BIOCHEMICAL GENETIC POPULATION STRUCTURE OF YELLOWFIN SOLE, *LIMANDA ASPERA*, OF THE NORTH PACIFIC OCEAN AND BERING SEA

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ABSTRACT

The gene products of 31 protein-coding loci were examined electrophoretically in samples of yellowfin sole from the North Pacific Ocean and Bering Sea to assess genetic population structure. Four loci, *Ada-2*, *Gpi-1*, *Pep-2*, and *Pgd*, were polymorphic where the frequency of the most common allele was <0.95 and were used to test for allele-frequency differences within and between stock areas defined by life history and tagging data. A nested contingency-table analysis of allelic frequencies showed that there were no genetic subdivisions either within the eastern Bering Sea or within the Gulf of Alaska. At the next higher nested level, genetic heterogeneity was detected for the Japan-Bering Sea comparison at two loci and for the Japan-Bering Sea-Gulf of Alaska comparison at four loci. Genetic distances between pairs of samples within each of the genetic units averaged 0.0005 (±0.0003), but averaged 0.0049 (±0.0026) between samples from these groups. The results of a gene-diversity analysis showed that 95.7% of the total genetic variation was contained on average within populations and that 3.6% was due to differences between Japanese, Bering Sea, and Gulf of Alaska fish. The remaining 0.7% of the genetic diversity was due to differences between populations within these groups. The genetic differences between Bering Sea and Gulf of Alaska fish are due probably to genetic isolation and divergence caused by coastal glaciation in the Pleistocene Period.

Population genetic data of four additional flatfishes are summarized in the form of a gene-diversity analysis and compared with the genetic structure of yellowfin sole populations. There is generally very little genetic differentiation among flatfish populations separated by <1,000 km. The potential for mixing over this distance is great because of adult migration and passive drift of pelagic eggs and larvae.

Yellowfin sole, *Limanda aspera*, can be found from Vancouver Island in the eastern North Pacific Ocean to Japan in the western North Pacific Ocean. However, the greatest densities are found in the eastern Bering Sea where it is one of the major demersal fishery resources. In the late 1950's and early 1960's it was the primary target of Japanese and Soviet distant-water fisheries. During the period 1959-62, total catches ranged from 185,000 to 554,000 metric tons (t) annually (Wakabayashi et al.[4]) but have since declined to range from 42,000 to 167,000 t. In 1975 the biomass of yellowfin sole was estimated to be 23% (1,000,000 t) of all demersal fishes sampled by trawl survey covering the continental shelf region of the eastern Berin Sea (Pereyra et al.[5]).

Yellowfin sole migrate from wintering areas on the outer continental shelf to shallow water of the inner shelf in summer where they feed and spawn. There are two major winter concentrations in the eastern Bering Sea (Fadeev 1970; Wakabayashi[6]); the largest is located in the vicinity of Unimak Island and the second largest west of St. Paul Island. Other smaller wintering concentrations have been recognized by Fadeev (1970) and by Wakabayashi et al. (footnote 4), but the results of tagging studies (Wakabayashi et al. footnote 4) indicate that these concentrations are part of the Unimak Island group.

Tagging studies by Japan indicated that the west St. Paul Island and Unimak Island groups tended to

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[4]Wakabayashi, K., R. Bakkala, and L. Low, 1977. Status of the yellowfin sole resource in the eastern Bering Sea through 1976. Unpubl. manuscr., 21 p. Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112.

[5]Pereyra, W. T., J. E. Reeves, and R. G. Bakkala. 1976. Demersal fish and shellfish resources of the eastern Bering Sea in the baseline year 1975. Processed rep., 619 p. Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112.

[6]Wakabayashi, K. 1974. Studies on resources of the yellowfin sole in eastern Bering Sea. I. Biological characters. Unpubl. manuscr., 77 p. Japan Fishery Agency, Far Seas Fisheries Research Laboratory, 5-7-1 Orido, Shimizu, Shizuoka, Japan.

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remain separate throughout the year (Wakabayashi footnote 6). These tagging data, together with distribution patterns and morphological differences between populations of the two main wintering concentrations, suggested that these groups may constitute independent northern and southern spawning stocks. Other evidence supporting a two-stock concept was 1) apparent differences in growth rate and length-weight relationships between samples from the two areas (Wakabayashi footnote 6), 2) differences in egg diameter between samples from north and south of Nunivak Island, where independent spawning areas for the two stocks might exist (Kashikina 1965), and 3) distribution patterns shown by research vessel surveys in spring and late summer (Chikuni 1971; Yamaguchi 1972), which indicated independent concentrations of fish in northern and southern stock areas.

However, results from other studies have not supported a two-stock concept. Fadeev (1970) found no significant differences in growth rates, length-weight relationships, body proportions, and meristic characters for samples from the two principal wintering groups near Unimak Island and west of St. Paul Island, and Wakabayashi (footnote 6) found no significant differences in the relationship between total body length and radius of the otolith for samples from the two areas. Moreover, winter concentrations of small yellowfin sole have only been found in Bristol Bay (Fadeev 1970), and tag recoveries since 1974 have shown more intermixing of fish between the proposed northern and southern stock areas than had earlier tagging data (Wakabayashi et al. footnote 4).

In the Gulf of Alaska, yellowfin sole are much less abundant and have not been targeted by directed fisheries. As a result much less is known about the geographic distributions of morphological and life history traits in this area.

In this paper, the geographic distributions of electrophoretically detectable protein variants was used to describe the genetic stock structure of yellowfin sole in the eastern Bering Sea and the Gulf of Alaska. Specifically, samples were collected in the inshore spawning areas of the eastern Bering Sea to determine whether the northern and southern stocks are genetically distinct. In addition to these data, the data from one sample of Japanese yellowfin sole provide an estimate of the amount of ocean-wide genetic differentiation among populations. Finally, the genetic population structure of yellowfin sole is compared with the genetic structures of four species of flatfish using the gene-diversity analysis as a summary statistic.

MATERIAL AND METHODS

Electrophoresis

Tissue samples or whole fish were collected at 12 locations in the southeastern Bering Sea, at 3 locations in the Gulf of Alaska, and at 1 location off Hokkaido, Japan, and shipped frozen to Seattle, Wash. (Table 1, Fig. 1). Samples were held at −25°C up to 5 mo until laboratory analysis. The tissues assayed for specific proteins using horizontal starch-gel electrophoresis were skeletal muscle, heart, stomach muscle, vitreous fluid of eye, brain, liver, spleen, kidney, gill, and gonad, but only skeletal muscle, heart, vitreous fluid, and liver were examined in all of the samples. Extraction procedures and electrophoretic methods followed May et al. (1979). Gels consisted of 13% hydrolyzed potato starch (Electrostarch, Madison, Wis., lot 307; Sigma starch, lot 39c-0459)[7]. The locations of specific enzymes were visualized in the gels using solutions described by Harris and Hopkinson (1976). The peptidase staining method A of Harris and Hopkinson (1976) was used to detect zones of activity of a number of peptidases, except that 0-dianisidine diHCl was used as a dye coupler. Three peptides, leu-ala, leu-gly-gly, and phe-pro were used as peptidase substrates.

