Selection, hybridization and genome manipulation in Siluroidei

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Abstract

Selection, hybridization, and genome manipulation in the Siluroidei is reviewed. Selection topics include strain evaluation, qualitative traits, quantitative traits, mass selection, family selection, inbreeding, and crossbreeding. Hybridization is discussed with reference to culture traits. Genome manipulation technologies – sex reversal, gynogenesis, androgenesis, polyploidization, and gene transfer – are considered with application to genetic improvement of Siluroidei. Strains of ictalurid catfish vary significantly for traits such as growth, disease resistance, harvestability, reproduction, body conformation and carcass yield. Growth of domestic strains can be 250% greater than that of wild strains. Strain differences have also been identified for clariid, bagrid, and silurid catfishes. Mass selection has increased growth rate by up to 30%. Heritability estimates predict a response to selection for the traits of disease resistance and tolerance to low oxygen levels. Crossbreeding strains of *Ictalurus punctatus* have improved growth, disease resistance and reproductive performance. Two generations of inbreeding depressed body weight of *I. punctatus* as much as 30%, and can reduce viability and reproductive performance. Hybridization of *I. punctatus* X *I. furcatus* has resulted in a 20% increase in growth. Traits for tolerance to oxygen deficit, feed conversion, disease resistance, harvestability, and carcass yield were also improved. Interspecific, intergeneric, and interfamilial hybrids have been made with clariid fishes; heterotic growth was indicated. Hybridization has also been used to combine desirable traits of parental species. Sex reversal of *I. punctatus* using estrogen treatment followed by progeny testing can result in monosex, all-male populations. Gynogenesis and androgenesis are approaches to production of inbred lines of catfish, but thus far have limited application in aquaculture. Polyploidization has been applied in several ictalurid, silurid, and clariid fishes, but predicted improvements in performance have not been realized. Gene transfer has been accomplished in *I. punctatus* and *Clarias gariepinus* using microinjection and electroporation; the foreign genes have been expressed and inherited. Transgenic *I. punctatus* containing salmonid growth hormone genes grew 20-40% faster than controls.

Keywords: Siluroidei, genetics, selection, hybridization, genome manipulation, *Ictalurus, Clarias, Chrysichthys, Pangasius, Silurus.*

Sélection, hybridation et manipulation du génome chez les Siluroidei.

Résumé

La sélection, l'hybridation et les manipulations du génome chez les Siluroídei sont passées en revue. La sélection inclut l'évaluation de la sélection en masse, des caractéristiques qualitatives et quantitatives des souches, la sélection des familles, des accouplements et des croisements. L'hybridation est discutée en référence aux souches d'élevage. Les techniques de manipulation du génome, l'inversion de sexe, la gynogenèse, l'androgenèse, la polyploïdie et les transferts de gènes sont considérés en fonction de l'amélioration génétique des Siluroidei. Les souches de poissons-chats ictaluridés varient significativement tels que la croissance, la résistance aux maladies, la reproduction, la conformation du corps, le rendement. La croissance des souches domestiques peut être de 250 % supérieure à celle des souches sauvages. Les différences de souches ont été identifiées pour les poissons-chats clariidés, bagridés et siluridés. La sélection en masse a accru le taux de croissance de plus de 30 %. Les estimations d'héritabilité permettraient une sélection pour la résistance des maladies et la tolérance à un faible taux d'oxygène. Les croisements

