SERUM ESTERASE VARIATION IN CHANNEL CATFISH: GENETIC AND POPULATION ANALYSIS

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ABSTRACT

Electrophoretic variation in channel catfish serum esterase was expressed as six phenotypes and determined by three codominant alleles at a single locus. Allelic frequencies varied considerably between populations. One allele, designated "fast", predominates in commercial stocks and may be associated with increased growth rate under intensive culture conditions.

INTRODUCTION

The channel catfish, *Ictalurus punctatus*, has been intensively cultured for about 20 years. During this period, fish culturists have developed numerous "domestic" stocks of channel catfish that are apparently superior to wild fish when grown under intensive culture conditions (Burnside et al. 1975). However, genetic improvement has been slow and the need for increased genetic research is apparent.

One of the central problems in channel catfish selective breeding programs is an inadequate knowledge of population structure of the species. The assumption underlying the acquisition of many of the present genetic stocks is that fish from different river systems are genetically distinct. This assumption may be valid, however, the rapid expansion of commercial catfish production and substantial stockings of natural waters by state and federal hatcheries have likely reduced genetic differences between wild populations.

Electrophoresis is a useful method for examining the genetic structure of populations. By this technique, functionally similar proteins (enzymes) are exposed to an electrical field and fractionated into multiple forms (isozymes) of different electrophoretic mobilities. Electrophoretic techniques are usually qualitative and population differences are indicated by divergent isozyme frequencies. De Ligny (1969) has reviewed electrophoretic studies of fishes.

The esterases are tissue specific enzymes that are prone to electrophoretic variation. As such, they are potentially useful for investigating genetic relationships between populations of channel catfish. Knowles et al. (1968) described electrophoretic patterns of channel catfish brain esterases but found no variation among individual fish. However, an infrequent serum esterase polymorphism has been found in channel catfish from Arkansas and subsequent progeny analysis indicated a correlation between parental esterase phenotypes and progeny growth rate (C. J. Biggers and D. Tackett, personal communications).

This paper describes the genetic basis and phenotypic frequencies of serum esterase isozymes in six populations of channel catfish. The described polymorphism is probably the same as found by Biggers and Tackett.

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MATERIALS AND METHODS

Serum esterase phenotypes were examined in six stocks of channel catfish from different areas. Wild fish were collected from the St. Louis River, Minnesota, and two Texas impoundments, Falcon Reservior and Lake Trinidad. These fish, designated Minnesota, Rio Grande, and Trinidad respectively, are maintained as separate stocks at the Aquaculture Research Center, Texas A&M University.

One stock of domestic fish was purchased as fingerlings from a commercial source in Arkansas. These fish were used in cage production experiments at Lake Trinidad, a cooling reservoir owned by Texas Power and Light Company. At termination of the production studies, fish were separated into

marketable size (> 350 g) and culls (< 350 g). Electrophoretic studies were conducted on fish of both size groups.

Blood samples from fish from a second domestic stock (Joe Glasner, Buckholts, Texas) were furnished by Dr. Jack Austen, Texas A&M University. In addition, Mr. Dewey Tackett provided blood samples from 40 fish maintained at the Fish Farming Experiment Station, U. S. Fish and Wildlife Service, Stuttgart, Arkansas.

Whole blood was collected by syringe from either the heart or caudal peduncle of channel catfish. Serum was removed after clotting. Occasionally, plasma was obtained by centrifugation of blood collected in syringes that contained heparin or lithium oxalate. No differences were observed in serum and plasma collected from the same fish.

Genetic analysis of esterase patterns was conducted on single fry homogenates. Approximately forty fry (late yolk sac stage) were removed from each of three select spawns and individually homogenized in two volumes distilled water. Particulate matter was removed by centrifugation.

Horizontal starch gel electrophoresis (15% Connaught starch) was performed as described by Kristjanssen (1960). A discontinuous buffer (Storemont and Suzuki 1970) was used throughout the experiment. Serum, plasma, or fry homogenates were absorbed on filter paper squares and inserted into the gels near the cathodal end. After 15 minutes electrophoresis at 200 V, paper squares were removed and electrophoresis continued for two hours at 375 V. Gel temperatures were maintained near 4 C by refrigeration. Following electrophoresis, the gels were sliced horizontally and stained for esterase activity (Markert and Hunter 1959) using α napthyl propionate as substrate.

