and 19% of the stomachs of late-lifted fish were empty.

Food of the striped bass at Holyoke Dam was dominated by the body parts of adult American shad, blueback herring, and sea lamprey when many individuals of these species were being lifted, and dominated by forage fish and insects, when the alosids and sea lampreys were scarce (Fig. 1b). The reduced incidence of feeding on the body parts of large fish by striped bass lifted after 21 June was probably the result of a dramatic reduction in the availability of this food that occurred when the run of anadromous alosids diminished.

Hollis (1952) found alosid scales in the stomachs of adult striped bass captured below Conowingo Dam on the Susquehanna River in Maryland, but he dismissed these as accidental. In our study, alosid body parts occurred in stomachs too frequently to be accidental. Many authors consider the food that is selected by striped bass to be directly related to the availability (Hollis 1952; Thomas 1967; Schaefer 1970). During the run of anadromous fish at Holyoke Dam, the most abundant food that is available for striped bass is likely the body parts of dead or injured American shad, blueback herring, and sea lamprey, although we were not able to confirm this by sampling below the dam. About 900,000 adult alosids were passed upstream in the fish lifts in 1982, and injuries and mortalities were commonly observed at the dam and fish lifts. Subadult striped bass may typically concentrate below hydroelectric dams and feed on the parts of fish (anadromous or freshwater species) that die or sustain injury while attempting to move upstream or downstream of the dam.

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GENETIC CONFIRMATION OF SPECIFIC DISTINCTION OF ARROWTOOTH FLOUNDER, *ATHERESTHES STOMIAS*, AND KAMCHATKA FLOUNDER, *A. EVERMANNI*

The uncertain taxonomic status of two morphological types of *Atheresthes* (family Pleuronectidae) has led to some problems in fisheries surveys and stock assessments. Although data collection would be simplified if these types were conspecific morphs, a single classification would mask differences of distribution and abundance if each type actually represented a distinct species. Each type is described as a separate species: arrowtooth flounder, *A. stomias*, and Kamchatka flounder, *A. evermanni*, based on morphological differences in gill raker count, dorsal and anal fin rays, caudal vertebrae number, eye-dorsal fin distance, and relative position of the upper eye (Norman 1934; Wilimovsky et al. 1967). However, the differences are subtle, and both types have generally been considered *A. stomias* in fisheries surveys (e.g., Smith and Bakkala 1982).

Atheresthes stomias occurs in the eastern Bering Sea and eastern North Pacific Ocean from about St. Matthew Island, southward through the eastern Bering Sea and Gulf of Alaska, and along the North American coast to central California (Hart 1973). *Atheresthes evermanni* is distributed in the western

Bering Sea and western North Pacific Ocean from the Anadyr Gulf, south along the Kamchatka Peninsula, through the Sea of Okhotsk, and to northern Japan (Andriyashev 1939; Wilimovsky et al. 1967). The geographic ranges of the two types overlap in some areas of the Aleutian Islands and eastern Bering Sea.

Biochemical data have recently provided valuable insights towards clarifying genetic relationships among fishes. Findings have ranged from identifying previously unknown species (e.g., Shaklee et al. 1982) to grouping taxa previously considered distinct (e.g., Wishard et al. 1984). Biochemical data were therefore used to clarify the taxonomic status of *A. stomias* and *A. evermanni* through an electrophoretic examination of known individuals of both types. The level of genetic difference observed in this study is compared with that found between two other groups of marine fishes of the Bering Sea and the North Pacific Ocean.