d'*Ictalurus punctatus* ont amélioré la croissance, la résistance aux maladies et les performances en reproduction. Deux générations de croisements consanguins diminuent le poids du corps de *I. punctatus* de plus de 30 % et peut réduire la viabilité et les performances de reproduction. L'hybridation de *I. punctatus* X par *I. furcatus* entraîne une augmentation de croissance de 20 %. Les caractères de tolérance au déficit en oxygène, du taux de conversion alimentaire, de la résistance aux maladies, et au rendement ont également été améliorés. Des hybrides entre espèces, entre genres, entre familles ont été réalisés avec de poissons clariidés. Une croissance hétérogène a été observée. L'hybridation a été aussi utilisée pour combiner des traits de caractère souhaitables des espèces parentales. L'inversion sexuelle de *I. punctatus* au moyen d'un traitement oestrogène suivi de progénie peut résulter en populations unisexes mâles. La gynogenèse et l'androgenèse sont des approches de production de lignées consanguines de poissons-chats mais ont des applications limitées en aquaculture. La polyploïdie a été appliquée chez plusieurs poissons ictaluridés, siluridés et clariidés mais les améliorations attendues n'ont pas été réalisées. Les transferts de gènes ont été effectués chez *I. punctatus* et *Clarias gariepinus* au moyen de microinjections et d'électroporation. Les gènes étrangers se sont exprimés et transmis aux générations suivantes. Les *I. punctatus* transgéniques contenant des gènes de croissance hormonale grandissent de 20 à 40 % plus rapidement que les témoins.

Mots-clés : Silurodei, génétique, sélection, hybridation, manipulation du génome, *Ictalurus, Clarias, Chrysichthys, Pangasius, Silurus.*

INTRODUCTION

The majority of genetic work on catfishes to date has been done with the ictalurid group of Siluroidei and has included strain evaluation, crossbreeding, mass and family selection, and hybridization. This work covered two decades and focused on improving production traits of the ictalurids for aquaculture, resulting in the first release of genetically improved aquatic animals to commercial fish farmers. Continuing efforts to improve performance of ictalurids include polyploidy induction, sex reversal, and gene transfer. Cytogenetic and isozyme variation identified in other Siluroidei has set the stage for additional genetic improvement efforts in the areas of selection, hybridization, and genome manipulation. Genetic reviews with information on Siluroidei include Smitherman *et al.* (1983), Dunham and Smitherman (1984, 1987), Smitherman and Dunham (1985), Thorgaard (1986), Marian (1987), Maclean and Penman (1990), and Houdebine and Chourrout (1991).

SELECTION

Strain evaluation

Strains of catfish differ for a variety of traits. A strain is a breeding population with a similar history and possessing unique characteristics. Acquisition of the best available strains is one of the quickest ways to improve the quality of brood stock.

Strains of *Ictalurus punctatus* (Ictaluridae) originating from different geographic locations within the United States differ in growth rate, and domesticated strains grow faster than wild strains (Smitherman and Pardue, 1974; Chappell, 1979; Green *et al.*, 1979; Youngblood, 1980; Dunham and Smitherman, 1981). The domestication process increases growth

2-6% per generation (Dunham and Smitherman, 1983 *a*) due to indirect selection pressures created by differences between the natural environment and the culture environment. Growth of domestic strains can be 250% greater than that of wild strains. Also, strain differences exist in resistance to viral, bacterial (Plumb *et al.*, 1975; Dunham, 1981), and parasitic infections (Shrestha, 1977). The oldest domestic strain of *I. punctatus*, Kansas, is one of the fastest growing and most disease resistant strains (Dunham and Smitherman, 1984). In contrast, the Rio Grande strain, recently domesticated, is susceptible to several diseases (Plumb *et al.*, 1975; Dunham and Smitherman, 1984).

The fast growth in some strains of *I. punctatus* is caused by a combination of increased feed conversion efficiency (Chappell, 1979) and increased feed consumption (Al-Ahmad, 1983). Dressing percentage varies among strains, and is correlated to differences in body conformation (Dunham *et al.*, 1983 *a*). Harvestability by seine or angling also varies, and may be reduced by inadvertent selection pressures applied when progenitors are obtained from fish remaining after the majority of the fish have been captured by either method.

Time of spawning is a dramatic example of strain variation in *I. punctatus*. In one study, a north-south trend in spawning date was apparent, with northern strains spawning earlier than strains from southern locations (Broussard and Stickney, 1981). The strain of female was important in determining spawning date, and may impede crossbreeding success (Smitherman *et al.*, 1984). Also, southern strains of *I. punctatus* mature younger than northern strains, with a range of from two to four years of age at maturation.