RESULTS

Two prominent regions of serum esterase activity were visualized with the described technique. Based on preliminary studies of tissue distributions, substrate preferences, and electrophoretic mobilities, these regions have been designated Est-5 and Est-6. Three zones of esterase activity were found in the Est-5 region and designated F (fast), I (intermediate), and S (slow). These zones produced six phenotypes and are diagrammatically illustrated in Figure 1. No electrophoretic variation was observed in the Est-6 region.

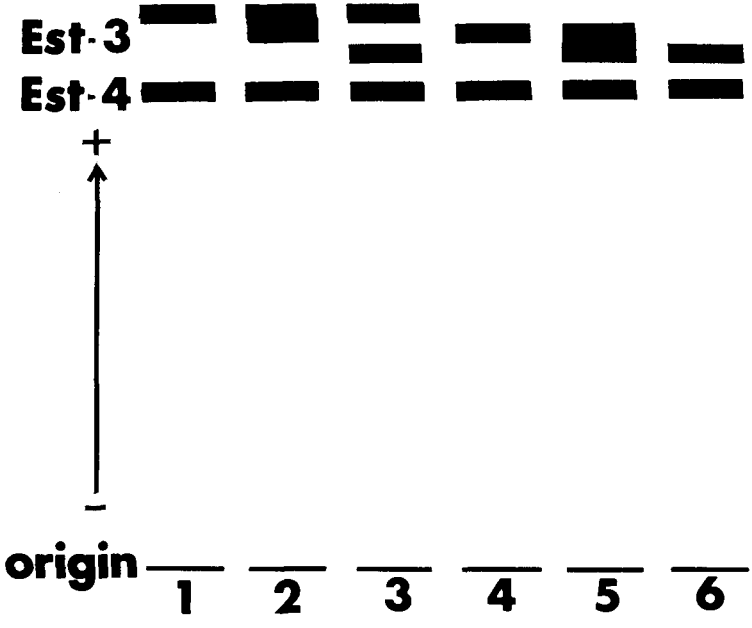

Figure 1. Electrophoretic patterns of channel catfish serum esterases. Slot 1: Est-5F/Est-5F; slot 2: Est-5F/Est-5I; slot 3: Est-5F/Est-5S; slot 4: Est-5I/Est-5I; slot 5: Est-5I/Est-5S; slot 6: Est-5S/Est-5S.

Genetic analysis of Est-5 isozymes were observed in fry homogenates is presented in Table 1. The parental genotypes were inferred from the progeny phenotype ratios. The observed progeny ratios indicate that the esterase variation is produced by three codominant alleles at a single locus. These alleles are designated Est-5F, Est-5I, and Est-5S corresponding to their respective gene products.

Distribution and analysis of Est-5 genotypes from the various stocks is presented in Table 2. Expected numbers of individuals of each genotype were calculated from observed gene frequencies according to the Hardy-Weinberg equation, $p^2+2pq+q^2=1$, where p and q are observed gene frequencies. This equation is derived from the Hardy-Weinberg law which states that gene frequencies within a population will remain constant assuming a large, random-mating population with no migration or mutation. Deviation from equilibrium indicates that one or more of the assumptions have been violated. All but one stock show agreement between observed and expected values. The Trinidad sample deviates greatly from Hardy-Weinberg equilibrium ($P<0.005$).

DISCUSSION

Genetic control of a channel catfish serum esterase by multiple alleles at one locus is similar to serum esterase inheritance reported in other fish species (Koehn and Rasmussen 1967; Fujino and Kang 1968; de Ligny 1969; Holmes and Whitt 1970; Koehn et al. 1971). Pair-wise χ^2 tests for homogeneity performed on the data in Table 2 indicated that all stocks except the Lake Trinidad and Arkansas culls are genetically different ($P<0.05$) with respect to the Est-5 alleles. Since the Trinidad sample is in genetic disequilibrium, the similar gene frequencies of this stock and the Arkansas cull stock are considered to be coincidental. In general, genetic divergence seems to be related to geographic divergence.

The gene frequencies for the Minnesota and Rio Grande samples are of particular interest. These fish represent extreme geographic and environmental divergence which is reflected at the Est-5 locus. The Minnesota fish are characterized by the singular presence of the Est-5S allele. It is possible that other alleles exist at low frequencies and these results should be verified in a larger sample. The Est-5I allele is apparently characteristic for the Rio Grande stock to the exclusion of Est-5F. This further indicates the genetic distinctiveness of the Rio Grande stock noted by Plumb et al. (1974).

