catfish performance leading to the development of improved lines.

5.4.1 Use in selective breeding

Because the development of improved animals through selective breeding methods and quantitative genetic analyses relies heavily on statistical variables and procedures, knowledge and understanding of some basic concepts are required. The following points are not comprehensive, but provide an understanding of the selective breeding process. As stated in the above paragraph, quantitative traits or important catfish production traits such as weight, length, and fecundity are recorded as measurements on individuals. These measurements are referred to as the phenotype, which provides the actual physical record of the genes controlling the trait. When a large number

of phenotypic measurements are recorded on individual fish, a number of statistical variables such as the mean, variance, standard deviation can be calculated for the trait. Quantitative genetics and selective breeding use this information to determine which animals have the best performance (e.g., fastest growth) and determine the appropriate way to select for these animals in a breeding program.

The mean phenotype and variation (differences among individuals) are two of the most important variables quantitative geneticists first evaluate. For example, most catfish culturists know that when juveniles or fingerlings are stocked into ponds at the same size, the sizes at harvest will vary greatly. Some fish will appear to have not grown at all, while others will have grown extremely rapidly. The growth of individual fish is controlled by the combination and interaction of the genes and the environment. Some fish grow fast because they have a good genetic basis; some grow fast because they have a favorable environment, perhaps providing access to more food, an environmental effect not related to genetics. In reality, the genetic control of traits like growth, reproduction, and disease resistance is extremely complicated. Implementation of a breeding program for catfish genetic improvement therefore requires measurement of many economically important traits and understanding the genetic and environmental control over those traits. After recording and analyzing the data on individual fish in a breeding program, specific fish are then selected with the best genetic merit to improve traits from one generation to the next. The method used to select the best fish in the breeding program is also based on information obtained from the statistical analysis and determines whether commonly used breeding methods such as individual or family selection, hybridization, crossbreeding or a combination are used to genetically improve the line. Future integration of molecular genetics, especially marker-assisted selection (MAS), with traditional quantitative genetics will increase the efficiency of breeding programs. Inheritance studies indicate that groups of major genes (quantitative trait loci or QTL) control the phenotype of probably all economically important traits (Dunham and Smitherman 1983, 1984, 1985, 1987, 1990; Bondari 1981, 1983; Cadieu, 1993; Argue, 1996). These basic inheritance studies of important QTL are the foundation for genetic linkage and QTL mapping.

5.4.2 Species traits relevant to culture

Breeding programs for channel catfish and blue catfish have been established for over 25 years. Channel catfish is the most important catfish in the industry because of its superior performance for growth and overall disease resistance. Channel catfish exhibit superior growth, feed conversion efficiency, resistance to the bacterial disease caused by *Flavobacterium columnare*, and tolerance to low oxygen. Blue catfish exhibit superior performance for processing yields, susceptibility to capture by seining, and greater resistance to the most serious bacterial disease, enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* (Wolters and Johnson 1994). White catfish *Ameiurus catus,* another species in the family Ictaluridae, is not common and is rarely cultured because of low fillet yield associated with a large head and small size at sexual maturity (Fig. 5.4).

5.4.3 Crossbreeding and hybridization

Crossbreeding between specific catfish strains has led to improved performance (Table 5.2) (Dunham and Smitherman 1985), but has not been widely practiced in commercial industry

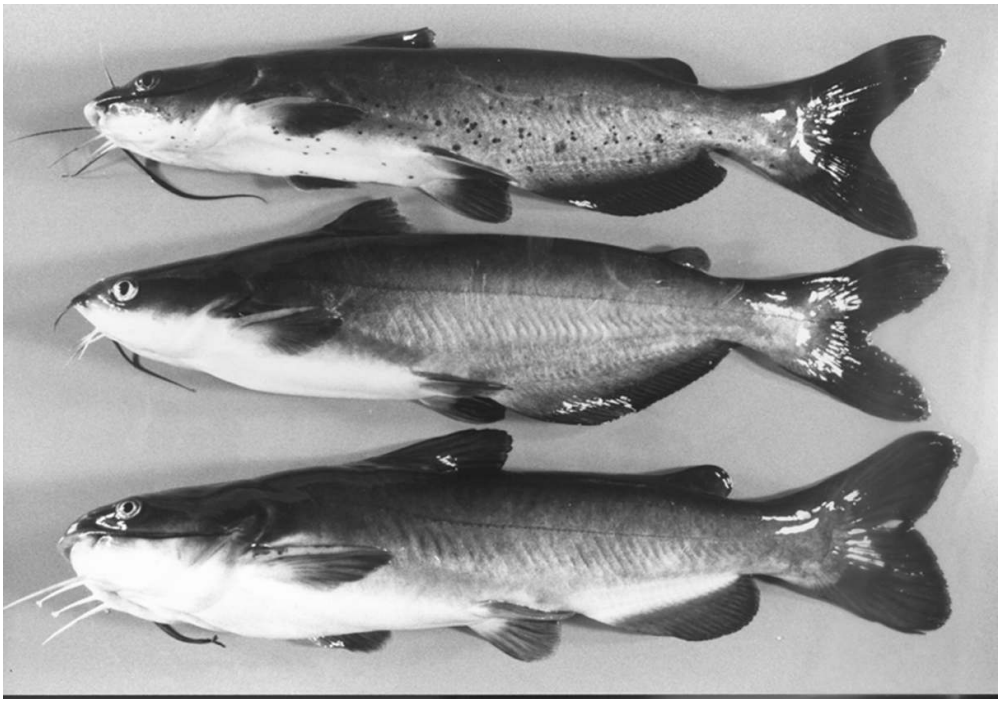

FIGURE 5.4. Three catfish species of the family Ictaluridae that have been evaluated for commercial culture and also used to evaluate interspecific crosses for heterosis: channel catfish, Ictalurus punctatus (top), blue catfish (middle), and white catfish (bottom).

TABLE 5.2. Effects of crossbreeding to increase growth and reproductive performance in two channel catfish strains (adapted from Dunham and Smitherman 1983).

Trait	Marion × Kansas	Marion	Kansas
Weight at 18 months	745 g	617 g	695 g
Spawning percentage at 3 years of age	62%	28%	4%
Spawning percentage at 4 years of age	53%	54%	49%
Number of eggs per kg of female at 3 years of age	7,784	5,116	6,952
Number of eggs per kg of female at 4 years of age	8,130	8,100	8,024
Number of fingerlings per kg of female at 3 years of age	2,427	441	44
Number of fingerlings per kg of female at 4 years of age	1,804	1,508	1,759

TABLE 5.3. Production traits (mean ±SEM) for channel catfish, blue catfish, and channel catfish × blue catfish hybrids cultured communally in replicated earthen ponds at the USDA-ARS Catfish Genetics Research Unit, Stoneville, Mississippi. Means within a column not followed by the same letter differ at the 0.05 level of probability.

Species or hybrid	Stocking weight (g)	Harvest weight (g)	Survival (%)	Yield (kg/ha)	Yield (% of total)	Total fillet yield (%)
Channel catfish	63.2 ± 4.7	391 ± 24b	90.2 ± 14.9	418 ± 25b	27.9 ± 1.8b	43.9 ± 0.5b
Blue catfish	46.4 ± 1.8	418 ± 33ab	98.5 ± 1.9	494 ± 81ab	33.0 ± 2.7ab	45.7 ± 0.8b
Channel × blue	65.3 ± 2.3	502 ± 21a	94.0 ± 6.1	586 ± 25a	39.1 ± 1.5a	49.6 ± 0.6a

TABLE 5.4. Production traits (mean ± SEM) for blue catfish, five groups of channel catfish (albino, Auburn, Kansas, Mississippi, and Norris) and four channel × blue hybrids cultured in replicate earthen ponds at the USDA-ARS Catfish Genetics Research Units in Stoneville Mississippi. Means within a column not followed by the same letter differ at the 0.05 level of probability.