In the clariid catfishes, most work to identify strain differences has been done electrophoretically; van der Bank *et al.* (1992) compared two domesticated populations and one wild population of *Clarias*

gariepinus (Clariidae). Based on a survey of 22 loci, average heterozygosities of 0.0759 and 0.0033 for the domesticated and 0.0467 for the wild populations were reported. In another study, groups of individuals from a progeny batch differed significantly in body weight at age 9 months, indicating potential for improving growth rate through mass selection (Grobler *et al.*, 1992). Finally, Walt *et al.* (1993) correlated glucose-6-phosphate isomerase phenotypes with growth performance. In an evaluation of 15 loci in four species of catfish in Malaysia, Ismail *et al.* (1989) found the highest variation in *Prophagorus cataractus*, followed by *Mystus nemurus* (Bagridae) and *C. batrachus*. No variation was found in *C. macrocephalus* for these isozymes.

Studies of the bagrid catfishes are limited primarily to *Chrysichthys nigrodigitatus*. Ezenwa *et al.* (1985) used the criteria of fecundity, egg size, hepatosomatic index, gonad index, and condition to identify strain differences in fish from three geographic areas, with possible potential for utilization in selection programs. Protein electrophoresis of products of 19 loci showed low genetic variation in populations of the *C. auratus* complex (Agnese, 1991), and a comparison of *C. nigrodigitatus* from two lagoons in Nigeria concluded that they were not genetically separable populations (Ikusemiju, 1975).

A review of Hungarian breeding programs involving *Silurus glanis* (Siluridae) was presented by Marian (1987).

Qualitative traits

Qualitative traits are those that exhibit discrete variation, "all or none" expression of a phenotype. Qualitative traits are controlled by single or few loci. An example is albinism in *I. punctatus*. Also, caudal and vertebral deformities are usually controlled by a few loci or can be environmentally induced congenital defects with no genetic basis (Dunham *et al.*, 1991). If deformities have a genetic basis and are recessive, crossbreeding should eliminate the abnormalities. If the deformities were dominant, all individuals possessing a copy of the gene would express it and could easily be culled, eliminating the gene from the population in a single generation.

Quantitative traits

Quantitative traits are those that exhibit continuous variation, a range of phenotypic expression. Quantitative traits are controlled by many loci, and include most production traits. Body weight, feed conversion efficiency, dressout percentage, fecundity, disease resistance, tolerance of oxygen deficit, and seinability are quantitative traits (Dunham and Smitherman, 1987).

Two basic breeding programs that are used to improve quantitative traits are selection (mass and family) and crossbreeding. The decision to utilize one or both of these breeding programs is based on the heritability of the trait and the amount of phenotypic variation expressed.

Mass selection

In mass selection, the individual's own performance is the basis of selection. Mass selection will be most effective when the individual's performance (phenotype) reflects the individual's true breeding value, that is, when heritability is high. Heritability (h^2) is a measure of the amount of additive genetic variation and is defined as the ratio of the additive genetic variation to the total phenotypic variability. Heritability ranges from zero to one and varies from population to population.

When heritability is moderate to high (greater than 0.2) additive genetic variation is usually deemed sufficient to make mass selection programs effective, providing there is significant phenotypic variation (selection differential). If heritability is less than 0.2, dominance genetic variation is usually considered more important and crossbreeding programs may be appropriate.

Realized heritability values for increased body weights were more conservative than heritability estimates (Reagan *et al.*, 1976; El-Ibiary and Joyce, 1978, Reagan, 1979; Bondari, 1983; and Dunham and Smitherman, 1983 *a*). Estimates of heritability for body weight of *I. punctatus* from half-sib analysis ranged from 0.61 to 0.75 (Reagan *et al.*, 1976). Realized heritabilities for body weight in Marion, Kansas, and Rio Grande populations were 0.50, 0.33, and 0.24, respectively (Dunham and Smitherman, 1983 *a*); Three generations of mass selection in the Kansas population at Auburn University has yielded 30% improvement in body weight. Positive responses to mass selection have also been obtained for other populations of *I. punctatus* (Bondari, 1983).

Generally, *I. punctatus* with shorter periods of domestication had greater response for body weight selection (Dunham and Smitherman, 1983 *a*). Mass selection in one generation improved growth rate two to six times faster than that which had occurred during three to fifteen generations of domestication (undirected selection).