Three buffer systems were used to achieve maximum resolution of the protein bands on the gels: (I) gel, TRIS 0.03 M, citric acid 0.005 M (pH 8.5), tray, lithium hydroxide 0.06 M, boric acid 0.3 M (pH 8.1) (Ridgway et al. 1970); (II) gel, 1:20 dilution of tray solution, tray, citric acid 0.04 M adjusted to pH 6.1

[7]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—Locations (see also Figure 1) and collection dates of samples of yellowfin sole used for electrophoretic analysis.

Location	Lat. N	Long.	Date
Western North Pacific Ocean			
1. Hokkaido, Japan	42°40′	145°10′E	Aug. 1981
Bering Sea			
North stock area			
2. Norton Sound	63°48′	164°14′W	Aug. 1979
3. Nunivak I.	62°20′	167°28′W	Aug. 1979
4. Nunivak I.	59°21′	167°56′W	Aug. 1979
5. Nunivak I.	60°30′	168°00′W	Oct. 1975
6. St. Paul I.	58°10′	170°40′W	July 1977
7. St. Paul I.	57°00′	171°10′W	Feb. 1978
South stock area			
8. Kuskokwim Bay	59°00′	163°57′W	July 1979
9. Bristol Bay	57°20′	160°24′W	July 1979
10. Bristol Bay	57°59′	161°39′W	July 1979
11. Bering Shelf	56°50′	164°30′W	Sept. 1975
12. Unimak I.	56°10′	164°40′W	Sept. 1975
13. Strogonof Pt.	56°55′	159°30′W	Aug. 1977
Gulf of Alaska			
14. Chirikof I.	56°21′	154°25′W	June 1980
15. Kodiak I.	57°51′	152°44′W	July 1980
16. Kayak I.	59°52′	154°45′W	July 1980

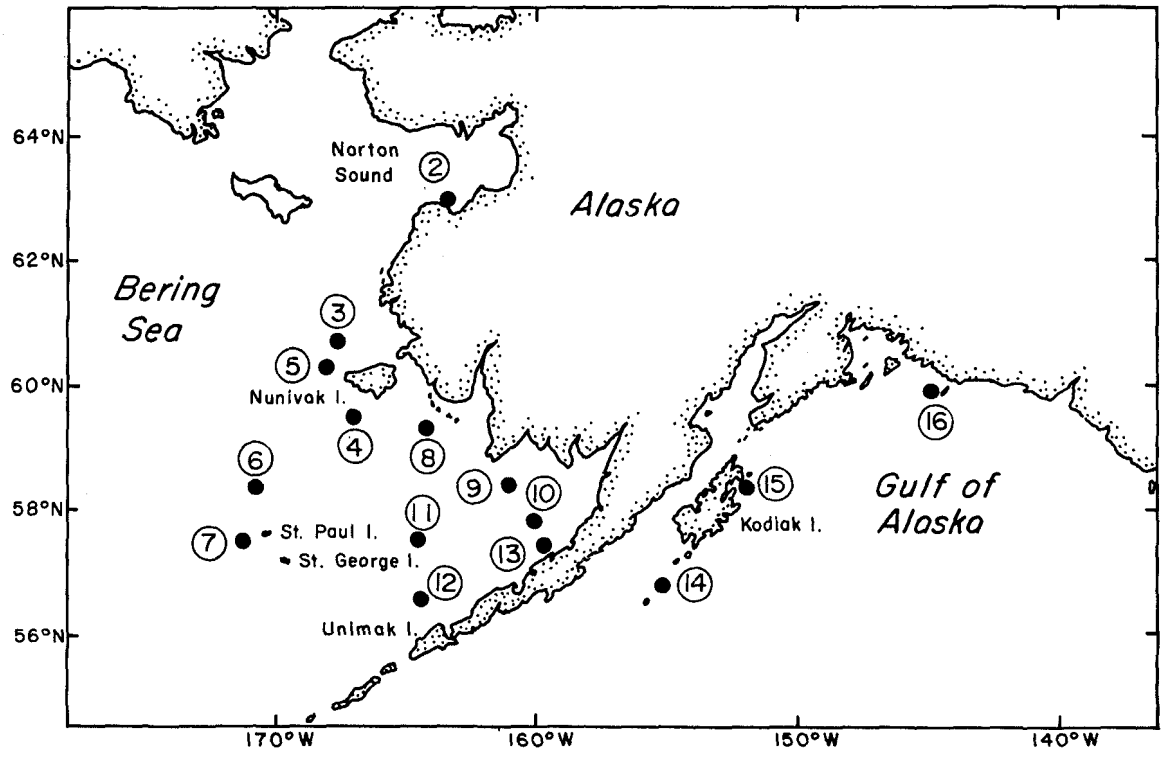

FIGURE 1.—Map of Bering Sea and Gulf of Alaska showing locations (see Table 1 for numbered locations) of samples of yellowfin sole used in this study.

with N(3-aminopropyl)-morpholine (Clayton and Tretiak 1972); (III) gel 1:4 dilution of tray solution, tray, TRIS 0.18 M, boric acid 0.1 M, EDTA 0.004 M (pH 8.7) (Markert and Faulhaber 1965).

The system of locus and allelic nomenclature suggested by Allendorf and Utter (1979) was used. Locus homologies with other fish (Whitt et al. 1975; Fisher and Whitt 1978) were designated with letters where they could be deduced from tissue distributions (Table 2). The enzymes examined in this study, their abbreviations, and Enzyme Commission (E.C.) numbers are listed in Table 2.

Statistical Procedures

Departures from Castle-Hardy-Weinberg proportions in each of the samples were detected using the log likelihood-ratio test for goodness of fit (Sokal and Rohlf 1969) with the degrees of freedom equal to the number of phenotypes minus the number of alleles for a codominant locus.