Species or hybrid	Stocking weight (g)	Harvest weight (g)	Survival (%)	Yield (kg/ha)	Yield (% of total)	Total fillet yield (%)
Blue	46.4 ± 1.8d	418 ± 33bc	98.5 ± 1.9	494 ± 81ab	10.2 ± 0.8ab	45.7 ± 0.8bc
Albino	57.6 ± 1.4c	239 ± 10d	92.0 ± 2.2	264 ± 22c	5.4 ± 0.2c	43.5 ± 0.7c
Auburn	76.4 ± 4.7ab	401 ± 29bc	90.5 ± 7.5	434 ± 46b	9.2 ± 0.5b	43.9 ± 0.8c
Kansas	26.6 ± 2.2e	351 ± 18c	97.0 ± 1.3	415 ± 52b	8.5 ± 0.5b	42.4 ± 0.3c
Mississippi	82.7 ± 3.3a	479 ± 46ab	93.5 ± 4.3	534 ± 92bc	11.0 ± 0.9bc	45.9 ± 05ab
Norris	72.5 ± 4.0b	484 ± 36ab	75.8 ± 14.4	437 ± 119b	9.0 ± 1.2b	44.0 ± 1.4c
Auburn × blue	67.5 ± 4.4b	533 ± 21a	93.0 ± 3.7	589 ± 34a	12.1 ± 0.3a	48.6 ± 0.4ab
Kansas × blue	67.1 ± 3.6b	517 ± 38a	94.5 ± 2.2	591 ± 102a	12.2 ± 1.0a	49.6 ± 2.9ab
Mississippi × blue	70.9 ± 4.0b	525 ± 23a	93.5 ± 3.3	575 ± 89a	11.8 ± 0.9a	50.4 ± 0.6a
Norris × blue	57.1 ± 1.2c	436 ± 49abc	97.5 ± 2.5	509 ± 114ab	10.5 ± 1.2ab	49.8 ± 0.8ab

probably because of the lack of true inbred lines and predictable performance among crossbreds (Tave 1986). Hybridization particularly between channel catfish females and blue catfish males, provides significant increases in production (Table 5.3). However, studies have also shown variability in production depending on the strains of channel catfish and blue catfish used to produce the hybrid cross (Table 5.4) (Bosworth et al. 1998). Hybrid vigor has been demonstrated for growth rate and processing yield. Hybrids of channel catfish × blue catfish seem to exhibit heterosis for processing yield because hybrids retain the small head size from blue catfish and the robust body shape from channel catfish (Fig. 5.5). Twenty-eight different interspecific hybrids

FIGURE 5.5. Channel catfish (top), hybrid of blue catfish (male) × channel catfish (female), and blue catfish (bottom) showing relative differences in body conformation that result in differences in processing yields.

have been produced from seven different channel catfish species (Dunham and Smitherman 1987); however, poor rates of natural spawning is the main constraint on the use of hybrids in commercial culture. Although some success in producing hybrids can be achieved with hormone injections using pen and aquarium spawning procedures, it is not practical on a large commercial operation. Strong paternal predominance has been reported in the interspecific hybrid of the channel and blue catfish, with the appearance of the F_1 more similar to its paternal parent (Dunham et al. 1982; Goudie et al. 1993).

Future research needs to be conducted on either developing procedures to increase hybridization rates for commercial culture or developing "synthetic lines" incorporating the best production characteristics from particular species into a line that will spawn naturally in ponds under conditions currently used by commercial producers. Two "synthetic" catfish lines have been produced using two different strains of channel catfish and blue catfish as part of the USDA-ARS breeding program in Stoneville, Mississippi. These lines have been under development since 1992, were produced through a series of backcrosses, and are being selected for body conformation (fillet yield), growth, disease resistance, and reproductive performance (Brian Bosworth, USDA-ARS, Stoneville, Mississippi, unpublished data).

5.4.4 Strain and trait evaluations

Performance evaluations on strains, and effects from inbreeding and intraspecific crossbreeding are available from a number of research studies conducted primarily at university and federal research locations in the southeastern United States (Dunham and Smitherman 1983; Argue, 1996; Wolters et al. 2000). Strains of channel catfish used in research and commercial culture are based on geographic origin rather than on performance records (Dunham and Smitherman 1984). Many of these strain evaluations were conducted more than 10 years ago on research stocks. Continued research needs to be repeated with recent stocks from genetic improvement programs under current high-density culture conditions in several geographic locations. The most extensive research and evaluation studies have been conducted on the catfish line, NWAC103, jointly released by the USDA-ARS and the Mississippi Agricultural and Forestry Experiment Station from the cooperative program at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, Mississippi.

Catfish strains that have been cultured in hatcheries or used in commercial culture generally have faster growth than wild stocks. Most comparative evaluations have focused on growth because of its obvious economic impact in commercial production. However, strains have also been shown to differ in feed consumption and efficiency, disease resistance, dressout percentage, environmental tolerances and reproduction. Research has provided evidence for phenotypic and genetic variation in commercially important traits among catfish strains (Broussard and Stickney 1981; Dunham and Smitherman 1987; Tomasso and Carmichael 1991; Silverstein et al. 1999; Li et al. 1998; Wolters et al. 2000). Overall, it is apparent that channel catfish stocks in breeding programs have improved growth performance over unselected wild or commercial stocks.

Studies have also evaluated catfish strains for differences in other economically important traits such as reproductive performance and disease resistance. In general, catfish germplasm has not been developed with improved performance for additional traits presumably because adequate information has not existed for these traits and no clear economic benefit will occur. Some differences in reproductive performance have been found between catfish strains (Table 5.5), and minimal selection pressure has been placed on this trait. Differences have been found in spawning

TABLE 5.5. Reproductive traits (mean ± SEM) for USDA103 and Kansas strain broodfish spawned in replicate earthen ponds at the USDA-ARS Catfish Genetcis Research Unit, Stoneville, Mississippi. Means in a row not followed by the same letter differ at the 0.05 level of probability.

Variable or trait	Kansas	USDA103
Female weight (kg)	1.38 ± 0.02a	3.63 ± 0.13b
Male weight (kg)	1.56 ± 0.05a	3.54 ± 0.20b
Broodfish standing crop (kg/ha)	2,011 ± 31a	1,850 ± 43a
Broodfish sex ratio (♂ to ♀)	1.5 to 1	1.25 to 1
Spawning success		
Female (%)	23.3 ± 5.1a	57.5 ± 7.6b
Male (%)	20.0 ± 2.9b	50.0 ± 0.0a
Egg hatchability (%)	51.6 ± 9.4a	42.4 ± 7.6a
Egg size (number per gram)	29.1 ± 1.1a	33.8 ± 1.3b
Absolute fecundity (eggs per pound)	7,398 ± 910a	8,685 ± 869a
Testosterone (males, ng/mL)	0.58 ± 0.18a	18.6 ± 1.54b
Estrogen (females, ng/mL)	0.95 ± 0.08a	9.68 ± 0.83b
Testosterone (females, ng/mL)	0.36 ± 0.11a	9.42 ± 1.88b

success, age at maturity, fecundity, and serum levels of reproductive steroids (Wolters et al. 2000). Increased emphasis will undoubtedly be placed on selection for disease resistance in future breeding programs, and there is evidence for genetic control over resistance to bacterial diseases and response to vaccination (Wolters and Johnson 1995; Wolters et al. 1996; Wise et al. 2000). Significant differences in bacterial disease resistance between catfish strains have been shown. However, in the case of enteric septicemia of catfish (ESC)—caused by the bacterium *Edwardsiella ictaluri*, the magnitude of the strain effect is not as great as the reduction in mortality achieved by managing the disease through vaccination or withholding feed (see Section 15.7.2) (Wise and Johnson 1998; Wise et al. 2000).

Heritability estimates from sibling analyses and realized heritabilities calculated from selection responses have been made for several production parameters in channel catfish (Tave 1986; Dunham and Smitherman 1983; Dunham and Smitherman 1987). Most estimates have been for body weight or length, and mass selection has been successful in increasing body weight (Table 5.6). Most selection programs developed at public research institutions have been hindered

TABLE 5.6. Response to selection and realized heritabilities for body weight in three channel catfish strains (adapted from Dunham and Smitherman 1983).