Family selection

Another genetic improvement program is family selection. The basis of family selection is the average performance of family members. This technique has had limited application in catfish production because of the need for increased manpower and facilities to rear the individual family lots separately (Bondari, 1983).

Inbreeding

Inbreeding is the mating of individuals more closely related to one another than the average of the population. It can be used to develop selected lines for crossbreeding, and selection programs are in fact mild forms of inbreeding. Inbreeding increases homozygosit, which can lead to reduced rate of growth, viability, reproductive performance, and increased biochemical disorders and deformities. Two generations of inbreeding depressed the body weight of *I. punctatus* by 30% (Smitherman and Dunham, 1985).

Crossbreeding

Crossbreeding is the opposite of inbreeding, and can produce immediate improvement through heterosis or hybrid vigor. By convention, the female is listed first in a designated cross. Crossbreeding has improved body weight in *I. punctatus*, but the tested strains had different combining abilities, with 55% of the crosses resulting in positive overdominant growth by the F_1 (Dunham and Smitherman, 1983 b). Domestic X domestic crosses were more likely to give positive heterosis (80%) than domestic X wild crosses (33%). Domestic X wild crosses were more likely to result in fish with growth rates intermediate to their parent strains. Nine of eleven crossbreeds grew better than at least one of their parents.

Reciprocal crossbreeds did not grow at the same rate, and males and females of specific strains had different combining abilities with other strains. A maternal effect for combining ability was evident for crossbreeds from females of the Auburn strain of *I. punctatus* (Dunham and Smitherman, 1983 b) and also for females of other selected strains (Bondari, 1983).

Age of the fish apparently has an effect on the amount of expression of heterosis for growth. The decrease with age of the body weight difference between crossbreeds and parents may have been caused by the relative growth rate of *I. punctatus* slowing with increased size or by the onset of early sexual maturity in crossbreeds. Another explanation might be a genotype-age interaction; crossbreeding may be more effective for improving growth of younger fish than older fish. The amount of heterosis exhibited decreases with age in most organisms (Lasley, 1978).

Crossbred fish usually spawned earlier than pure-strain *I. punctatus* (Dunham *et al.*, 1983 b). As three-year-olds, crossbred fish had higher spawning rates and fecundity than purebred fish, and their fingerling output per kilogram of female parent was greater. As was the case for growth, heterosis decreased with age. A benefit of strain crossbreeding is production of fish that mature earlier in life and spawn earlier in the season than pure-strain fish. Although F_1 crossbreeds often make excellent brood stock with high reproductive performance, their fingerlings generally have performance intermediate to that of the original two parental strains.

HYBRIDIZATION

Ictaluridae

Different species of ictalurid catfish have been evaluated and exhibit different culture traits. *Ictalurus punctatus* grew more rapidly to harvestable size (Chappell, 1979) and were the most disease resistant species, but were difficult to capture by seining. *Ictalurus furcatus* had superior dressing percentage (Chappell, 1979), were very seinable (Chappell, 1979), and were more uniform in length (Brooks *et al.*, 1982 a), but were prone to disease. *Ictalurus catus* tolerated low dissolved oxygen levels and had the fastest growth during winter (Dunham and Smitherman, 1981), but had slow growth to harvest size and poor dressing percentage (Chappell, 1979) caused by a large head (Benchakan, 1979). *Ictalurus punctatus* and *I. catus* became sexually dimorphic in size (males larger) at six months (Brooks *et al.*,1982 b), but *I. furcatus* exhibited no sexual dimorphism in size until they were older than three years (Dunham, 1979).

Hybridization between species, a form of cross-breeding, has been done in an attempt to take advantage of these specific characteristics and find hybrids expressing heterotic growth rates. Twenty-eight interspecific hybrids from seven species of Ictaluridae have been produced and evaluated for growth rate (Giudice, 1966; Dupree and Green, 1969; Yant *et al.*, 1975; Dunham and Smitherman, 1984). Only hybrids of *I. punctatus* female X *I. furcatus* male have shown overdominant growth in the pond environment, with an average increase in body weight of 20% above that of *I. punctatus* (Giudice, 1966; Yant *et al.*, 1975; Smitherman *et al.*, 1983). Also, feed conversion of this hybrid was 11-14% more efficient than that of *I. punctatus*. The reciprocal hybrid did not exhibit heterotic growth (Dunham and Smitherman, 1987). Dupree and Green (1969) indicated that the *I. punctatus* X *I. catus* had superior growth in aquaria, but Chappell (1979) found that they grew slowly to harvestable size in ponds.