Stock structure was analyzed using a nested contingency-table analysis where the total heterogeneity among allelic frequencies at each locus was partitioned into orthogonal, regional comparisons in a manner analogous to ANOVA. The log likelihood-ratio test criterion, G, was used to test each comparison with the degrees of freedom equal to the number of alleles minus one, times the number of areas or samples minus one. Only loci having variant-allele frequencies of 0.05 or greater were used in this analysis to avoid low expected frequencies. Rare alleles at these loci were pooled into the next least-frequent allelic class until the pooled class reached a frequency of at least 0.05. The significance level of each comparison was modified to account for the increase in type I error, when multiple tests of the same comparison are made (Cooper 1968). Comparisons at each locus were considered significant if G exceeded the value in a chi-square table associated with a probability of $0.05/4 = 0.012$, where n was the number of polymorphic loci. In this way the overall probability of rejecting H_0 by chance was $1 - (1 - 0.05/4)^4 \approx 0.05$. Only data of samples taken in 1979, 1980, and 1981 were used in all of the statisical analyses, because the six earlier samples were not all taken from spawning areas and because not all of the loci were examined in these samples. However, allelic

TABLE 2.—Protein-coding loci surveyed in yellowfin sole. Multiple loci are numbered beginning at the cathodic end of a gel. Letter designations after locus abbreviations show homologies with proteins in other teleosts. Tissue abbreviations: M = skeletal muscle, H = heart, L = liver, E = vitreous fluid of eye, B = brain, Sp = spleen, K = kidney, St = stomach muscle, Gi = gill, Go = gonad.

Protein (Enzyme Commission number)	Locus abbreviation	Used for study	Buffer	Tissue
Adenosine deaminase (3.5.4.4)	*Ada-1*		III	Sp
	Ada-2	X	III	H, M
Adenylate kinase (2.7.4.3)	*Ak*	X	II	M, Gi
Alcohol dehydrogenase (1.1.1.1)	*Adh*	X	II	L
Creatine kinase (2.7.3.2)	*Ck-1* (C)		II	H, St
	Ck-2 (A)	X	III	M
	Ck-3 (D)		III	Go
	Ck-4 (B)	X	III	B, E
General protein	*Gp*	X	I	M
Glucosephosphate isomerase (5.3.1.9)	*Gpi-1*	X	I	M
	Gpi-2	X	I	H, E, M
Glutamate dehydrogenase (1.4.1.3)	*Gdh*	X	III	L
Glyceraldehyde phosphate dehydrogenase (1.2.1.12)	*Gap-1*	X	II	M, H, B
	Gap-2	X	II	E, H, B
Glycerole-3-phosphate dehydrogenase (1.1.1.8)	*G3p-1*	X	II	L, M
	G3p-2		II	M
	G3p-3		II	L
Isocitrate dehydrogenase (1.1.1.42)	*Idh-1*	X	II	H, M
	Idh-2	X	II	L
Lactate deydrogenase (1.1.1.27)	*Ldh-1* (B)	X	I	M, B
	Ldh-2 (A)	X	I	M, H, B
	Ldh-3 (E)	X	I	E
Malate dehydrogenase (1.1.1.37)	*Mdh-1*	X	II	L
	Mdh-2	X	II	L, E, St
	Mdh-3	X	II	M
Malic enzyme (1.1.1.40)	*Me-1*	X	II	M
	Me-2		II	H, M, Go
Mannosephosphate isomerase (5.3.1.8)	*Mpi*	X	III	M
Peptidase (3.4.11)	*Pep-1*[1]		III	M
	Pep-2[1]	X	III	M, H, B
	Pep-3[2]	X	III	M, K, H
	Pep-4[3]	X	III	M
	Pep-5[2,3]	X	III	M
Phosphoglucomutase (2.7.5.3)	*Pgm-1*	X	I	M
	Pgm-2	X	I	H, L
Phosphogluconate dehydrogenase (1.1.1.44)	*Pgd*	X	II	M, H, K, Go
Superoxide dismutase (1.15.1.1)	*Sod*	X	I	L
Xanthine dehydrogenase (1.2.3.2)	*Xdh*	X	III	L

[1] Substrate: Phenylalanyl-proline.
[2] Substrate: Leucyl-glycyl-glycine.
[3] Substrate: Leucyl-alanine.

frequencies in the six earlier samples were compared with allelic frequencies of the samples taken from the same general areas in 1979 using the contingency-table analysis.

The standard genetic distance, D, (Nei 1972) and its standard error (Nei and Roychoudhury 1974) were calculated for each pair of samples which were examined for all 31 loci. D is an estimate of the number of codon differences in DNA between each pair of samples.

Total gene diversity (H_T) of allelic frequencies, pooled over samples, was partitioned into its components at three levels of population subdivision, regions (R), stocks (S), and populations (P) such that

$$H_T = H_P + D_{PS} + D_{SR} + D_{RT}$$

where H_P is the average heterozygosity over samples, D_{PS} is the diversity due to differences between populations within stocks, D_{SR} is the diversity due to differences between the north and south Bering Sea stocks, and D_{RT} is the diversity due to differences between the western North Pacific Ocean, the Bering Sea, and the eastern North Pacific Ocean. Genetic differentiation relative to H_T was estimated for each subdivision. Thus, $G_{SR} = D_{SR}/H_T$ was the proportion of gene diversity due to subdivision into stocks within regions. The model of population subdivision for both the gene-diversity and the contingency-table analyses is presented in Table 3.

TABLE 3.—Model of population subdivision in yellowfin sole used for contingency-table and gene-diversity analyses. Location codes correspond to numbers in Table 1 and Figure 1.

Total	1	2	3	4	8	9	10	14	15	16
Regions	1	2	3	4	8	9	10	14	15	16
Stocks	1	2	3	4	8	9	10	14	15	16
Populations	1	2	3	4	8	9	10	14	15	16

RESULTS

Electrophoretic Variation

Nineteen protein systems were examined, and 31 zones of enzymatic activity appeared to represent gene products of single locus, which could be reliably scored for population data (Table 2). In the absence of breeding data, the Mendelian nature of the electrophoretic variants may be inferred from the banding patterns. Four guidelines were useful in formulating genetic models: 1) Banding patterns had to be consistent with the subunit structures of homologous proteins in related teleosts, 2) models were formulated by considering gene expression in other teleosts, 3) whenever the same locus was expressed in two or more tissues, the banding patterns of the variants had to be consistent among tissues, and 4) the frequencies of the phenotypes had to fit Castle-Hardy-Weinberg proportions in most of the spawning-area samples. This last criterion has been criticized by Fairbairn and Roff (1980) because of the low power of statistical tests to distinguish among alternative hypotheses (e.g., random distribution of phenotypes) with samples sizes normally used in population studies.

Twenty-one invariant bands appeared on the gels and each was interpreted to reflect the gene products of a monomorphic locus. Although these loci provided no information about differences among populations, they were routinely scored to provide a basis for computing average heterozygosities and genetic distance, both of which require a large sample of randomly selected loci. The following proteins were controlled by one or more polymorphic loci.

Adenosine Deaminase (Ada)

Two loci with different tissue expressions were observed. The last anodal locus, *Ada-2*, had a number of single- and double-banded phenotypes which reflected homozygotes and heterozygotes of seven alleles (Fig. 2). Double-banded heterozygotes suggest that the subunit structure of this enzyme is monomeric. Similar phenotypes have been observed in Pacific herring (Grant 1981) and in North Atlantic pliace, *Pleuronectes platessa* (Ward and Beardmore 1977).