Strain	Mean weight (g)	Response (g)	Selection differential (g)	Realized heritability
Rio Grande				
Select	431	63	263	0.24 ± 0.06
Random	368			
Marion				
Select	486	73	145	0.50 ± 0.13
Random	413			
Kansas				
Select	513	54	163	0.33 ± 0.10
Random	459			

by the lack of long-term funding to sustain continuity. Because of the 3- to 5-year generation interval, a long-term funding commitment must be made for significant progress to occur. Selection of high-level performers, along with moderate heritability estimates for some traits (Tave 1986), will permit breeders to realize greater genetic gains in early generations of select populations.

Selective breeding, especially directional mass selection and family selection have been used to improve catfish growth and feed consumption. Although growth traits affect economic returns in commercial production, breeding programs must also focus on multiple traits in lines planned for release to commercial producers. The breeding program conducted by the USDA-ARS in Stoneville, Mississippi, has developed a multi-trait selection program based on family means in the USDA103 catfish research line. Adult catfish have been stocked into earthen ponds and allowed to mate at random. Full and half-sib families have been evaluated for nine economically important traits (such as disease resistance, growth rate, feed conversion, and fillet yield), and parentage of families determined from microsatellite genotypes of parents and offspring. Family ranking was determined for each trait, and the 20 families with the highest composite ranking were saved as a selected line. Coefficients of variation for the nine traits ranged from 0.87 for carcass yield to 78.0 for juvenile feed consumption, and mean differences between traits for the 99 families and the 20 selected families ranged from 0.29% for shank fillet yield to 60.9% for harvest weight. The 2-year-old spawning success in this line was 42%, and offspring obtained from the selected population are being evaluated for the same nine traits. Selection differentials would have been larger for selection solely on individual traits, but the selection index implemented is designed to improve all traits simultaneously.

5.5 MOLECULAR AND CELLULAR GENETICS

The goal of an agricultural genomics program is to provide useful molecular tools for the identification of genes that control economically important traits. Molecular markers that identify beneficial alleles within these genes can be used to increase the efficiency of broodstock selection. Traditional selection by performance is difficult for many traits. A trait may be sex-limited, such as identifying males that will pass on beneficial traits for the spawning success of their daughters. Exposure of broodstock to test disease resistance depends on the ability to perform controlled experiments with pathogens, often difficult for catfish diseases. In some cases performance testing is lethal, such as measurement of carcass quality. A rapid molecular test, for example a DNA test or protein assay, can be used to identify broodstock that have inherited useful genetic variants without the need for performance testing. The tools needed to perform these tests in catfish include molecular DNA and protein markers, genetic linkage and physical maps, reference and resource families, and bioinformatics capacity.

5.5.1 Genome size

The double helix of deoxyribonucleic acid (DNA) is the storage mechanism for genetic information in all animals. For the most part, every cell nucleus contains DNA which provides the blueprint for the development and activities of that cell. Body cells typically have double the amount of DNA (termed diploidy) than that of sex cells (haploid). The quantity of DNA contained within a cell is called genome size and can be determined by a number of methods, of

which flow cytometry is one of the most accurate. Genome size of channel catfish blood cells (a standard cell type for this analysis) determined by flow cytometry is consistently found to be around 2 pg (i.e., 2×10^{-12} g; the combined DNA of 500 billion cells would yield one gram of DNA) (Tiersch and Goudie 1993). This is a common value for fishes, but is small compared to other vertebrates with genome size values typically between 5 and 10 pg, and ranging as high as 100 pg (Wachtel and Tiersch 1993). Mammals, such as humans, for example, typically have around 7 pg per cell. The variation in genome size among channel catfish is small. A comparison of more than 100 channel catfish representing 12 populations from different locations revealed a total of 2% variation in genome size, which is among the lowest values reported for vertebrates (Tiersch et al. 1990). Other fishes such as cyprinids (Gold et al. 1990) and salmonids (Lockwood and Derr 1992) can exhibit variation larger by an order of magnitude. In addition, the genome is stable and inherited with great fidelity in channel catfish, even in crosses made with catfish of other species and genera (Tiersch and Goudie 1993).

5.5.2 Cytogenetics

Studies focusing on catfish chromosomes began 25 years ago (Hudson 1976) although reports addressing catfish can be found earlier (Muramoto et al. 1968). Most of these studies used chromosomal data for analysis of phylogenetic relationships (e.g., LeGrande 1981). The earliest studies of channel catfish reported 56 chromosomes in diploid cells (Muramoto et al. 1968; Hudson 1976), but later studies have found the chromosome number to be 58 (LeGrande 1981; Wolters et al. 1981b; Zhang and Tiersch 1998a). Although differences exist in chromosome number and structure among species within the family Ictaluridae, hybrids have been studied between channel catfish and representatives of the genera *Ictalurus*, *Ameiurus*, and *Pylodictis* (LeGrande et al. 1984; Zhang and Tiersch 1997). The cross between channel catfish and white catfish (which has 48 chromosomes) represents an extreme example yielding a hybrid with 53 chromosomes (LeGrande et al. 1984). The diploid numbers of hybrid fish were found to be the average of the diploid numbers of the parental species (LeGrande et al. 1984; Zhang and Tiersch 1997). This information supports the observation that the genome of ictalurid catfishes segregates as a function of haploid chromosome number and nuclear DNA content, and is stable in interspecific and intergeneric hybrids.

As in most fishes, channel catfish do not possess sex chromosomes (LeGrande 1981; Tiersch et al. 1992). In humans, for example, there exists a pair of chromosomes (the X and Y) that are dissimilar in size and shape, and one of these (the Y) carries the gene (*SRY*) that triggers the pathway that leads to male sexual development (Sinclair et al. 1990; Tiersch et al. 1992). Thus mammals such as humans have in comparison with channel catfish fewer chromosomes (46) that are larger (comprising 7 pg of DNA), and possess natural indicators of particular genes (e.g., sex chromosomes). The small, numerous and essentially anonymous chromosomes of channel catfish have posed challenges for genetic study. Differential staining by chemical treatment is a standard technique for identification and localization of certain chromosomal structures. Staining techniques are more straightforward in mammalian chromosomes for reasons such as their larger size, but also because they are naturally more heterogeneous, enabling visualization of light and dark areas (bands) along the length of the chromosomes.

Because of the difficulties inherent in studying catfish chromosomes, only certain structures have been identified at present. One of these is the nucleolus organizer region (NOR), which are active areas of RNA synthesis for the ribososmes, the cellular organelle involved in protein

synthesis. Several methods will identify NOR including fluorescent DNA dyes and protein staining. These techniques have mapped the NOR, and the associated ribosomal RNA genes, to a chromosome identified as D-11 (Zhang et al. 1999), and the NOR phenotype was stable among different tissue types (Zhang and Tiersch 1998a). Another useful technique has involved staining of non-coding DNA called constitutive heterochromatin (C-banding). The constitutive heterochromatin of channel catfish chromosomes was found to be sparse and limited to centromeric regions (Zhang and Tiersch 1998a). Distinct secondary bands were absent on channel catfish chromosomes unlike results observed in cyprinids (Gold et al. 1986) and many salmonid fishes (Phillips and Hartley 1988). The C-bands were prominent and useful for identifying homologous (paired) chromosomes. The low abundance of heterochromatin may explain the stable genome size found in the ictalurid catfishes as described above and reflect evolutionary conservatism within the ictalurid genome.

The NOR-staining and C-banding techniques are useful to identify chromosomes with special structures and to identify homologous pairs. However, these techniques do not readily distinguish among chromosomes of similar size and shape (centromeric position). Staining methods such as Giemsa (G-banding) and reverse (R-banding) banding (Schwarzacher and Wolf 1974) are useful with mammalian chromosomes, but application of standard treatments for these staining methods—such as trypsin, heat, or fluorochromes—were not successful with catfish chromosomes (Zhang 1996).

