EXAMINATION OF BIOCHEMICAL GENETIC VARIATION IN SPAWNING POPULATIONS OF YELLOWFIN SOLE (LIMANDA ASPERA) OF THE EASTERN BERING SEA

by

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INTRODUCTION

This report is a followup to an earlier report (Grant et al., 1978) which attempted to identify genetically distinct stocks of yellowfin sole (Limanda aspera) using the geographic distributions of electrophoretically detectable protein variants. The samples analyzed in that study were collected, for the most part, in offshore areas not associated with the spawning areas of this fish, and were, therefore, not appropriate to test for genetically distinct stocks. The present report presents the results of the analysis of samples taken inshore in summer when the fish would be segregated into discrete stocks, if they exist.

There are two major winter concentrations of adult fish on the outer continental shelf located west of St. Paul Island and northwest of Unimak Island (Fadeev, 1970; Wakabayashi, 1974). These groups of fish move into the shallow waters of the shelf in April and May, and by summer the main body of yellowfin sole is in waters of less than 100 m. Spawning occurs at this time. Tagging studies by Japan through 1973 indicated that the west St. Paul Island and Unimak Island groups largely remained separate throughout the year (Wakabayashi, 1974). These tagging data, coupled with distribution patterns and morphological differences between populations of the two main wintering groups, suggested that these groups may constitute separate northern and southern spawning stocks.

MATERIALS AND METHODS

Six samples of about 50 fish each were collected in July and August 1979 on the inner continental shelf (Figure 1) and held in frozen storage until laboratory analysis. Starch gel electrophoresis as outlined by Utter et al. (1974) was used to detect protein mobility variants. Tissue samples of muscle, heart, eye, and liver from 50 fish were assayed for several enzymatic proteins in an attempt to detect additional polymorphic loci that might be useful for stock identification. The remaining fish were assayed only for the polymorphic loci. Nine polymorphic loci could be reliably scored, but only five loci had variants of 0.04 or greater which were useful for the statistical analysis of stock structure.

Bands on the starch gels reflecting allelic products were designated by their mobilities relative to the bands reflecting the common allele which was designated 100. Where multiple forms of the same functional enzyme were expressed, the loci were numbered beginning with the least anodal locus. Since breeding studies were lacking, the genetic natures of the protein variants were inferred from the observed banding patterns. Two guidelines were useful in formulating interpretations: (1) banding patterns had to be consistent with known molecular structures of similar proteins in other fish, and (2) whenever the same locus was expressed in two or more tissues, the banding patterns of the variants had to be consistent among tissues.

Allelic ("gene") frequencies were estimated from genotype frequencies, since all of the variants observed in this study were interpreted to reflect products coded by codominant loci. Departures from Hardy-Weinberg proportions were detected with the likelihood-ratio test for goodness of fit (Sokal and Rohlf, 1969) with (number of genotypes)-(number of alleles) degrees of freedom. Stock structure, as reflected in the geographic distributions of