Glucosephosphate Isomerase (Gpi)

The most common phenotype of *Gpi* had three bands reflecting the gene products of two loci where the central band represented the heterodimeric product between the two loci (Fig. 2). Several single- and triple-banded phenotypes were observed at each locus along with corresponding interlocus heterodimeric bands.

Isocitrate Dehydrogenase (Idh)

Two zones of activity having different tissue distributions appeared to reflect the products of two loci where heterodimeric bands between the loci did not form (Fig. 2). The more anodic oocus, *Idh-2*, was expressed predominantly in liver was invariant. *Idh-2* had a number of single- and triple-banded phenotypes reflecting the products of four alleles.

Malate Dehydrogenase (Mdh)

The following genetic model of *Mdh* is based on the observation of several low-frequency variants (Fig. 2). The most common phenotype consisted of four bands, the least anodic of which is designated as the homodimeric band of *Mdh-1*. The products of this locus do not form heterodimeric bands with the products of other *Mdh* loci. An analogous locus appears in salmonids (May et al. 1979) and in Pacific herring (Grant 1981) and is considered to be mitochondrial. The next anodic band of the common phenotype reflects the products of a single locus, *Mdh-2*, having single- and triple-banded variants. The third band of the common phenotype represents a heterodimeric band between *Mdh-2* and *Mdh-3*. At *Mdh-3* there were triple- and broad-banded heterozygotes with corresponding heterodimeric bands with *Mdh-2*.

Mannosephosphate Isomerase (Mpi)

One zone with rare two-banded heterozygotes was observed for this monomeric enzyme.

Peptidase (Pep)

Three polypeptide substrates were used to detect the products of five peptidase loci. *Pep-2* was segregating for five alleles that produced single-banded homozygotes and triple-banded heterozygotes (Fig. 2). The remaining peptidase loci appeared to be monomorphic.

Phosphoglucomutase (Pgm)

There appeared to be two loci with different tissue expressions. The locus, which was predominantly expressed in skeletal muscle tissue, had several different single- and double-banded phenotypes reflecting the products of seven alleles (Fig. 2). *Pgm-2*, which was best visualized with extracts of heart and liver tissues, was monomorphic.

Phosphogluconate Dehydrogenase (Pgd)

The products of *Pgd* were interpreted to be coded by a single locus having five alleles. Heterozygotes were triple- or broad-banded depending upon the relative mobilities of the variant alleles (Fig. 2).

Population Structure

The proportion of polymorphic loci was similar to that observed for other teleosts. The frequency of the most common allele was 0.99 or less for 10 loci (32%), including 4 loci—*Ada-2*, *Gpi-1*, *Gpi-2*, and *Pgd*—which were polymorphic at the 0.95 frequency criterion. The allelic frequencies of these 10 polymorphic loci are presented in Table 4. A complete set of data was not available for samples collected between 1975 and 1978, so these data were only used to test for differences between these samples and those collected in the same areas in the eastern Bering Sea in 1979 and 1980. The remaining statistical analyses were applied only to data collected in 1979,

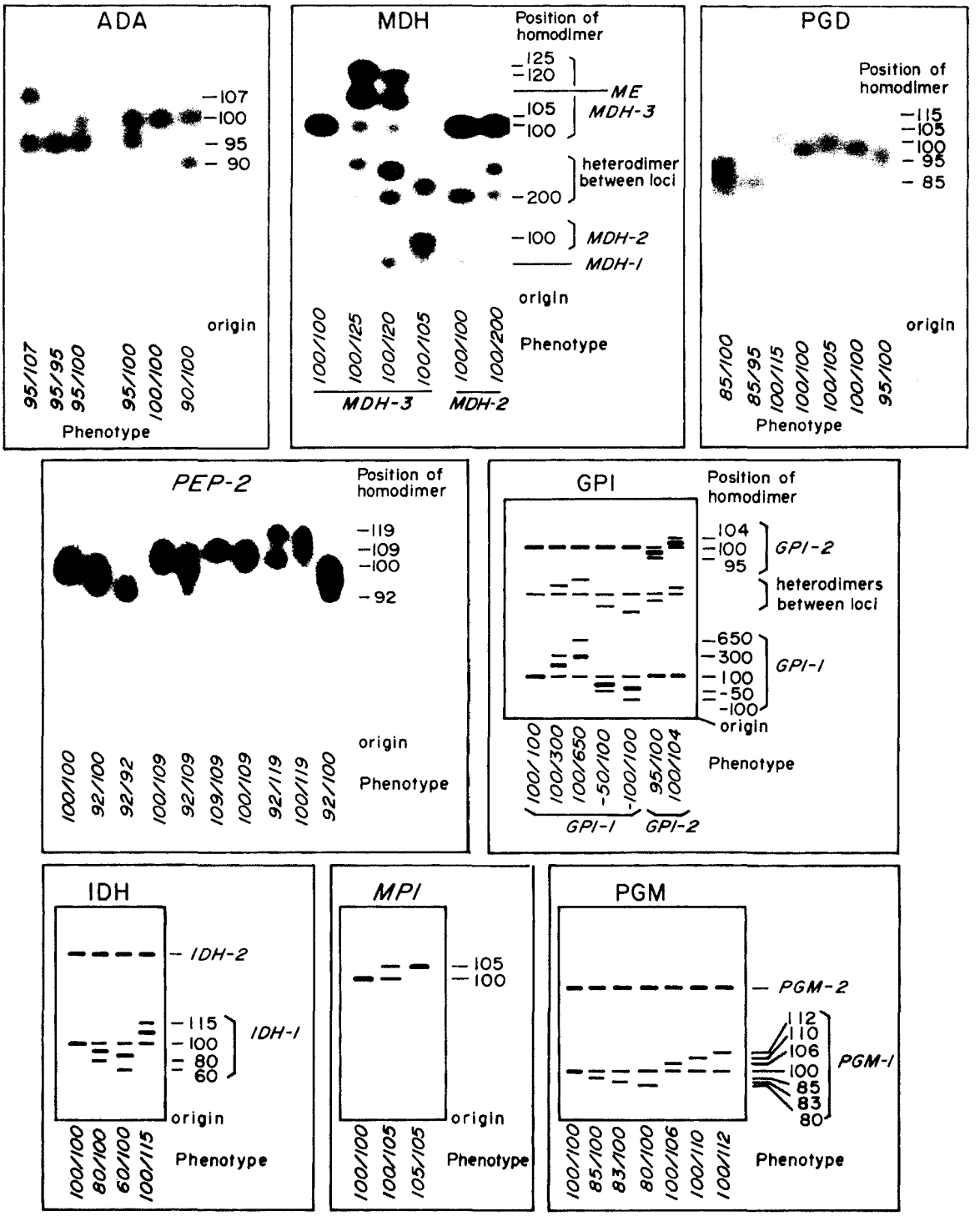

FIGURE 2.—Photographs and diagrammatic representations of electrophoretic gel banding patterns of yellowfin sole.