Fortunately, other techniques, such as digestion of chromosomes with restriction enzymes, causing removal of DNA fragments followed by Giemsa staining, are capable of generating multiple bands, allowing differentiation of morphologically similar chromosomes (Zhang and Tiersch 1998b). Another useful technique is replication banding which is based on the incorporation of the base analogue bromodeoxyuridine during DNA replication resulting in regions detectable by ultraviolet irradiation coupled with fluorochrome and Giemsa staining. In channel catfish, five to 13 bands were generated on each chromosome by this technique, with a total of 215 bands on the entire haploid set (Zhang et al. 1998). It should be noted that these techniques did not produce uniformly strong staining and analysis of the banding patterns required assistance of a computer-based densitometric method (Zhang and Tiersch 1998b). Integration of information from various banding techniques can be used to characterize individual chromosomes (Fig.5.6).

The banding procedures described above are useful for laying out a general map of the chromosomes based on DNA sequences that generally are not involved in coding for proteins. Other techniques are necessary in conjunction with banding procedures to identify the location of specific genes. Often, portions of the genes themselves are synthesized, labeled, and used as probes to identify the presence or location of a complementary sequence. Such techniques are referred to as in-situ hybridization (ISH), and have worked successfully for identification of highly repeated sequences and genes with multiple copies in the haploid genome (Phillips and Reed 1996). However, these techniques have a limited capability to identify target sequences existing as a single copy in haploid genomes. A new technology called in-situ polymerase chain reaction (ISPCR) allows multiplication of target DNA sequences to increase efficiency of hybridization and detection (Gu 1994). In this technique, short pieces of single-stranded DNA called primers are used to direct the copying of target DNA sequences by the polymerase chain reaction (PCR). Labeling of the copied DNA allows detection (mapping) of the target sequence on chromosomes. This technique has been applied to mapping of the channel catfish *Ig H* gene (Wilson et al. 1990), which encodes a portion of the immunoglobulin heavy chain, and the

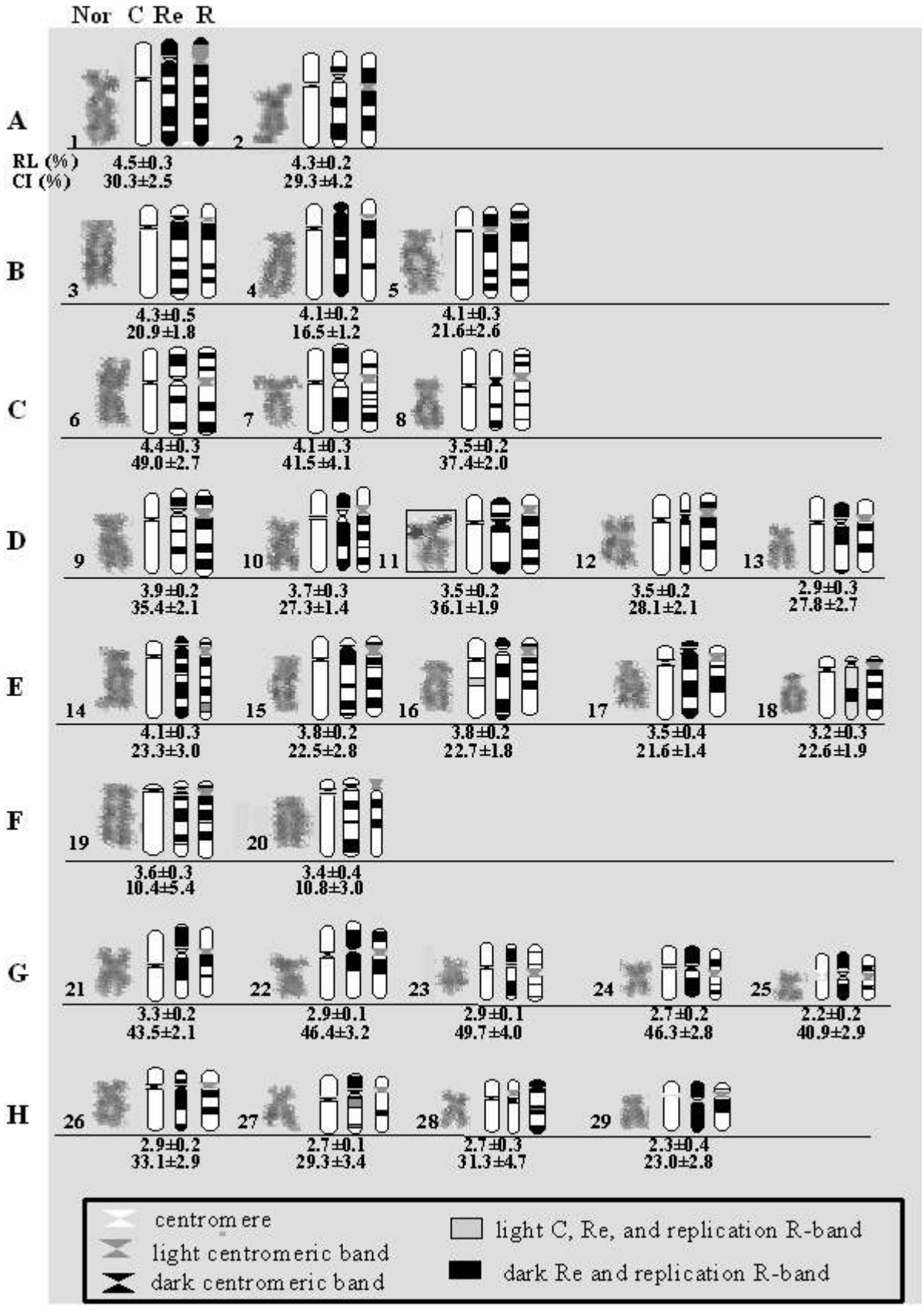
Nor C Re R
A
RL (%)
CI (%)
1 4.5±0.3 30.3±2.5
2 4.3±0.2 29.3±4.2
B
3 4.3±0.5 20.9±1.8
4 4.1±0.2 16.5±1.2
5 4.1±0.3 21.6±2.6
C
6 4.4±0.3 49.0±2.7
7 4.1±0.3 41.5±4.1
8 3.5±0.2 37.4±2.0
D
9 3.9±0.2 35.4±2.1
10 3.7±0.3 27.3±1.4
11 3.5±0.2 36.1±1.9
12 3.5±0.2 28.1±2.1
13 2.9±0.3 27.8±2.7
E
14 4.1±0.3 23.3±3.0
15 3.8±0.2 22.5±2.8
16 3.8±0.2 22.7±1.8
17 3.5±0.4 21.6±1.4
18 3.2±0.3 22.6±1.9
F
19 3.6±0.3 10.4±5.4
20 3.4±0.4 10.8±3.0
G
21 3.3±0.2 43.5±2.1
22 2.9±0.1 46.4±3.2
23 2.9±0.1 49.7±4.0
24 2.7±0.2 46.3±2.8
25 2.2±0.2 40.9±2.9
H
26 2.9±0.2 33.1±2.9
27 2.7±0.1 29.3±3.4
28 2.7±0.3 31.3±4.7
29 2.3±0.4 23.0±2.8
centromere
light centromeric band
dark centromeric band
light C, Re, and replication R-band
dark Re and replication R-band

FIGURE 5.6 (Opposite page). A comprehensive summary of cytogenetic information, referred to as an ideogram, (used with permission from Zhang et al. 2000) of channel catfish chromosomes treated by silver staining to reveal the nucleolus organizer region (NOR), C-banding (C) to reveal constitutive heterochromatin, and the restriction enzyme *Hind* III (Re) and replication R-banding (R) to reveal banding patterns that are unique for each chromosome. The chromosomes were organized by shape (centromeric index; CI) and relative length (RL), and divided into eight groups: A, large submetacentric (the centromere is located off-center); B, large subtelocentric (centromere at end); C, large and medium metacentric (centromere in middle); D, medium submetacentric; E, medium subtelocentric; F, telocentric; G, small metacentric, and H, small submetacentric chromosomes. The inset box (D-11) shows the NOR-bearing chromosome.

location of 28S ribosomal RNA gene (28S rDNA) associated with the NOR. These results demonstrate the validity of the ISPCR techniques for use in chromosomal mapping (Zhang et al. 1998a; 2000).