Ictalurus punctatus X *I. furcatus* hybrid exhibits heterosis for resistance to critically low oxygen levels (Dunham *et al.*, 1983 c). Therefore, culture of this hybrid could reduce losses to oxygen depletion. When 90% of a population of *I. punctatus* succumbed due to low dissolved oxygen, only 50% of the hybrids in the same pond died. When 50% of a population of *I. punctatus* expired from low dissolved oxygen, only 10% of the hybrids died.

Generally, *I. punctatus* X *I. furcatus* is more disease resistant than *I. punctatus*. This hybrid was more resistant to columnaris than *I. punctatus* (Ella, 1984). Also, although susceptibility of the hybrids to channel

catfish virus was similar to that of *I. punctatus* when injected intraperitoneally, they appear to be refractive to the virus by natural routes of transmission (Plumb and Chappell, 1978).

Reciprocal hybrids of *I. punctatus* and *I. furcatus* were more vulnerable to angling than their parent species (Dunham *et al.*, 1986), and the *I. punctatus* X *I. furcatus* was the more catchable reciprocal. The parent species did not differ. This hybrid was also much easier to catch by seining than *I. punctatus* (Tave *et al.*, 1981). All interspecific hybrids that have been evaluated are easy to catch by seine compared to their parents except *I. furcatus*, which are as seinable or more seinable than all of the hybrids evaluated.

The average dressout percentage for the hybrid *I. punctatus* X *I. furcatus* was higher (64.5) than for the *I. punctatus* (61.2) (Yant *et al.*, 1975). *Ictalurus furcatus* X *I. punctatus* did not exhibit heterosis for this trait, and had a lower dressout percentage than its reciprocal (Chappell, 1979).

Interspecific reciprocal hybrids differ from each other for several additional traits (Dunham *et al.*, 1982). The male parentage of interspecific crosses between *I. furcatus* and *I. punctatus* has controlling influence on several growth patterns. Paternal predominance exists if the external appearance or if the values for morphometric, meristic, or behavioral traits for one hybrid are different from its reciprocal hybrid and more similar to its male parent than its female parent. Paternal predominance also exists when a mean for one hybrid is not different from that of its male parent, and a mean for the male parent is different from that of the reciprocal hybrid (Dunham *et al.*, 1982).

Several traits are paternally predominate in hybrids of *I. punctatus* and *I. furcatus*, including external appearance, swim bladder shape, and anal fin ray number (Dunham *et al.*, 1982). Paternal predominance was also evident in both growth and morphometric uniformity. Susceptibility to capture by seine was influenced more by the male parent than the female parent. The *I. punctatus* X *I. furcatus* hybrid inherits the desirable traits of growth uniformity, body conformation, morphometric uniformity, and seinability from its *I. furcatus* sire. However, preliminary data from both intra- and interspecific crosses of *I. punctatus* (Dupree and Green, 1969; Broussard, 1979; Chappell, 1979) indicate that maternal influences for growth rate may exist, possibly due to maternally inherited mitochondrial DNA.

The hybridization rate between female *I. punctatus* and male *I. furcatus* has been variable, 0-100% in pens and 30% in ponds, and remains the major obstacle to the commercial production of hybrid fingerlings. Hormone injection of females increased the hybridization rate (Tave and Smitherman, 1982). Also, the use of crossbred female *I. punctatus* increased the hybridization rate with *I. furcatus* (Smitherman and Dunham, 1985). Hatchability of

hybrid eggs and viability of hybrid fry was as high as that of parental species (Tave and Smitherman, 1982).