TABLE 4.—Allelic frequencies of protein variants in samples of yellowfin sole of the North Pacific Ocean and Bering Sea. Sample numbers correspond to location numbers in Table 1 and Figure 1.

		Japan	Bering Sea												Gulf of Alaska		
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ada-2	80	0.007	—	—	—				—	0.008	—				—	—	—
	90	0.051	0.033	0.030	0.046				0.010	0.025	0.020				0.031	0.023	0.040
	93	—	—	—	—				0.021	—	—				0.011	0.011	0.040
	95	0.246	0.325	0.370	0.375				0.365	0.358	0.420				0.213	0.157	0.080
	97	—	—	—	0.011				—	0.017	—				—	—	0.020
	100	0.609	0.575	0.580	0.534				0.583	0.542	0.500				0.745	0.809	0.820
	107	0.087	0.067	0.020	0.034				0.021	0.050	0.060				—	—	—
	N	69	60	50	44				48	60	50				47	89	25
Gpi-1	−150	0.007	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	−100	0.053	0.025	0.050	0.023	0.082	0.022	0.071	0.021	0.025	0.010	0.020	0.065	0.034	—	—	—
	− 50	0.027	—	—	—	0.015	0.008	—	—	0.008	0.010	—	—	0.011	—	—	—
	100	0.733	0.750	0.770	0.796	0.796	0.848	0.772	0.896	0.808	0.770	0.870	0.740	0.764	0.958	0.949	1.000
	250	0.007	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	300	0.160	0.208	0.180	0.182	0.107	0.112	0.157	0.083	0.142	0.210	0.110	0.193	0.185	0.042	0.051	—
	650	0.013	0.017	—	—	—	0.006	—	—	0.017	—	—	—	0.006	—	—	—
	N	75	60	50	44	98	189	35	48	60	50	50	85	100	48	[1]89	25
Gpi-2	95	0.027	0.017	0.010	0.057				0.042	0.050	0.020				—	—	—
	100	0.967	0.975	0.990	0.943				0.958	0.950	0.980				0.989	0.959	0.980
	104	0.006	0.006	—	—				—	—	—				0.011	0.041	0.020
	N	75	60	50	44				48	60	50				47	86	25
Idh-1	60	—	—	—	—	—	—	—	—	—	—	—	—	—	0.010	—	—
	80	—	0.025	—	0.011	—	—	0.014	0.021	0.017	0.020	—	—	—	—	—	—
	100	0.986	0.950	0.990	0.955	1.000	1.000	0.986	0.969	0.975	0.970	1.000	1.000	1.000	0.990	1.000	1.000
	115	0.014	0.025	0.010	0.034	—	—	—	0.010	0.008	0.010	—	—	—	—	—	—
	N	69	60	49	44	98	189	35	48	60	50	50	85	100	48	89	25
Mdh-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	—	0.008	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	N	75	60	50	44	98	189	35	48	60	50	50	85	100	48	89	25
Mdh-3	60	—	—	—	—	0.005	—	0.015	—	—	—	—	0.006	—	—	—	—
	100	0.974	1.000	0.990	0.977	0.980	1.000	0.971	0.990	0.992	0.990	0.990	0.982	0.960	1.000	1.000	1.000
	105	0.013	—	—	—	—	—	—	—	—	0.010	—	—	—	—	—	—
	120	0.013	—	0.010	0.023	0.015	—	0.014	—	0.008	—	0.010	0.012	0.020	—	—	—
	125	—	—	—	—	—	—	—	0.010	—	—	—	—	0.020	—	—	—
	N	75	60	50	44	98	189	35	48	60	50	50	85	100	48	89	25
Mpi	100	1.000	0.992	1.000	1.000				0.990	0.992	0.980				1.000	0.994	1.000
	105	—	0.008	—	—				0.010	0.008	0.020				—	0.006	—
	N	75	60	50	44				48	60	50				48	89	25
Pep-2	92	0.132	0.148	0.180	0.114				0.125	0.178	0.120				0.073	0.062	0.080
	100	0.772	0.708	0.720	0.761				0.792	0.712	0.800				0.635	0.640	0.660
	102	0.007	0.008	—	—				—	—	—				—	—	—
	109	0.073	0.092	0.090	0.114				0.073	0.093	0.080				0.292	0.287	0.260
	116	0.015	0.008	0.010	0.011				0.010	0.017	—				—	0.011	—
	N	74	60	50	44				48	59	50				48	89	25
Pgm-1	80	0.014	0.008	—	—	—	—	—	—	—	—	0.010	0.012	0.018	—	—	—
	83	—	—	—	—	—	—	—	0.010	—	—	—	—	—	—	—	—
	85	0.021	—	0.010	—	0.005	0.006	—	0.021	0.017	0.030	0.030	0.039	—	—	—	—
	100	0.917	0.950	0.960	0.966	0.964	0.964	0.971	0.948	0.958	0.940	0.940	0.947	0.976	0.958	0.994	1.000
	106	0.034	0.008	—	0.011	—	—	—	—	—	—	—	—	—	—	—	—
	110	0.014	0.025	0.030	0.023	0.026	0.024	0.014	0.021	0.025	0.030	0.020	0.012	0.006	0.042	0.006	—
	112	—	0.009	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	N	73	60	50	44	98	189	35	48	60	50	50	85	100	48	89	25
Pgd	85	0.013	0.025	0.040	0.114	0.056	0.014	0.029	0.063	0.067	0.030	0.070	0.010	0.037	0.196	0.191	0.292
	95	—	—	—	—	—	—	—	—	0.008	—	—	—	—	—	—	—
	100	0.987	0.967	0.960	0.863	0.908	0.969	0.957	0.917	0.900	0.950	0.920	0.990	0.944	0.804	0.803	0.708
	105	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.006	—
	115	—	0.008	—	0.023	0.036	0.017	0.014	0.021	0.025	0.020	0.010	—	0.019	—	—	—
	N	75	60	50	44	98	189	35	48	60	50	50	85	100	46	89	24
H%		0.055	0.056	0.050	0.060				0.049	0.058	0.053				0.044	0.043	0.041

[1]Deviation from Castle-Hardy-Weinberg proportions, $P < 0.01$.

1980, and 1981. One significant ($P < 0.05$) departure from Castle-Hardy-Weinberg proportions was detected for *Gpi-1* in sample number 15 collected in the Gulf of Alaska.