5.5.3 Protein loci variation

Electrophoretic analyses of enzyme and protein variation have been extensively studied in channel catfish, blue catfish and white catfish (Dunham and Smitherman 1984; Hallerman et al. 1986; Carmichael et al. 1992). These studies have demonstrated the ability to differentiate between species and stocks of catfish, and measure changes in gene frequencies caused by selection. Genetic variability within and between channel and blue catfish were measured at 70 isozyme loci (Dunham and Smitherman 1984, Hallerman et al. 1986, Carmichael et al. 1992). Allozyme frequencies at 9 of 13 polymorphic loci were found to change in response to selection for growth rate, indicating these isozyme loci influenced growth directly or were linked to other loci related to growth (Hallerman et al. 1986). Unfortunately, these relationships were not further explored. The small number of isozyme alleles and difficulty in measurement limits their use in a large mapping effort, and low levels of polymorphism coupled with lack of correlation with phenotypes limits the use of isozyme markers in catfish selective breeding (Dunham and Smitherman, 1984; Hallerman et al., 1986; Carmichael et al., 1992). Although these fixed and polymorphic loci can be used to determine unique genetic markers of some populations and different catfish species, future research will focus on identifying unique DNA sequences between strains and populations (Lloyd et al. 1989; Turner et al. 1989; Turner et al. 1991).

5.5.4 DNA marker variation

There are several advantages for molecular genetic analyses and genetic mapping of catfish. The genome size of channel catfish is approximately 1×10^9 base pairs per haploid nucleus, which is equal to 447 kbp per centimorgan (cM), smaller than most domestic mammalian and avian species (Tiersch et al. 1990; Tiersch and Goudie 1993). The genome size in terms of recombination is not known. However, assuming the recombination genome size to be 2500 cM (1 cM = ~ 400,000 bp), 500 evenly distributed genetic markers would be required for a 5 cM resolution, and can be accomplished in a reasonable time (2 to 3 years) and subsequently incorporated into an applied breeding program.

A more efficient and useful approach for utilizing molecular genetic information for catfish breeding will utilize DNA marker technology. The molecular maps that have been formulated for domestic animal genomes are providing powerful tools for selective breeding. The initial DNA

marker-based linkage maps for aquatic species like tilapia and rainbow trout relied primarily on random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995; Kocher et al. 1998; Young et al. 1998). These markers are anonymous DNA sequences that are dominant markers and may be specific to the mapping population. Both RAPD and AFLP markers developed for channel catfish have low levels of polymorphism (Liu et al. 1998a, b). Although these markers are abundant and can be identified rapidly, they are likely to be useful only for mapping interspecific hybrid populations and less useful for intraspecific mapping.

The most useful genetic linkage maps developed for catfish will be based on microsatellite loci (Waldbieser and Bosworth 1997); however, the channel catfish mitochondrial genome has recently been sequenced and may also yield useful genetic information applicable to breeding programs (Waldbieser et al. 2003). Microsatellite loci are polymorphic DNA sequences containing short tandem repeats. Microsatellite loci appear to be distributed throughout the genome and demonstrate high levels of intraspecific allelic polymorphism. Unique DNA sequences flanking microsatellites can be used to identify and further characterize regions around these loci. Genetic linkage maps based on micosatellite markers have been produced for agriculturally important species such as cattle, swine, sheep and chickens (Rohrer et al. 1996; Kappes et al. 1997; DeGortari et al. 1998; Groenen et al. 2000). Microsatellite linkage maps have also been produced for rainbow trout, zebrafish, and some microsatellite loci were included in the tilapia linkage map (Shimoda et al. 1999; Sakamoto et al. 2000; Gates et al. 1999; Kocher et al. 1998). Compared to AFLP and RAPD markers, microsatellite markers require a large amount of experimental work (sequencing and PCR optimization) before they can be used in linkage mapping. Minisatellite loci have also been cloned and a family of AT-rich *Xba* elements or repetitive sequences is estimated to cover 5 to 6% of the catfish genome (Liu et al. 1999b).

5.5.5 Status of genetic mapping in catfish

There are two options for production of mapping populations for construction of genetic linkage and QTL mapping in catfish: (1) the intraspecific mating plan, and (2) the channel catfish x blue catfish hybrid system. In either case, broodstock fish have been produced and are being used as mapping populations for linkage analysis and QTL measurements at the USDA-ARS Catfish Genetics Research Unit (Stoneville, Mississippi) and at Auburn University. The USDA researchers have focused on intraspecific matings with channel catfish families, while Auburn researchers have focused on matings of channel catfish × blue catfish.

An initial genetic linkage map has been produced from two families made by crosses between NWAC 103 and Norris strains (USDA) to potentially increase the heterozygosity of markers to maximize the number of informative inheritance events (meioses) in the offspring. This map consists of 263 microsatellite markers arrayed in 32 linkage groups covering 1958 cM of the channel catfish genome, providing an average distance of 9 cM between markers (9% recombination between markers) (Waldbieser et al. 2001; Fig. 5.7). An additional 24.5 cM is contained in three two-point linkage groups and 252 cM to include the teomeric regions increases the estimated total genome size to 2234.5 cM. As more markers are developed and placed on the map there will presumably be 29 linkage groups, one for each chromosome in the haploid genome. Only 8% of these markers came from sequences encoding genes, the rest were developed from non-coding genomic DNA unique to channel catfish. Many catfish cDNA sequences contain short tandem repeats in the 5′ or 3′ untranslated region, therefore some cDNA libraries have now

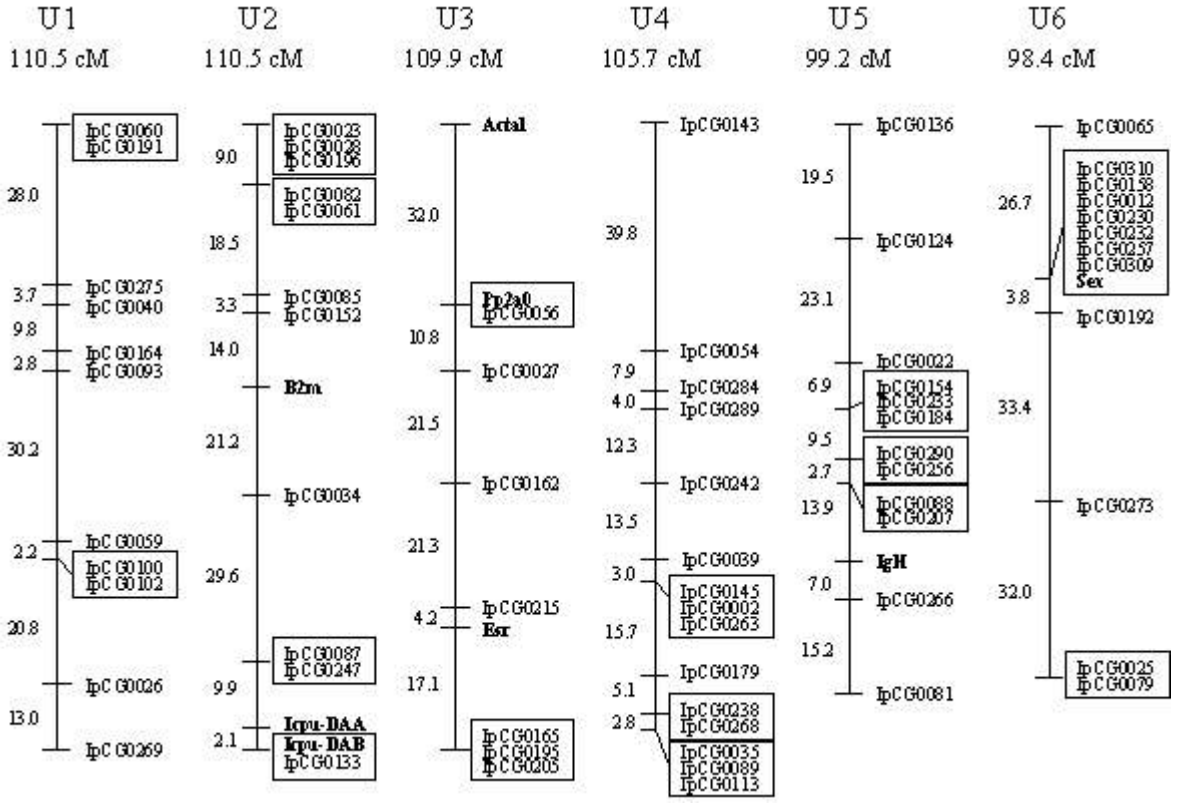

FIGURE 5.7. A comprehensive summary of molecular genetic information, referred to as a linkage map (adapted from Waldbieser et al. 2001), was prepared from the channel catfish genome by cutting the DNA with enzymes to yield multiple analyzable segments. The DNA sequence of these segments is identified and overlapping sequences are interpreted to reassemble the DNA sequence of entire chromosomes. At the beginning of this process, the sequences are referred to as linkage groups (i.e., these sequences are linked). Eventually linkage groups would be resolved into knowledge of the genetic material on the chromosomes. In this example, we present the six largest (of 32) linkage groups. The letter U refers to putative linkages, and the unit centiMorgan (cM) refers to relative genetic distances among the labeled (e.g., IpCG or Acta1) segments arrayed in a linear display.