Currently, research is examining different strains of *I. furcatus* and types of hormone injection to increase the rate of hybridization. Artificial spawning and fertilization are an alternative approach to produce hybrids. However, the procedure is labor intensive and requires the sacrifice of the males to obtain the testes and milt. The incision used to remove the testes can be sutured and high survival of the males attained, but it is not yet known if the testes will regenerate sufficiently after removal. If the reproductive barriers are overcome, *I. punctatus* X *I. furcatus*, superior for several traits, will be available for the catfish industry.

The initial production of F_2 hybrid catfish and three-way crosses have been unsuccessful (LeGrande *et al.*, 1984). Chappell (1979) found that the number of fertile and viable eggs of *I. punctatus* X *I. catus* and *I. catus* X *I. furcatus* was extremely low. Chromosome incompatibilities appear to be the primary block to the success of the crosses. Three parental species and four hybrid combinations among them have the following diploid numbers (LeGrande *et al.*, 1984):

I. catus 2N=48; *I. punctatus* and *I. furcatus* 2N=58; *I. punctatus* X *I. catus* and *I. furcatus* X *I. catus* 2N=53. Karyotypes of *I. punctatus*, *I. furcatus*, and the reciprocal hybrid combinations were not distinguishable from one another, yet F_2 eggs had minimal hatch. The karyotype of *I. catus* differed from those of *I. punctatus* and *I. furcatus* in diploid number and in arm number. Hybrids between *I. catus* and either *I. punctatus* or *I. furcatus* were intermediate in karyotype, and the most obvious parental marker chromosomes were five chromosomes of *I. catus* which were considerably larger than any element in the karyotype of *I. punctatus* or *I. furcatus*.

Clariidae

There have been a number of interspecific, intergeneric, and even interfamilial hybrids made with clariid catfishes. Unfortunately, little work has been done to thoroughly evaluate these hybrids as to their suitability for aquaculture.

Hecht and Lublinkhof (1985), Legendre *et al.* (1992), and Teugels *et al.* (1992) artificially hybridized *Clarias gariepinus* and *Heterobranchus longifilis*. Both reciprocal hybrids had chromosome numbers (2N=54) intermediate of the parental numbers (2N=56 and 52, respectively) (Teugels *et al.*, 1992). Legendre *et al.* (1992) reports numerous abnormalities in gonadal development of these hybrids, which nevertheless produced viable larvae from F_2 fertilizations. Increased growth in the hybrids may have been a result of heterosis, or a result of somatic growth replacing gonadal development. Sexual dimorphism for growth observed in *C. gariepinus* was not observed in *H. longifilis* nor in reciprocal hybrids.

Salami *et al.* (1993) artificially hybridized *C. gariepinus* and *Heterobranchus bidorsalis*. Evaluation of growth in concrete tanks indicated that the female *C. gariepinus* X male *H. bidorsalis* grew faster than the reciprocal cross, and both hybrids grew faster than the parental species. Survival of hybrids of *Heteropneustes fossilis* X *C. batrachus* was studied by Mukhopadathy and Dehadrai (1987). Wembiao *et al.* (1988) reported hybridization of male *C. lazera* (synonym *gariepinus*) with female *C. fuscus* to combine paternal growth characteristics with maternal flesh quality. Hybrids of *C. lazera* X *C. fuscus* were also made by Wu *et al.* (1988), who found that the hybrids were closer to the female parents in three quantitative and six morphological traits, and closer to the male parents in one quantitative and eight morphological traits. Ratios of body depth and width to body length in the hybrids were closer to those of the female than those of the male species. The males of this cross were reported to be sterile. Hybrids of *C. gariepinus* X *C. macrocephalus* have been made to combine the growth of the former with the yellow flesh color of the latter (personal communication, Uthrait Na-Nakorn, Kasetsart University, Bangkok, Thailand). Growth and survival of hybrids of *C. macrocephalus* X *C. batrachus* were studied by Alam *et al.* (1993). The hybrid *C. macrocephalus* X *Pangasius sutchi* (Pangasiidae) has been made; the hybrids possess unusual karyotypes (Na-Nakorn *et al.*, 1993). Tarnchalanukit (1986) crossed both *C. macrocephalus* and *C. batrachus* with *P. sutchi*.