Contingency-Table Analysis

The results of the nested contingency-table analysis are presented in Table 5. Within the eastern Bering Sea, the allelic frequencies of *Gpi-1* and *Pgd* for samples collected from 1975 to 1978 were not significantly different from samples collected in 1979 from the same stock areas. Likewise, no significant allele-frequency differences were detected for *Ada-2*, *Gpi-1*, *Pep-2*, and *Pgd* for either the within- or between-Bering Sea stock comparisons. Similarly,

TABLE 5.—Contingency-table analysis of allelic frequencies of yellowfin sole of the North Pacific Ocean and eastern Bering sea. Parentheses indicate non-orthogonal tests not included in the totals.

Source of variation	*Ada-2*		*Gpi-1*		*Pep-2*		*Pgd*		Sum	
	df	*G*	df	*G*	df	*G*	df	*G*	df	*G*
Total	18	[1]73.63	9	[1]79.21	18	[1]70.61	9	[1]94.00	54	[1]317.45
Among Bering Sea, Gulf of Alaska, Japan	4	[1]60.29	2	[1]68.89	4	[1]63.74	2	[1]81.62	12	[1]274.54
(Between Japan and Bering Sea)	(2	[1]10.36)	(1	2.74)	(2	0.49)	(1	[2]6.32)		
Between Bering Sea stocks	2	0.45	1	2.74	2	1.08	1	0.43	6	4.70
Within Northern stock	4	2.66	2	0.60	4	2.41	2	7.68	12	13.75
(Between years)		—	(1	3.73)		—	(1	0.43)		—
Within Southern stock	4	2.87	2	5.95	4	3.19	2	2.02	12	14.03
(Between years)		—	(1	0.09)		—	(1	3.98)		—
Among Gulf of Alaska stocks	4	7.36	2	1.03	4	0.19	2	2.25	12	10.83

[1] $P<0.01$.
[2] $0.05 \geq P \leq 0.01$.

none of the among-sample comparisons for the Gulf of Alaska were significant. At the next nested level, the allelic frequencies of *Ada-2* and *Pgd* were significantly different ($P<0.05$) between the pooled Bering Sea samples and the sample from Japan. The three-way comparison between the pooled frequencies of the Bering Sea, the Gulf of Alaska, and Japan was significant ($P<0.01$) for each of the four polymorphic loci.

Genetic Distance

Among the Bering Sea and Japanese samples, D ranged from 0.0002 to 0.0012 and averaged 0.0005 with an average standard error of 0.0003 (Table 6). Similarly, for the Gulf of Alaska samples D ranged from 0.0002 to 0.0008 and averaged 0.0005 with an average standard error of 0.0003. However, the D's between the samples from these two areas were much greater than those within each region; they ranged from 0.0029 to 0.0086, averaged 0.0049, and had an average standard error of 0.0026.

Gene-Diversity Analysis

The results of the gene-diversity analysis are presented in Table 7. The average within-population diversity (heterozygosity) ranged from 0.041 to 0.056 and averaged 0.051. This represented 95.7% of the total gene diversity. Of the remaining gene diversity, 3.6% was due to regional differences between the Gulf of Alaska, the Bering Sea, and the Japanese samples. The proportion of the total gene diversity due to differences among populations within each stock was 0.6% and that due to differences between the north- and south-stock areas in the Bering Sea was 0.1%.

DISCUSSION

The results of this study show that there is little genetic structuring of yellowfin sole populations within the eastern Bering Sea or within the Gulf of Alaska. Although tagging studies in the eastern Bering Sea reported by Wakabayashi et al. (footnote 4) demonstrated that fish from the northern and southern stock areas largely remained separated during their annual inshore-offshore migration, there appears to be sufficient migration between these areas to prevent genetic differentiation. A similar degree of migration between areas in the Gulf of Alaska can be inferred from the lack of allele-

TABLE 6.—Standard genetic distance (below diagonal) and standard errors (above diagonal) between samples of yellowfin sole based on 31 protein-coding loci. Location numbers correspond to those in Table 1 and Figure 1.

1	—	0.0002	0.0004	0.0006	0.0007	0.0004	0.0008	0.0021	0.0021	0.0035
2	0.0004	—	0.0002	0.0004	0.0006	0.0001	0.0003	0.0020	0.0023	0.0037
3	0.0006	0.0002	—	0.0002	0.0004	0.0001	0.0001	0.0018	0.0022	0.0036
4	0.0011	0.0007	0.0006	—	0.0003	0.0002	0.0002	0.0017	0.0024	0.0033
8	0.0012	0.0010	0.0007	0.0006	—	0.0002	0.0006	0.0016	0.0021	0.0030
9	0.0008	0.0004	0.0003	0.0003	0.0004	—	0.0002	0.0016	0.0023	0.0033
10	0.0010	0.0005	0.0005	0.0005	0.0008	0.0005	—	0.0026	0.0033	0.0048
14	0.0041	0.0040	0.0037	0.0032	0.0029	0.0032	0.0051	—	0.0002	0.0005
15	0.0044	0.0046	0.0044	0.0040	0.0035	0.0039	0.0060	0.0002	—	0.0004
16	0.0068	0.0071	0.0069	0.0059	0.0054	0.0060	0.0087	0.0008	0.0006	—
	1	2	3	4	8	9	10	14	15	16

TABLE 7.—Gene-diversity analysis of yellowfin sole of the North Pacific Ocean and Bering Sea.

H_P Mean within population diversity (SE)	D_{PS} Between populations within stocks (SE)	D_{SR} Between Bering Sea stocks (SE)	D_{RT} Between regions (SE)	H_T Total (SE)
Average absolute gene diversity				
0.0507 (0.0223)	0.0003 (0.0001)	0.00005 (0.00001)	0.0019 (0.0009)	0.0530 (0.0233)
Average relative gene diversity				
0.9575 (0.0065)	0.0057 (0.0013)	0.0009 (0.0003)	0.0359 (0.0049)	1.0000

frequency differences among the samples from that region. In addition to adult migration, the passive transport of pelagic eggs and larvae may also contribute to gene flow between stocks within these two regions.

In contrast to the genetic homogeneity within these regions, significant allele-frequency differences were detected for the four polymorphic loci between the Bering Sea and the Gulf of Alaska. This genetic subdivision across the Alaska Peninsula was also reflected in the genetic distances between samples and in the gene-diversity analysis.