Materials and Methods

Collections were made in the Bering Sea near Unalaska Island by National Marine Fisheries Service research vessels *Oregon* (lat. 53°45'N, long. 166°56'W, August 1980) and *Miller Freeman* (lat. 54°44'N, long. 166°29'W, February 1981). The 12 Kamchatka flounder (4 taken in 1980 and 8 in 1981) included males and females with fork lengths ranging from 24 to 43 cm. The 13 arrowtooth flounder, taken only in 1981, also included both sexes and ranged in fork lengths from 33 to 43 cm. Morphological types were distinguished by the gill raker counts and position of the upper eye. In specimens identified as Kamchatka flounder, the upper eye did not reach the edge of the head and the mean total gill raker count was 12.4. The upper eye of specimens identified as arrowtooth flounder reached the edge of the head, breaking the dorsal profile, and the mean total gill raker count was 15.3. Fish were frozen intact at −20°C following collection and remained frozen up to 30 mo until tissues were removed for electrophoretic analysis.

Sample preparation and electrophoresis followed methods given by Utter et al. (1974). Buffer systems included 1) a discontinuous tris-citric acid (gel pH 8.2), lithium hydroxide-boric acid (tray pH 8.0) buffer, described by Ridgway et al. (1970); 2) a tris-boric acid - 0.004 M EDTA (pH 8.5) buffer, described by Markert and Faulhaber (1965); and 3) an aminopropylmorpholine-citric acid - 0.01 M EDTA (pH 6.5) buffer, described by Clayton and Tretiak (1972).

Procedures of visualizing enzyme activities following electrophoresis were those outlined by May et al. (1979). We followed the criteria of Allendorf and Utter (1979) for the inference of Mendelian inheritance in the absence of breeding data. Genetic data were collected from 22 protein systems (Table 1). A system of nomenclature suggested by Allendorf and Utter (1979) was used where the most common allelic form of a locus was designated as 100, and other allelic forms were assigned values based on their mobility relative to the common form. Alleles migrating cathodally were given negative values.

Phenotypic frequencies of the overall sample (all specimens of both presumed species pooled together) at each polymorphic locus were tested for expected binomial (i.e., Hardy-Weinberg) distributions using a G statistic for goodness of fit (Sokal and Rohlf 1969; Goodenough 1978). Multiple allelic cases were collapsed to two allelic classes to allow for small sample sizes. A contingency table analysis of allelic frequencies testing the null hypothesis of no difference between the two groups also used the G statistic,

TABLE 1.—Protein systems used in this study including tissues and appropriate buffer systems for detection of suitable activity.

Protein system	Enzyme commission number	Tissues[1]	Buffer[2]
Acid phosphatase (ACP)	3.1.3.2	M,L,H	1,2,3
Adenosine deaminase (ADA)	3.5.4.4	M,E	1
Alcohol dehydrogenase (ADH)	1.1.1.1	L	3
Aldolase (ALD)	4.1.2.13	M	1,3
Aspartate aminotransferase (AAT)	2.6.1.1	M	1,2
Creatine kinase (CK)	2.7.3.2	M	1,3
Esterase (EST)	3.1.1.1	L,H,E	3
General protein (GP)		M,E	2,3
Glucosephosphate isomerase (GPI)	5.3.1.9	M,E	1
Glyceraldehydephosphate dehydrogenase (GAP)	1.2.1.12	E,M	1,3
Glycerol-3-phosphate dehydrogenase (G3P)	1.1.1.8	M	3
Glycylleucine peptidase (GL)	3.4.11	E,M	1,2
Isocitrate dehydrogenase (IDH)	1.1.1.42	M,H,E	3
Lactate dehydrogenase (LDH)	1.1.1.27	M,E	3
Leucylglycylglycine peptidase (LGG)	3.4.11	M	1
Malate dehydrogenase (MDH)	1.1.1.37	H,L,E.M	3
Malate dehydrogenase (ME) (decarboxylating - NADP+)	1.1.1.40	M	2
Mannosephosphate isomerase (MPI)	5.3.1.8	M	2
Phosphoglucomutase (PGM)	2.7.5.1	M	1
6-phosphogluconate dehydrogenase (PGD)	1.1.1.44	M,E	3
Phosphoglycerate kinase (PGK)	2.7.2.3	M	3
Superoxide dismutase (SOD)	1.15.1.1	M,H	1,3

[1]M = muscle, L = liver, H = heart, E = eye.
[2]1 = discontinuous tris citrate, lithium borate; 2 = continuous tris, borate, EDTA; 3 = continuous amine citrate, EDTA.

with Yates correction for small sample sizes (Sokal and Rohlf 1969). Nei's (1978) measure of genetic distance for small sample sizes was calculated between the two groups.