been enriched for these clones to develop markers specific for these genes (Liu et al. 1999a; Nonneman et al. 2001). Increasing the marker density on the linkage map with genes that are conserved between species will allow researchers to compare catfish with better characterized species, especially zebrafish *Danio rerio* (Gates et al. 1999; Shimoda et al. 1999; Woods et al. 2000).

5.5.6 Application of molecular genetics

Genomics has already been applied to genetic improvement of catfish. Microsatellite markers have been used to determine parentage of spawns collected from communal ponds (Waldbieser and Wolters 1999). This marker technology allows breeders to use large half-sib and full-sib families from natural spawning conditions to estimate genetic components of phenotypic variation. The markers have also been used to provide a genetic fingerprint of the NWAC103

catfish line. Coincident with the release of this catfish line was the development of a strain certification program by the catfish industry. Genetic markers will help producers maintain the genetic integrity of the NWAC103 line, and other lines of catfish released in the future (MAFES 1996; MSIA 2000).

The relative abundance of catfish microsatellite loci found in gene-encoding regions demonstrates the potential for application of molecular genetics into breeding programs. Successful mapping of QTL loci will require several basic requirements (Mauricio 2001). The primary requirement is to initially choose parents with differences in alleles that control traits of interest. There does not need to be differences in the mean phenotypes between the parents, although different allelic combinations are important. A detailed linkage map allowing differences between parents or strains is required, and more markers will increase mapping resolution.

A number of different crossing schemes can be used to create and measure the different combinations of phentoypes and genotypes in individual offspring. Large family sizes in channel catfish will facilitate the ordering of closely linked markers and increase the power of statistical methods used to detect QTL controlling economically important traits. Molecular markers and a genetic linkage map are currently available, however, the map will need approximately 1,000 evenly distributed markers to be efficiently used for localization of genomic regions controlling important traits. Because QTL mapping is essentially a statistical approach using both phenotypic and genotypic data, the accuracy and power of QTL analyses will depend on knowing trait heritabilities, precise trait measurement, and the size of the mapping population.

Research in genomics will be expensive and time-consuming, but must be incorporated into current breeding programs because ultimately the benefits will extend beyond the current limitations of quantitative genetics and selective breeding (Waldbiesser and Wolters 2001). Short-term goals in catfish genomics include increasing the number of markers on the genetic linkage map to improve map density and genomic coverage, development of a comparative genetic map with zebrafish, development of QTL resource populations, and improvement of bioinformatics capacity. Microsatellite alleles within known (candidate) genes can be tested for association with performance. Molecular markers should be used for family and individual identification to assist traditional breeding programs and strain identification. Also, assays for measurement of quantitative traits such as reproductive performance, disease resistance and carcass quality need to be refined.

Mid-term goals will include QTL scans to identify regions of the genome involved in important traits. Regions must then be “fine mapped” with more markers to identify candidate genes. The development and utilization of large genomic clone maps based on bacterial artificial chromosomes (BAC) will assist this effort (Quiniou et al. 2003). Development of large genomic clone maps based on bacterial artificial chromosomes (BAC) will assist this effort. Bioinformatics methods should be developed for efficient identification of superior broodstock and efficient use of marker-assisted selection to identify catfish lines. Gene transfer in model species such as zebrafish will assist breeders in evaluating catfish gene constructs and the potential use of this technology. Long-term goals could include the determination of the complete DNA sequence of the catfish genome. Finally, molecular markers should be used from QTL studies to select superior broodstock and assist breeders in the introduction of beneficial alleles from other ictalurid species.

5.6 SEX REVERSAL

Traditional selective breeding approaches, specifically directional mass selection and family selection, generally can realize gains in performance traits of 10 to 15% per generation (Tave 1986). In addition to these traditional approaches, specific breeding aids such as sex reversal may also be used in a breeding program to further increase performance. The ability to produce single-sex populations has been proposed in many fish species to control reproduction and also to allow culturists to select fish of a specific sex that grows fastest (Hunter and Donaldson 1983; Goudie et al. 1983).

As stated above, channel catfish do not possess morphologically distinct sex chromosomes like the X and Y chromosomes of mammals. Despite this difference, sex determination (the developmental process leading to phenotypic sex) in channel catfish follows a pattern consistent with an XX-XY model (Davis et al. 1990; Tiersch et al. 1992). This condition, referred to as male heterogamety (or sometimes as female homogamety), indicates that the male parent produces two types of sperm that determine if the offspring will be male or female. It is possible to manipulate this natural mode of sex determination by addition of hormones during specific stages of development. Monosex female populations of channel catfish have been produced by feeding hormone-treated feed to newly hatched fry (Goudie et al. 1983). Oral adminstration of 17α-estradiol and 17α-testosterone, and other steroid sex hormones including estrogens, aromatizable androgens (susceptible to enzymatic conversion to estrogens), non-aromatizable androgens, and aromatase inhibitors have consistently produced sex ratios in channel catfish biased towards or completely female (Davis et al. 1990). This has been described as paradoxical feminization because typically masculinization of teleosts results from treatment with androgens (Goudie et al. 1983). Because male catfish generally grow faster than females in mixed-sex populations, all-male culture would provide an increase in production efficiency (Simco et al. 1989). Given that hormone treatments yielded females, an indirect approach was necessary to produce all-male populations. The sex-reversed XY females were identified and mated with normal XY males to yield a sex ratio of 3 males to 1 female (Davis et al. 1991). Male catfish are normally heterogametic (capable of producing offspring of either sex), and 33% of the males produced in this cross would be YY (homogametic). The YY males are viable and can be mated with normal

TABLE 5.7. Body weight, gonad weight (as a percent of body weight = gonadosomatic index, GSI), yield, pond survival, and fillet yield of control and trenbolone acetate-treated channel catfish cultured in earthen ponds and mortality of juvenile catfish challenged with the bacterium *Edwardsiella ictaluri*. Treated fish were fed 50 mg of trenbolone acetate per kg of food for first 60-days of feeding. Means in a row not followed by the same letter differ at the 0.05 level of probability.

Variable	Untreated control	Trenbolone acetate-treated
Body weight at 18 months (g)[a]	875a	650b
Gonadosomatic index (%)[a]	0.16a	0.05b
Yield (kg/ha)[2]	645 ± 7	587 ± 48
Survival in ponds (%)[b]	83.3 ± 1.2	81.9 ± 10
Total fillet yield (shank plus nugget, %)[b]	43.8 ± 1.2	41.9 ± 0.8
Survival after exposure to *E.ictaluri*[2]	93.7 ± 1.8a	73.7 ± 9.0b

[a] Davis et al. (2000)

[b] W.R. Wolters (unpublished).

XX females to produce monosex XY male populations without the need for feeding sex hormones. Progeny testing of sex-reversed fish leading to the production and maintenance of these YY male lines would be an important long-term activity of breeding programs.

A synthetic androgen, trenbolone acetate (TBA), which induces anabolic weight gain in beef cattle, has been shown to masculinize blue tilapia *Oreochromis aureus* and channel catfish (Roche and Quirke 1986; Galvez et al. 1995, 1996). Presumably, feeding of TBA to newly hatched fry would produce 100% male offspring in a single generation and allow for integration of this breeding aid into a selection program to provide an additional gain above that achieved through selective breeding. However, channel catfish treated with TBA did not result in all-male populations, and growth of TBA-treated fish was slower than controls, resulted in abnormal gonadal development, and increased susceptibility to infection with *Edwardsiella ictaluri* (Table 5.7). Other genetic manipulations of normal developmental processes are possible in channel catfish and are described in the next section.