The reason for the observed genetic structure of yellowfin sole populations cannot be due to isolation by distance, because the greatest genetic differences were detected between nearby populations and not between more distantly separated populations. The two major genetic groups most likely reflect past periods of isolation and genetic divergence caused by coastal glaciation during the Pleistocene. The first of four major glacial periods in Alaska began about 2 million yr ago and the last major period of glaciation ended only 11,000 yr ago (Ericson and Wollin 1964; Péwé and Roger 1972). During most of these periods glacial ice covered the coastline of the Alaska Peninsula and central Alaska. Since yellowfin sole are rarely found at depths >100 m (Hart 1973) and since juveniles use shallow bays and estuaries as nursery areas, populations would be greatly influenced by coastal glaciation. There are similar genetic subdivisions across the Alaska Peninsula or across the Bering Sea for Pacific herring, *Clupea pallasi* (Grant in press); walleye pollock, *Theragra chalcogramma* (Iwata 1975; Grant and Utter 1980); and Pacific cod, *Gadus macrocephalus* (Grant et al.[8]).

How similar is the genetic population structure of yellowfin sole to that of other flatfishes? Two statistics can be used to make this comparison, genetic distance and relative gene diversities. The former statistic cannot be used for most of the available flatfish data because only a few loci were examined in these studies. The gene-diversity analysis is more appropriate in cases where only a few loci have been examined because the analysis can be computed for each locus. Nonetheless, the best estimates of population structure are averages over loci because random effects can produce different results for different loci, even though each locus experiences the same population events. Caution must be used when comparing the results of these analyses between species because the results depend, in part, on the geographic extent of the study and, hence, on the number of genetic subdivisions included in the data. A summary of all of the available biochemical data for five species of flatfishes is presented in Table 8 in the form of a gene-diversity analysis.

Thirteen loci (2 polymorphic loci) were examined in Greenland halibut, *Reinhardtius hippoglossoides*, collected from four coastal areas of eastern Canada and from the Bering Sea (Fairbairn 1981a). If only the Canadian samples are considered, 99.93% of the gene variation was contained within populations, and

[8]Grant, W. S., C. I. Zhang, and T. Kobayashi. 1982. Biochemical genetics of *Gadus*: II Population structure of Pacific cod (*Gadus macrocephalus*). Processed rep., 27 p. Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112.

TABLE 8.—Summary of relative gene diversities in five species of flatfishes.

Species	No. of polymorphic loci	Geographic range of samples (km)	Within population diversity	Between populations within areas	Between areas within regions	Between regions within seas	Between seas	References
Atlantic Ocean								
Greenland halibut, *Reinhardtius hippoglossus*	2	1,000 (+Bering Sea)	0.9993 0.9308	— —	0.0001 0.0001	0.0006 0.0004	— 0.0687	Fairbairn (1981a)
Witch, *Glyptocephalus cynoglossus*	2	1,500	0.9923	0.0011	0.0066	0.0001	—	Fairbairn (1981b)
Plaice, *Pleuronectes platessa*	5	1,000	0.9959	—	—	0.0013	0.0018	Ward and Beardmore (1977); Purdom et al. (1976)
Pacific Ocean								
Pacific halibut, *Hippoglossus stenolepis*	17 1	6,200 2,000	0.9871 0.9962	— 0.0039	— —	0.0040 0.0009	0.0089 —	Grant et al. (in press); Tsuyuki et al. (1969)
Yellowfin sole, *Limanda aspera*	10	6,200	0.9597	0.0057	0.0009	—	0.0359	This paper

0.07% was due to differences between the Gulf of St. Lawrence and the shelf populations, and to population differences on the continental shelf. No significant allele-frequency differences were detected among these samples. When the Bering Sea sample is included in the analysis, 6.9% of the gene diversity was due to subspecific differences between the two areas.

Also in eastern Canada, the gene products of 15 loci (2 polymorphic loci) were examined in samples of witch flounder, *Glyptocephalus cynoglossus*, (Fairbairn 1981b). The gene-diversity analysis of these data also shows low levels of genetic differentiation among witch flounder populations; 99.2% of the total gene diversity was contained on average within populations and 0.8% was due to all population differences combined. In spite of the low level of genetic differentiation among populations, significant allele-frequency differences were detected between some of the areas. Tag-and-recovery and distribution data show that adults tend to be sedentary and that populations tend to be separated by weak barriers to migration such as the cold shallow waters of the Grand Bank (Bowering 1976; Bowering and Misra 1982). However, the potential gene flow at the egg and larval stages is great (Evseenko and Nevinsky[9]) and no doubt counters genetic divergence of the partially isolated adult populations.

For plaice, *Pleuronectes platessa*, in the eastern North Atlantic Ocean, there are data for the five loci in common in the studies of Ward and Beardmore (1977) and Purdom et al. (1976). Allele-frequency differences were not detected either within the Irish Sea, within the North Sea, or between these seas for these loci. The results of the diversity analysis reflected this degree of homogeneity; 99.6% of the total gene diversity was contained within populations and only 0.4% was due to differences between populations.

Grant et al. (in press) examined the gene products of 17 polymorphic loci in three widely separated samples of Pacific halibut, *Hippoglossus stenolepis*, in the North Pacific Ocean and Bering Sea. The gene-diversity analysis showed that 98.7% of the gene diversity was contained within populations, that 0.4% was due to differences between the Bering Sea and the Gulf of Alaska, and that 0.9% was due to trans-Pacific Ocean differences. Tsuyuki et al. (1969) examined genetic variation at one locus in samples from 10 locations in the Bering Sea and in the eastern North Pacific Ocean. The gene-diversity analysis of these results estimated the within-population diversity to be 99.6%, that due to differences between regions to be 0.09%, and that due to differences among populations within regions to be 0.04%. This high degree of genetic homogeneity which was detected in both studies reflects the long distance migrations that Pacific halibut are known to make. For instance, tagging studies have demonstrated migrations of at least 3,200 km (Skud 1977).

The results of these studies show that there is very little genetic differentiation among populations of flatfishes located over areas of about 1,000 km. For Pacific halibut, areas of genetic homogeneity appear to be even larger because of its ability to migrate long distances. These areas of genetic homogeneity probably cannot be considered randomly mating populations in the strict sense, because it is unlikely, for example, that fish located on one edge of a genetic unit have an equal chance of mating with fish on the other side of the genetic unit. Rather, these units reflect long-term processes that influence population size and migration over several generations. The division of yellowfin sole into two genetic groups by coastal glaciation in the Pleistocene is an excellent example of the importance of interpreting present-day allele-frequency distributions in terms of past population events.

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LITERATURE CITED

ALLENDORF, F. W., AND F. M. UTTER.
1979. Population genetics. *In* W. S. Hoar, D. S. Randall, and J. R. Brett (editors), Fish physiology, Vol. 8, p. 407-454. Acad. Press, N.Y.

BOWERING, W. R.
1976. Distribution, age and growth, and sexual maturity of witch flounder (*Glyptocephalus cynoglossus*) in Newfoundland waters. J. Fish. Res. Board Can. 33:1574-1584.

BOWERING, W. R., AND R. K. MISRA.
1982. Comparisons of witch flounder (*Glyptocephalus cynoglossus*) stocks of the Newfoundland-Labrador area, based upon a new multivariate analysis method for meristic characters. Can. J. Fish. Aquat. Sci. 39:564-570.