Results and Discussions

Data were collected from 22 enzyme systems encoding the following 32 presumed loci (polymorphic loci having one or more variant alleles are indicated by *): AAT*, ACP-1, ACP-2*, ADA*, ADH*, ALD, G3P-1*, G3P-2, CK-1, CK-2, EST, GAP-1*, GAP-2, GL-1, GL-2, IDH*, LDH-1*, LDH-2, LDH-3, LGG*, MDH-1, MDH-2, MDH-3, ME, PGD*, PGM-1, PGM-2, GPI-1, GPI-2*, PGK*, MPI, SOD.

Allelic distributions for the 13 polymorphic loci indicate considerable similarity for most of the systems but some distinct differences as well, based on the contingency analysis (Table 2). The nonsignificant differences observed at nine of the loci are not highly informative given the limited number of individuals that were sampled.

However, the differences that were statistically significant provide considerable information. The most striking difference is at the ADH locus, where no alleles were shared between the 12 arrowtooth and the 10 Kamchatka flounders. These data alone confirm the genetic distinctness of the two types. The allelic distribution between the two forms is almost as distinct at the GAP-1 locus; a lesser, but significant difference also exists at the ACP-2 locus. Gel banding patterns observed for these three loci are shown in Figure 1.

Not surprisingly, the genotypic frequencies at the ADH and GAP-1 loci also deviated significantly ($P < 0.001$) from the ratios of a binomial expansion of allelic frequencies (Hardy-Weinberg equilibrium expected in a single, randomly mating population). This difference resulted from excesses of homozygous and deficits of heterozygous classes, a situation expected in population mixtures (i.e., the Wahlund effect, see Futuyma 1979).

The distinct genotypic distribution of the two forms at the ADH and GAP-1 loci, coupled with their sympatric occurrence and subtle but consistent morphological identities, support their present taxonomic status as distinct congeneric species. However, the value of genetic distance observed, 0.052, is rather low for distinct species suggesting recent speciation (Avise 1976).

Recent genetic studies of two other pleuronectid species sampled from the same geographic region indicate only conspecific variation. The Alaska Peninsula separates two population groups of yellowfin sole, *Limanda aspera*, at a mean genetic distance of 0.005 (Grant et al. 1983). No significant differences of allelic frequencies were detected in Pacific halibut, *Hippoglossus stenolepis*, sampled in the Bering Sea and the North Pacific Ocean (Grant et al. 1984). These various outcomes among confamilial groupings undoubtedly reflect the past and present actions of numerous variables; two major factors are differing capabilities for gene flow based on distinct life history patterns, and differing times and degrees of isolation imposed by glaciation events within the past 2 million years (discussed by Grant and Utter 1984).

Finally, the possibility of hybridization and introgression between the two species of *Atheresthes* should be examined through more extensive sampling of these two forms over a broader geographic range. The distinct distribution of ADH alleles excluded a hybrid origin of any individuals in this study.

TABLE 2.—Observed number and (in parentheses) within group frequency of alleles of 13 polymorphic loci in samples of arrowtooth and Kamchatka flounder.