5.7 POLYPLOIDY, GYNOGENESIS, AND ANDROGENESIS

Shortly after fertilization in fishes, an extra set of maternal chromosomes is eliminated from the egg (a process referred to as polar body extrusion following the second meiotic division). Application of heat, cold, pressure or specific chemicals can interfere with this normal process and cause retention of the polar body, yielding an embryo with three sets of chromosomes (a condition referred to as triploidy) instead of the normal two (diploidy). In a similar fashion, the first cell division of the developing embryo can be interrupted to yield four sets of chromosomes in a single cell (instead of two sets in each of two cells) yielding a condition referred to as tetraploidy. Triploidy and tetraploidy have been induced in channel catfish by cold shocking or heat shocking of fertilized eggs at appropriate times after fertilization (Wolters et al. 1981b; Bidwell et al. 1985). Triploid fish were found to be sterile and exhibit faster growth than diploids in tank culture (Wolters et al. 1982). Subsequent studies showed no production improvement when triploid fish were grown in earthen ponds (Wolters et al. 1991). Because of the practical limitations on collecting large numbers of fertilized catfish eggs through artificial fertilization, it is unlikely that polyploid catfish will be used in commercial culture. The development of tetraploid broodstock would theoretically enable spawning of tetraploid females with diploid males (or the reverse cross) using conventional pond spawning procedures, however, this accomplishment has not been demonstrated. Another more labor-intensive approach would be to use cryopreserved sperm from tetraploid males to fertilize eggs collected for artificial spawning. This approach would be most useful for limited production of sterile triploid fishes for research purposes, and would be especially useful for security of transgenic catfish lines, which will be addressed in the next section.

It is also possible to utilize the methods for production of polyploidy in combination with genetic inactivation by irradiation of the genome of the egg or sperm to produce diploid offspring that inherit chromosomes only from a single parent. For example, if the male genome is eliminated, but the sperm is able to activate further development in the egg, a haploid embryo (with one set of chromosomes) would result. Application of cold, heat, pressure or chemicals in a fashion similar to triploidy or tetraploidy induction can restore diploidy, yielding an embryo with two sets of chromosomes from the mother, a condition referred to as gynogenesis which offers potential benefits for research and genetic improvement programs (see, for example, Mair

1993). Gynogenesis has been produced in channel catfish by the use of ultraviolet irradiation of sperm cells and application of pressure shocks after fertilization (Goudie, 1987; Goudie et al. 1991) and has provided highly inbred lines for research purposes such as genome mapping (Liu et al. 1992).

Androgenesis is another form of chromosome set manipulation that is well studied in fishes (Stoss, 1983; Ihssen et al., 1990), and yields offspring that carry only two sets of paternal chromosomes. It is induced in a manner similar to tetraploidy, except that maternal (egg) chromosomes are inactivated by irradiation (typically gamma or ultraviolet), which yields a doubling of the paternal (sperm) nucleus at first mitosis, and produces homozygosity (two exact copies of each gene). If cryopreserved sperm was available from a particular line or species, androgenesis could be used to produce homozygous offspring from irradiated eggs from another line or species. This process raises the possibility of recovering lost research lines or extinct species (Thorgaard et al., 2000). Androgenesis has been produced in channel catfish by the use of gamma irradiation of eggs and application of cold or heat shocks after fertilization (Lee et al. 2000).

5.8 GENE TRANSFER

Gene transfer technology is an established tool in molecular genetics. The ability to transfer individual genes from one organism to another has led to the development of new biotechnology industries (Pursel et al. 1989). Over the past few years, the technology has been applied to produce transgenic fish in several species (Maclean and Penman 1990). Foreign DNA has been successfully integrated into the channel catfish genome (Dunham et al. 1987). Research is currently underway to determine the contribution that transgenic fish could make in aquaculture. For example, faster growth and increased disease resistance are likely to be exhibited by transgenic fish and could improve production efficiency. Silverstein et al. (2000) recently demonstrated increased growth, feed intake, and IGF-I levels in two catfish strains administered bovine growth hormone, suggesting similar results in transgenic catfish carrying the gene for bovine growth hormone. Overall, transgenic fish may eventually be utilized if public perception changes and becomes favorable towards their production and consumption. This change in perception will be difficult to realize with the persistent attacks by advocacy groups on genetically modified foods and fish farming in general (e.g., Goldberg et al. 2001, Tiersch and Hargreaves 2002).

The use of transgenic fish, and also genetically improved hatchery stocks, in aquaculture has ecological implications (Kapuscinksi and Hallerman 1990; Hew and Gong 1992; Hallerman and Kapuscinski 1992). Production of channel catfish is almost exclusively in large earthen ponds, often built in areas prone to flooding. The accidental release of transgenic fish from commercial ponds is a possibility, and transgenic fish could breed with wild fish transferring transgenes into the native population. The genetic and ecological structures of native channel catfish populations have not been as well studied as those of salmonids. Guidelines on the use of transgenic catfish in aquaculture systems need to be developed to ensure against accidental release. Future studies should focus on characterization of the total phenotype of transgenic catfish including all aspects of physiology, reproduction, and behavior. Transgenic fish will be excellent models for animal research, however, and considerable research is still needed to determine how introduced genes modify the phenotype of transgenic fish, and for regulatory approval and application of transgenic fish into production systems.

5.9 CRYOPRESERVATION

Sperm cryopreservation has been studied in as many as 200 species of fish (Tiersch 2000; 2001) including three families of catfishes (Ictaluridae, Clariidae, and Pangasiidae). Work with channel catfish began in the 1970s and yielded limited motility in thawed sperm (Guest et al. 1976). Studies were resumed in the 1990s and yielded fertilization with thawed sperm (Tiersch et al. 1994). Subsequent work has refined protocols using 10% methanol as a cryoprotectant, standard 0.5-mL French straws (used for bull semen), and a cooling rate of 40°C per minute (Christensen and Tiersch 1997). Procedures such as these have been standardized for research in fish species (Wayman and Tiersch 2000), although there are species-specific considerations. For example, sperm cannot be stripped from male channel catfish and testis must be removed to collect samples (Sneed and Clemens 1963; Bart and Dunham 1990). The testes are crushed to suspend the sperm in an extender solution. Best results for cryopreservation and refrigerated storage of sperm have come from the use of Hanks' balanced salt solution with an osmolality of 270 to 300 mOsmol/kg (Bates et al. 1996; Christensen and Tiersch 1996).

Long-term gamete storage would permit reduction of the facilities needed for broodstock maintenance, increase the numbers of genetic stocks available, facilitate shipment of improved germplasm or reference stocks to widespread locations, and provide a year-round supply of sperm for commercial production and research. Application of this technology beyond research is constrained, however, by the requirement for collection of unfertilized eggs for artificial spawning. Beyond this, there are needs for automated filling of straws or use of larger containers (Wheeler and Thorgaard 1991), inventory and record databases for frozen samples (Kincaid 2000), bulk storage capacity, sample security, protection from pathogen transmission (Jenkins and Tiersch 1997; Tiersch and Jenkins 2003), and distribution capabilities, before application can occur at the commercial scale. Infrastructure of this type could be developed for channel catfish by adding the technology to existing facilities (Caffey and Tiersch 2000a, 2000b), or by adopting existing technology from other industries. Commercial equipment and procedures used for dairy bull sperm have been shown to yield fertilization by channel catfish sperm that was not different between fresh (non-frozen) and thawed samples (Roppolo 2000; Lang et al. 2003b). This technology would be especially useful for the development of commercial-scale production of hybrids by use of cryopreserved sperm from blue catfish. Cryopreservation has become essential within the dairy industry for the production of genetic improvement by acceleration of selective breeding efforts, maintenance of improved lines, and distribution of superior genetic material. Indeed, global billion-dollar industries exist for cryopreserved livestock sperm and thus provide useful models for corresponding development within aquaculture industries such as channel catfish (Johnson 2000; Lang et al. 2003b).