CHIKUNI, S.
1971. Report on the biological research of groundfish in the Bering Sea and the northeastern Pacific by Inase Maru No. 3 in 1970. Jpn. Fish. Agency, Far Seas Fish. Res. Lab., Shimizu, Jpn., Data Rep., 175 p.

[9]Evseenko, S. A., and M. M. Nevinsky. 1973. Breeding and development of witch flounder (*Glyptocephalus cynoglossus*) in the Northwest Atlantic Ocean. Int. Comm. Northwest Atl. Fish. Res. Doc. 73/49, Ser. No. 2990, 23 p.

CLAYTON, J. W., AND D. N. TRETIAK.
1972. Amino-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169-1172.

COOPER, D. W.
1968. The significance level in multiple tests made simultaneously. Heredity 23:614-617.

ERICSON, D. B., AND G. WOLLIN.
1964. The deep and the past. Knopf, N.Y.

FADEEV, N. W.
1970. Promysel i biologicheskaya kharakteristika zheltoperoi kambaly v vostochnoic chasti beringova morya (The fishery and biological characteristics of yellowfin sole in the eastern part of the Bering Sea). [In Russ.] Tr. Vses. Naucho-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 70 (Izv. Tikhookean. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 72):327-390. (Transl. *in* P. A. Moiseev (editor), Soviet fisheries investigations in the northeastern Pacific, Part 5, p. 332-396, by Isr. Program Sci. Transl., 1972, avail. U.S. Dep. Commer., Natl. Tech. Inf. Serv., Springfield, Va., as TT 71-50127.)

FAIRBAIRN, D. J.
1971a. Biochemical genetic analysis of population differentiation in Greenland halibut (*Reinhardtius hippoglossoides*) from the Northwest Atlantic, Gulf of St. Lawrence, and Bering Sea. Can. J. Fish. Aquat. Sci. 38:669-677.
1981b. Which witch is which? A study of the stock structure of witch flounder (*Glyptocephalus cynoglossus*) in the Newfoundland Region. Can. J. Fish. Aquat. Sci. 38:782-794.

FAIRBAIRN, D. J., AND D. A. ROFF.
1980. Testing genetic models of isozyme variability without breeding data: Can we depend on the x^2? Can. J. Fish. Aquat. Sci. 37:1149-1159.

FISHER, S. E., AND G. S. WHITT.
1978. Evolution of isozyme loci and their differential tissue expression Creatine kinase as a model system. J. Mol. Evol. 12:25-55.

GRANT, W. S.
1981. Biochemical genetic variation, population structures, and evolution of Atlantic and Pacific herring. Ph.D. Thesis, Univ. Washington, Seattle, 149 p.
In press. Biochemical genetic population structure of Pacific herring (*Clupea pallasi*). Can. J. Fish. Aquat. Sci.

GRANT, W. S., AND F. M. UTTER.
1980. Biochemical genetic variation in walleye pollock, *Theragra chalcogramma*: Population structure in the southeastern Bering Sea and the Gulf of Alaska. Can. J. Fish. Aquat. Sci. 37:1093-1100.

GRANT W. S., D. J. TEEL, T. KOBAYASHI, AND C. SCHMITT.
In press. Biochemical population genetics of Pacific halibut (*Hippoglossus stenolepis*) and comparison with Atlantic halibut (*H. hippoglossus*). Can. J. Fish. Aquat. Sci.

HARRIS, H., AND D. A. HOPKINSON.
1976. Handbook of enzyme electrophoresis in human genetics. Am. Elsevier Publ. Co., N.Y.

HART, J. L.
1973. Pacific fishes of Canada. Fish. Res. Board Can. Bull. 180:1-740.

IWATA, M.
1975. Population identification of walleye pollock, *Theragra chalcogramma* (Pallas), in the vicinity of Japan. Mem. Fac. Fish. Hokkaido Univ. 22:193-258.

KASHKINA, A. A.
1965. O razmnozhenii zheltoperoi kambaly Limanda aspera (Pallas) v vostochnoi chasti beringova morya i ob izmenenii ee nerestovogo stada (Reproduction of yellowfin sole (*Limanda aspera*, Pallas) and changes in its spawning stocks in the eastern Bering Sea). [In Russ.] Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 58 (Izv. Tikhookean. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 53):191-199. (Transl. *In* P. A. Moiseev (editor), Soviet fisheries investigations in the northeastern Pacific, Part 4, p. 182-190, by Isr. Program Sci. Transl., 1968, avail. U.S. Dep. Commer., Natl. Tech. Inf. Serv., Springfield, Va., as TT 67-51206.)

MARKERT, C. L., AND I. FAULHABER.
1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159:319-332.

MAY, B., J. E. WRIGHT, AND M. STONEKING.
1979. Joint segregation of biochemical loci in salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114-1128.

NEI, M.
1972. Genetic distance between populations. Am. Nat. 106: 283-292.

NEI, M., AND A. K. ROYCHOUDHURY.
1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379-390.

PÉWÉ, T. L., AND R. D. ROGER.
1972. Modern and Wisconsinan snowlines in Alaska. Proc. 24th Int. Geol. Congr., Montreal, Sect. 12:187-197.

PURDOM, C. E., D. THOMPSON, AND P. R. DANDO.
1976. Genetic analysis of enzyme polymorphisms in plaice (*Pleuronectes platessa*). Heredity 37:193-206.

RIDGWAY, G. J., S. W. SHERBURNE, AND R. D. LEWIS.
1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99:147-151.

SKUD, B. E.
1977. Drift, migration, and intermingling of Pacific halibut stocks. Int. Pac. Halibut Comm., Sci. Rep. 63, 42 p.

SOKAL, R. R., AND F. J. ROHLF.
1969. Biometry. Freeman and Co., San Franc., 776 p.

TSUYUKI, H., E. ROBERTS, AND E. A. BEST.
1969. Serum transferrin systems and the hemoglobins of the Pacific halibut (*Hippoglossus stenolepsis*). J. Fish. Res. Board Can. 26:2351-2362.

WARD, R. D., AND J. A. BEARDMORE.
1977. Protein variation in the plaice, *Pleuronectes platessa* L. Genet. Res. 30:45-62.

WHITT, G. S., J. B. SHAKLEE, AND C. L. MARKERT.
1975. Evolution of the lactate dehydrogenase isozymes of fishes. *In* C. L. Markert (editor), Isozymes IV genetics and evolution, p. 381-400. Acad. Press, N.Y.

YAMAGUCHI, H.
1972. Report on the biological research of ground fish in the Bering Sea by Chōsui maru in 1968. Far Seas Fish. Res. Lab., Shimizu, Jpn., Data Rep., 139 p.