Locus	Allele	Observed alleles (frequencies) Arrowtooth	Kamchatka	P[1]	Subunit structure[2]
AAT	92	2(0.100)	no data		d
	100	10(0.500)			
	106	8(0.400)			
ACP-2	100	20(0.769)	22(1.000)	<0.05	m
	109	6(0.231)	0(0.000)		
ADA-1	100	24(0.923)	19(0.792)	ns	m
	108	2(0.077)	5(0.208)		
ADH	−100	24(1.000)	0(0.000)	<0.001	d
	−75	0(0.000)	1(0.050)		
	−13	0(0.000)	19(0.950)		
G3P-1	100	24(1.000)	19(0.950)	ns	d
	150	0(0.000)	1(0.050)		
GAP-1	13	0(0.000)	9(0.375)	<0.001	t
	70	0(0.000)	12(0.500)		
	100	26(1.000)	3(0.125)		
GPI-2	100	25(0.962)	24(1.000)	ns	d
	107	1(0.038)	0(0.000)		
IDH	70	0(0.000)	3(0.125)	ns	d
	100	26(1.000)	21(0.875)		
LDH-3	100	26(1.000)	22(0.917)	ns	t
	117	0(0.000)	2(0.083)		
LGG	86	1(0.038)	0(0.000)	ns	d
	100	25(0.962)	22(1.000)		
PGD	75	4(0.154)	0(0.000)	ns	d
	100	22(0.846)	22(1.000)		
PGM-1	84	0(0.000)	1(0.042)	ns	m
	100	23(0.885)	22(0.916)		
	105	3(0.115)	0(0.000)		
	113	0(0.000)	1(0.042)		
PGK	100	26(1.000)	19(0.950)	ns	m
	133	0(0.000)	1(0.050)		

[1]Contingency tests of allelic frequencies using the G-statistic with Yates correction for small sample sizes, assuming all samples drawn from the same population; ns = not significant.

[2]Protein subunit structure based on observed banding patterns of variants; m = monomer, d = dimer, t = tetramer.

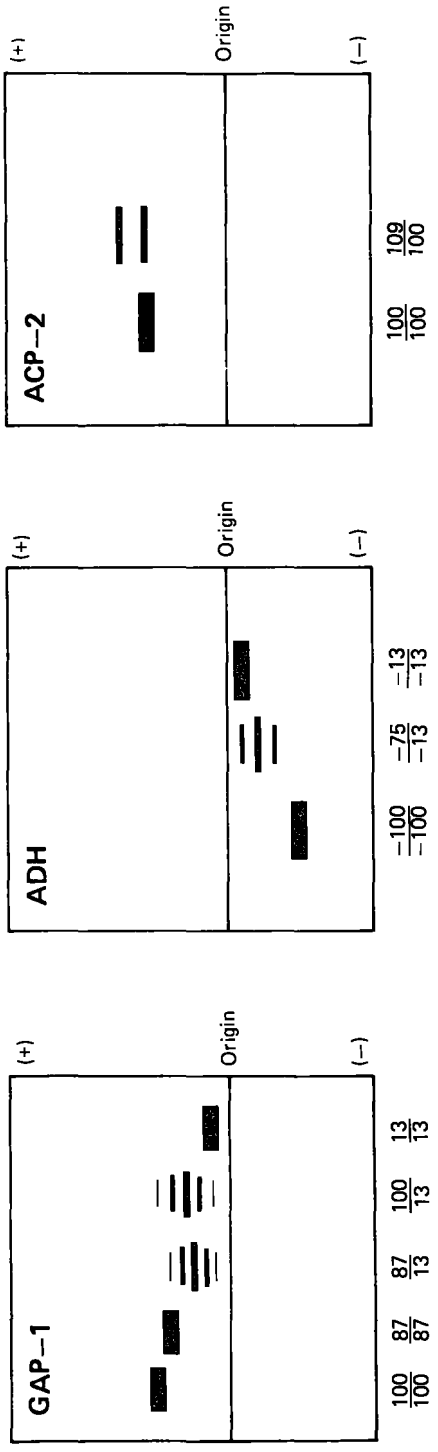

FIGURE 1.—Starch gel banding patterns of variants observed for three loci in *Atheresthes*: GAP1, ADH, and ACP.

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