GENOME MANIPULATION

Sex reversal

Sex reversal is used to limit reproduction in fish and ultimately improve the rate of growth by directing energy used for reproduction into the production of flesh. The direction of reversal should take advantage of any sexual dimorphism for size. For example, in ictalurid catfishes an all-male population is desirable, because these males grow faster than females (Benchakan, 1979). Sex reversal to monosex female populations has been successful using estrogens in *I. punctatus* (Goudie *et al.*, 1983). However, attempts to produce monosex male populations have not been successful due to the phenomenon of paradoxical feminization, where the application of androgens unexpectedly produces female fish (Davis *et al.*, 1990).

A combination of sex reversal and breeding allows production of all-male populations of *I. punctatus*. Females are homogametic (XX) and males are heterogametic (XY) (Davis *et al.*, 1990). When *I. punctatus* are sex reversed to all females with hormones, their natural hormone system is swamped by the artificial hormone, causing their phenotypic sex to change without changing their genotype. Therefore, when catfish are sex reversed to all females, one-half have the female genotype (XX) and one-half, although female in appearance, have the male genotype (XY).

These females with the male genotype (XY) can be mated with normal males (also XY) to produce three genotypes, YY and XY which are males and XX which are females. This produces a sex ratio of three males (1/3 are YY) to one female. The YY males will produce 100% male (XY) fry when mated to normal females. The YY males are viable, and can be identified by progeny testing.

The progeny testing to identify commercial quantities of YY males requires considerable facilities, testing, and time. Research has been initiated to find DNA markers that would identify gender (Tiersch *et al.*, 1992), enabling testing of fingerlings and facilitating commercialization of sex reversal and breeding programs.

Gynogenesis

Gynogenesis is all-female inheritance. Irradiated sperm is used to fertilize the egg which still allows the sperm to penetrate the eggs, triggering cell division and development, but destroying the sperm DNA, eliminating any paternal genetic contribution. The maternal genome is then doubled by blocking expulsion of the second polar body or blocking the first cell division. These processes are difficult, and only a few gynogenetic embryos survive. The primary purpose for producing gynogenetic fish is development of homozygous control lines for experiments or inbred lines for use in crossbreeding programs. Gynogenesis should also result in all-female populations when the females are homogametic.

Gynogenetic channel catfish have been produced at Auburn University. Also, gynogenetic *Silurus glanis* have been produced, but only 20-30% were viable (Krasznai and Marian, 1987).

Androgenesis

Androgenesis, or all-male inheritance, is done by irradiating eggs and then doubling the paternal genome as described for gynogenesis (Thorgaard, 1986). The viability of androgenetics is even less than that of gynogenetics due to destruction of mitochondrial DNA along with the maternal nuclear genome. In addition to production of homozygous lines, this may also have utility for comparing mitochondrial genotypes, if mitochondrial DNA transfer can be accomplished. To date, this technique has not been applied in catfish culture.

Polyploidization

Thorgaard (1986) provides a review of ploidy manipulation in fish. Polyploidy refers to individuals with extra sets of chromosomes. The normal chromosome complement is two sets (diploid). Triploidy refers to individuals with three sets of

chromosomes and tetraploidy refers to individuals with four sets. Both of these conditions can be induced in fertilized fish eggs by temperature shock, hydrostatic pressure, or chemical treatment.

Both triploidy (Wolters *et al.*, 1982) and tetraploidy (Bidwell *et al.*, 1985) have been induced in channel catfish and in hybrids among *I. punctatus*, *I. furcatus*, and *I. catus* (Rezek, 1988; Bury, 1989). Induction of triploidy was 100% and induction of tetraploidy was 62% in channel catfish using temperature shocks. Survival of triploid *I. punctatus* and hybrid catfish was as good or better than survival of diploids through two years of age (Bury, 1989), but survival of tetraploids was extremely low (Rezek, 1988).

Triploid *S. glanis*, have been produced by cold shock (Krasznai *et al.*, 1984). Treated eggs had reduced hatching rate, but triploid and diploid fry had equivalent viability. Also, karyotyping has been done for *S. asotus* in preparation for ploidy manipulation (Kim *et al.*, 1988).