5.10 INTEGRATION OF TECHNOLOGIES INTO BREEDING PROGRAMS

Genetic improvement programs for channel catfish offer considerable potential for commercial production. Research areas should incorporate traditional animal breeding approaches and new biotechnologies (Fig. 5.8). Programs that coordinate industry and research while addressing priority areas of importance to commercial production will have the greatest chance for success. Integration of technologies will be essential for this process. For example, genetic mapping can yield DNA markers that assist in selection of broodstock and improved lines. Genetic screening

FIGURE 5.8. Genetic improvement is a process that requires interaction of research and production. New and improved genetic tools are developed by research and are applied to genetic improvement of specific lines. Improved lines are distributed to the industry and comprehensive data on production and processing yields are used to evaluate practices and to focus future research efforts. A strong information base and many genetic tools are available for application in the catfish industry, but the benefits of genetic improvement will not be realized without industry-wide monitoring and evaluation of production and processing yields.

would be used to ensure purity of these lines and to assist in development of broodstock certification programs. Sperm from these lines could be cryopreserved to assist in conservation and distribution of the genetic resources.

5.10.1 Considerations for breeding programs

Because of current management constraints, genetic improvement programs for channel catfish will be most effective if located at federal or university research stations. However, a comprehensive program for transfer of results of genetic research to private industry needs to be developed. Previous releases of catfish strains have been by research universities directly to commercial farmers (Dunham and Smitherman 1987). A protocol for release, testing, stock multiplication, and stock verification needs to be developed for channel catfish stocks. Procedures for releasing and testing plant varieties are well established and effective, and could serve as a model for future catfish releases (Poehlman 1959). A process of stock multiplication and release of improved germplasm independent of the research or breeder organization and reviewed by industry needs to be developed and followed by all cooperating agencies. The genetic identity of catfish germplasm should be maintained and available. Technology enabling genetic identification should be standardized and include protein or DNA polymorphisms (MSIA 2000). Without research and industry cooperation, minimal realized benefit will result from genetic research.

5.10.3 Commercial releases of improved Lines

There have been six different releases of improved catfish lines from research institutions since 1983 (Table 5.8), and do not include sales of fish in breeding programs from commercial producers (Roger Yant, Harvest Select Farms, Inc., Inverness, Mississippi, personal communication). The six releases to commercial producers have consisted of Kansas select lines (second, third, and fourth generation lines), Marion select lines (second generation), Auburn line, and Marion × Kansas (third generation) line from Auburn University catfish breeding program. The largest release with the greatest potential impact from Auburn University's breeding program occurred in 1984 when approximately 60,000 Kansas line fish were released to 63 producers in 7 states. The Kansas line is still cultured by commercial producers and generally considered one of the fastest growing catfish lines, however, reproductive performance (sexual maturity at 4 to 5 years and low spawning success) may have limited spread across commercial producers. No system was available or developed to insure the genetic integrity of the line following release. Mississippi State University Agricultural and Forestry Experiment Station also released improved catfish, the MSU2 line, selected for growth and nitrite tolerance in 1991. These fish are also still available on a limited scale from a few commercial producers.

TABLE 5.8. Releases of genetically improved catfish lines from university and federal research laboratories to commercial producers (data obtained from personal communications with Rex Dunham, Craig Tucker, and William Wolters).

Organization making the release	Fish released	Year	Number of fish released	Number of commercial recipients
Auburn University	Kansas select (2 generations)	1983	< 1,000	< 10
	Kansas	1984	60,000	63
	Auburn select	1989	2,000	3
	Kansas select (4 generations)	1994	2,000	3
Mississipi State University	MSU-2	1991	Unknown	< 10
USDA-ARS	NWAC103 (2 generations)	2001	217,000	35
		2002	35,000	

The largest release of an improved catfish line to commercial producers has been the NWAC103 line. These fish were developed through the breeding program in the USDA-ARS Catfish Genetics Research Unit in Stoneville, Mississippi, evaluated for multiple traits, and jointly released with Mississippi Agricultural and Forestry Experiment Station through the Thad Cochran National Warmwater Aquaculture Center. Beginning in February 2001, over 200,000 2- and 3-year-old fish were released to 35 producers in six states (Alabama, Arkansas, Louisiana, Mississippi, North Carolina, and Georgia) and approximately 35,000 fish were released in 2002.

5.10.3 Future Goals

Only one program specifically developed for catfish genetic improvement has existed in private industry, but this program no longer has genetic improvement as a primary emphasis (Roger Yant, Harvest Select Farms, Inc., Inverness, Mississippi, personal communication). Although most commercial producers recognize the value of genetic research to solve production problems, practical management considerations dictate an emphasis on short-term problems. Shultz (1986) outlined and discussed important elements in developing a commercial breeding program (Table 5.9).

These ten elements involve production assessment, establishing goals, recording data, determination of selection methods to be used, monitoring progress, and continual evaluation of the program. These goals should be considered in research and commercial breeding programs.

The first element, industry assessment, involves an understanding of production systems, processing, and marketing, and is important when considering applications of genetic improvement in commercial culture. The current production system used in commercial pond culture will be a major constraint to the implementation of breeding programs on commercial farms and involves raising multiple size-classes of fish in a single pond without a complete harvest (Tucker et al. 1992). In this management system, the average harvest size increases because the frequencies of fish in different size categories change over time. A mass selection program with culling for weight above a certain size would be difficult to implement in a multiple-batch culture system because the age of fish selected is unknown and the phenotype will be measured inaccurately. Because of differences in management on individual farms and practical constraints with large-scale aquaculture operations, channel catfish genetic improvement and breeding programs will be most effective if located at federal or university research stations.

TABLE 5.9. Elements to be considered in an applied breeding program leading to the development of improved lines of aquatic animals for commercial production (information adapted from Schultz 1986).

Breeding program element	Significance
Industry assessment	Geneticist must understand species biology, production system, specific problems to be addressed, consumer preferences
Establish goals	Determine specific traits to be improved, develop realistic goals based on time considerations, understand constraints
Measure goals	Requires accurate measurement of traits or phenotypes, consider important correlated traits, subdivide complex traits
Initial stocks	Success and progress of breeding program may depend on selection of initial stocks; finding and identifying stocks can be a problem
Estimate parameters	Develop a thorough knowledge of published and unpublished literature and other information
Mating system	Choice of mating system must be evaluated, it can be simple or complex and must incorporate all possible tools (e.g., genomics or other breeding aids)
Selection criteria	Evaluate as many traits as possible, avoid improving traits with no economic value, research is not the goal of the program: improved animals are the goal
Ancillary studies	Collect as much information on important traits as possible to understand correlations and environmental sources of variation
Monitoring procedures	Use controls to evaluate performance gains (or loss) and genetic change in each generation
Periodic reconsideration	Successful breeding programs continually evaluate the methods and progress of the program, and continue to visit producers and scientists

A process to transfer results of genetic research to private industry needs to be standardized and could follow the detailed process already developed by the USDA and Mississippi State University (MAFES 1996). The monitoring and verification of released lines, independent of the research or breeder should also be followed by cooperating agencies (MSIA 2000). Without communication and cooperation of research and industry, development of genetically improved catfish lines will not continue nor will their use provide beneficial impact.

In summary, we have provided in this chapter an overview of the current knowledge in major genetic topics concerning channel catfish. It is evident that much background information is available, and that especially within the past 10 years, a powerful group of modern technologies have become available for application in this species. Future advances will combine research efforts of government, university and private-sector facilities to apply the genetic tools discussed in this chapter to the improvement of channel catfish (Table 5.9). This will provide the first half of the process necessary for genetic improvement. Improved lines will have to be distributed to the industry for growth, harvest and evaluation. The catfish industry of the future will need to establish harvesting and processing evaluation programs similar in intensity and organization to those employed in livestock industries. With effective yield monitoring programs in place, the production information can be used to develop rational directions for genetic improvement programs, which will direct new research or additional uses for existing tools. Thus, genetic improvement is an interactive process that links research and production; it cannot function without inputs from each.

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