The Est-5F allele was restricted to the four remaining stocks. However, the original Trinidad stock has been contaminated with large numbers of commercial fish from the cage experiments. This may account for the presence of the Est-5F allele in the Trinidad sample. Significant deviation from Hardy-Weinberg equilibrium ($P<0.005$) probably reflects the presence of escaped commercial catfish in the Lake Trinidad population.

Much higher frequencies of Est-5F in the commercial stocks (Arkansas and Buckholts) indicate that these fish are genetically different from the wild and hatchery stocks. Common stock origin probably does not account for the more frequent occurrence of Est-5F in commercial stocks since I have commonly found Est-5F in limited samples from the Auburn University strain and a commercial stock in Mississippi.

The higher frequency of Est-5F in the larger of the two size groups from the Arkansas stock (Table 2) implies that the Est-5 locus is sensitive to selection under intensive culture conditions. This observation is in substantial agreement with results of Biggers and Tackett (personal communications) and may provide a biochemical explanation for the superior growth rate of domestic channel catfish strains compared to wild strains (Burnside et al. 1975).

These findings may have important implications for catfish culture. Since isozymes commonly differ from each other in chemical properties other than electrophoretic mobility (Johnson 1973), the presence of certain Est-5 isozymes in channel catfish may be physiologically beneficial. The physiological roles of esterases are not completely understood. However, esterase activity is commonly found in liver, pancreatic, and intestinal tissues and may be involved in lipid metabolism (de Migne 1974). Channel catfish possessing the Est-5F allele may be better able to utilize artificial diets and cope with increased deposits of body fat common in cultured catfish.

Certain pesticides are potent inhibitors of esterases (Holmes et al. 1968). Isozymes of acetylcholine esterase in rats are differentially inhibited by Parathion (Vijayan and Brownson 1975). If the Est-5 isozymes are also differentially sensitive to pesticides, chronic exposure to low pesticide levels might produce subtle physiological effects evidenced by altered growth rates, etc.

Divergent isozyme frequencies may be produced by environmental factors peculiar to different geographic regions. In some fishes, esterase isozyme frequencies are correlated with temperature (Koehn 1969; Baldwin and Hochachka 1970). Information on the distribution of Est-5 alleles in

additional populations of channel catfish may indicate clinal changes associated with environmental conditions.

Additionally, the behavior of the Est-5 alleles under apparent selection pressures could be explained by close chromosomal linkage with other gene(s) that influence genetic fitness. Such a linkage group would usually be inherited as a single unit and selection would not discriminate between Est-5 alleles and the selected trait.

All of these hypotheses are testable and represent an important area for future research.

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Table 1. Inferred parental genotypes based on phenotypic ratios of progeny

Parental phenotypes ♀ ♂	Inferred parental genotypes	Total No.	Progeny phenotypes F	FI	FS	I	IS	S	χ^2	Probability
FS X S	FS X SS	32 obs.			18			14	0.50	.50>P>.25
		exp.			16			16		
S X IS	SS X IS	39 obs.					17	22	0.61	.50>P>.25
		exp.					19.5	19.5		
S X S	SS X SS	36 obs.						36	0	1.00>P>.99
		exp.						36		

Table 2. Distribution of genotypes and gene frequencies of the Est-3 alleles within six stocks of channel catfish.

Stock	Total No.		Genotype FF	FI	FS	II	IS	SS	Gene frequency F	I	S	X^2 (2 df)	Probability
Minnesota	20	obs.	0	0	0	0	0	20			1.00	0	1.00>P>.995
		exp.	0	0	0	0	0	20					
Rio Grande	61	obs.	0	0	0	25	27	9		0.631	0.369	0.15	.95>P>.90
		exp.	0	0	0	24.28	28.41	8.31					
Trinidad	98	obs.	7	0	15	0	0	76	0.148		0.852	15.09	P<0.005**
		exp.	2.15	0	24.71	0	0	71.14					
Stuttgart	39	obs.	0	0	2	0	0	37	0.026		0.974	0.02	.995>P>.90
		exp.	0.02	0	1.95	0	0	36.9					
Buckholts	64	obs.	25	0	34	0	0	5	0.656		0.344	2.02	.50>P>.25
		exp.	27.56	0	28.86	0	0	7.56					
Arkansas													
marketable	38	obs.	10	0	17	0	0	11	0.487		0.513	0.41	.90>P>.75
		exp.	9.02	0	18.98	0	0	10.00					
culls	120	obs.	6	0	24	0	0	90	0.150		0.850	5.58	.10>P>.05
		exp.	2.70	0	30.60	0	0	86.70					