Cold shock has also been used to produce triploid *Clarias gariepinus* (Richter *et al.*, 1987; Henken *et al.*, 1987). Hatching rate for treated eggs was reduced compared to controls. Growth rate and feed conversion efficiency were not significantly different, but triploid fish had more product remaining after gutting, compared to diploid fish.

Theoretically, the energy not utilized for sexual maturation in sterile triploid fish is diverted to increased somatic growth. Also, polyploid cell nuclei are larger than diploid cell nuclei and some organisms respond to this increase in nuclear size with a corresponding increase in cytoplasmic volume, maintaining a constant cytoplasm/nucleus ratio. This results in larger cell size and increased body size in some species. However, no growth improvement for triploid *I. punctatus* or *I. punctatus* X *I. furcatus* hybrids has been observed relative to diploids in ponds (Bury, 1989; Dunham, 1990 *a*). Polyploidy may also be used to overcome chromosome incompatibilities in hybrids, thus increasing their viability (Thorgaard, 1986).

Manipulation of ploidy on a commercial scale is constrained by the inability to strip large quantities of catfish eggs. Theoretically, the development of tetraploid broodstock would enable the production of triploid offspring by matings to diploid broodstock. However, the low viability of tetraploid catfish may prevent production of large broodstock populations.

Gene transfer

Genetic engineering techniques have been developed which may complement traditional breeding programs for improvement of culture traits (Houdebine and Chourrout, 1991). Individual genes from one species are isolated, linked to promoter genes (regulatory DNA sequences or on/off switches), cloned, and grown in hosts such as bacteria, bacteriophages, cosmids, and plasmids. These genes are then transferred into genomes of other species by vector, microinjection, electroporation, or gene gun. Organisms containing these foreign genes are termed transgenic. Five objectives must be met to have successful gene transfer. The foreign gene must be transferred to the fish and integrate (combine with and become part of) the fish's chromosome or genetic make-up. The gene must express itself; transfer of the gene does not guarantee that it will function. A positive biological effect must result from expression of the foreign DNA, and no adverse biological or commercial effect occur. Finally, the foreign gene must be inherited by subsequent generations.

The mouse metallothionein (promoter) – human growth hormone gene, the Rous sarcoma virus-long terminal repeat (RSV, avian virus, promoter) –rainbow trout growth hormone gene and the RSV–coho salmon growth hormone gene have been transferred into *I. punctatus* via microinjection and electroporation (Dunham *et al.*, 1987; Dunham, 1990b; Dunham *et al.*, 1992). *Ictalurus punctatus* containing the salmonid growth hormone genes expresses these genes. These F_1 transgenic fish produce rainbow trout or salmon growth hormone, resulting in 20-40% growth improvement. Also, transgenic *I. punctatus* have transmitted the salmonid growth hormone genes to their progeny (Dunham *et al.*, 1992).

Muller *et al.* (1993) successfully transferred luciferase (Luc) and the *Escherichia coli* β-galactosidase (lacZ) genes, driven by cytomegalovirus (CMV) IE1 promoters, into *C. gariepinus* by electroporation. Reproducible transient expression of both genes was documented. Also, Muller *et al.* (1992) demonstrated transfer and expression of reporter genes in *C. gariepinus* by electroporating sperm.

OPPORTUNITIES FOR FURTHER GENETIC ADVANCEMENT IN SILUROIDEI

Efforts for further genetic enhancement in Siluroidei will combine population genetics and classical animal breeding programs with genome manipulation to improve performance traits. Preservation of extant variation in these fishes must be done, while genome mapping and manipulation techniques including gene transfer are refined.

Germplasm resources centers are needed to:

(1) Document ancestry and breeding histories for cultured populations along with data banks on strain characterization and related performance information; and

(2) Serve as repositories of critical breeding populations with sufficient numbers to assure conservation of unique alleles and;

(3) Develop technology for cryopreservation or otherwise storing gametes or embryos for future genetics research and development. Currently, maintenance of

selected lines, strains, and wild, unaltered populations requires a large commitment of resources to keep genetically effective breeding populations. Economical cryopreservation techniques would provide a much needed alternative to maintaining live fishes.

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