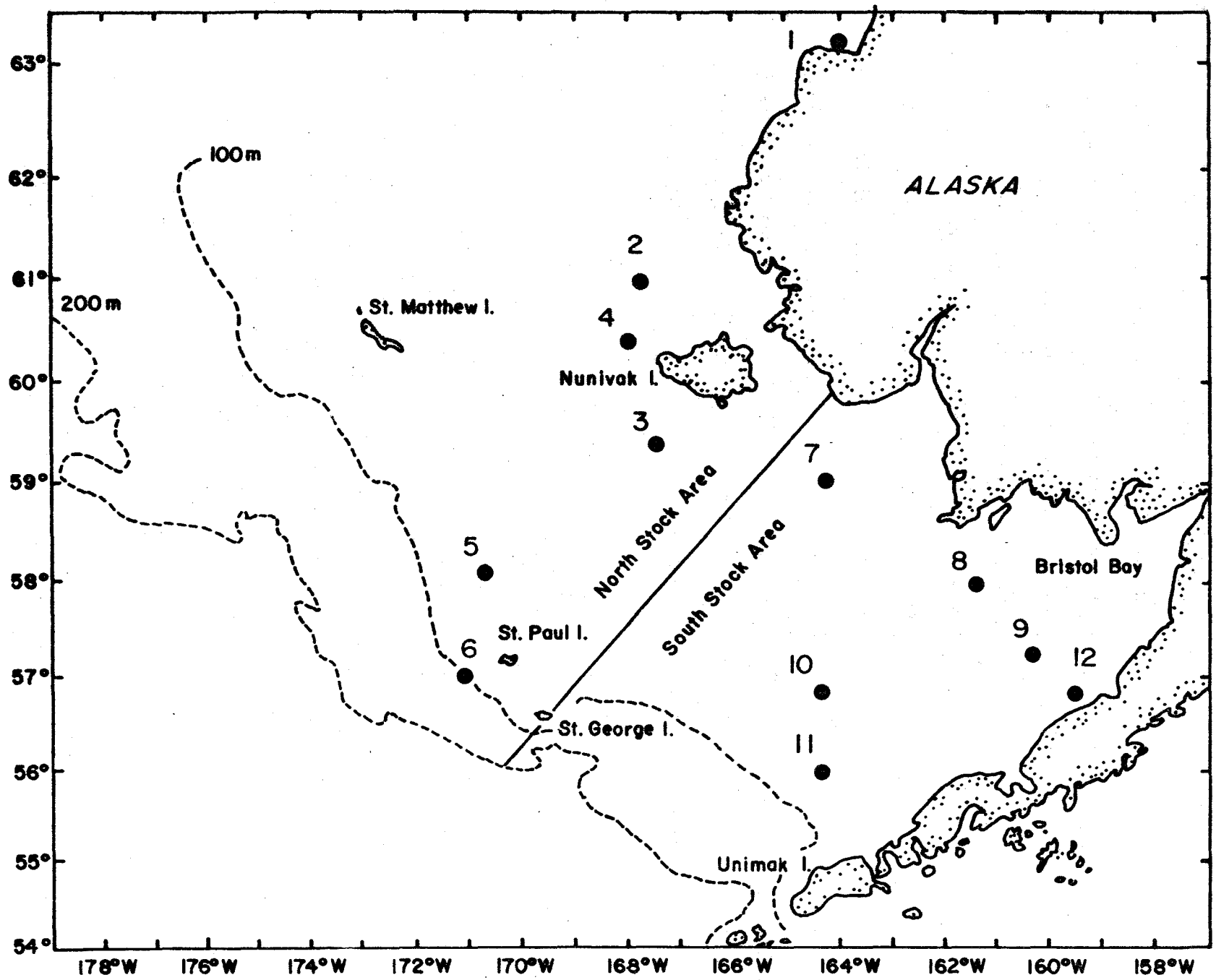

Figure 1.--Map of the southeastern Bering Sea showing locations of the samples of yellowfin sole used in this study. Sample numbers 1, 2, 3, 7, 8, and 9 were collected in 1979.

allelic frequencies, was analyzed using contingency table analysis where the total heterogeneity among the samples was partitioned into between and within stock components in a manner analogous to ANOVA. The likelihood-ratio test (Sokal and Rohlf, 1969) was used to test for significance with (allelic classes-1)X (areas-1) degrees of freedom. These test values were compared to critical X^2 values found in a chi-square table. Alleles having frequencies of less than 0.04 over all the samples were pooled into the next smallest allelic class. The significance level of the tests was modified to account for the increase in type I error when several tests are made simultaneously (Cooper, 1968). Accordingly the desired alpha, 0.05, was divided by the number of tests (11) made at each locus and the critical value was located under this column in the chi-square table for rejecting the null hypothesis of no heterogeneity.

RESULTS

Genetic Variation

Allelic frequencies of nine polymorhic loci are presented in Table 1. Frequencies at two of these loci, ADA (adenosine deaminase) and PEP-2 (peptidase; substrate, phenyalanyl-proline) are reported here for the first time. Only 5 of the loci had allelic frequencies which were great enough to be of use for stock structure analysis: PGI-1 (phosphoglucose isomerase), PGM (phosphoglucomutase), 6PG (6-phosphogluconate dehydrogenase), ADA, and PEP-2. Four additional loci, MDH-3 (malate dehydrogenase), IDH-1 (isocitrate dehydrogenase), PMI (phosphomannose isomerase), and PGI-2 had frequencies of variants of 0.04 or less and were not included in the subsequent statistical analyses.

Stock Structure

No significant departures from Hardy-Weinberg proportions were detected. This suggests that each of the samples were drawn from populations in which the fish were randomly mating but does not necessarily imply that the samples were drawn from the same population.

For the statistical analysis of stock structure the samples were divided into two groups representing the two hypothesized north-south stocks. Each of these groups was further subdivided into data from Grant et al. (1978) and data collected in 1979, and are referred to as offshore and inshore subareas, respectively. The total heterogeneity among the samples was partitioned into within-stock and between-stock comparisons and are presented in Table 2.

Heterogeneity ($P < 0.05$) in the overall test was detected at only one locus, 6PG, and was attributable to heterogeneity among the northern stock area samples. However, neither of the individual tests for heterogeneity in the inshore or offshore samples was significant. In the northern stock area the frequencies of the most common allele ranged from 0.88 to 0.97, but there

Table 1. Allelic frequencies of enzymatic mobility variants in samples of yellowfin sole collected from the Bering Sea. Alleles are designated by their mobilities relative to the common allele (100).

				PGI-1					PGM					
Location	Sample	Date	N	100	300	-100	-50	400	100	110	85	80	106	112
Northern Stock Area	1	8- 2-79	60	0.75	0.20	0.03	—	0.02	0.95	0.02	—	0.01	0.01	0.01
	2	8- 1-79	50	0.77	0.18	0.05	—	—	0.96	0.03	0.01	—	—	—
	3	8-14-79	44	0.80	0.18	0.02	—	—	0.97	0.02	—	—	0.01	—
	4	10-12-75	98	0.80	0.10	0.08	0.02	—	0.96	0.02	0.01	0.01	—	—
	5	7-22-77	189	0.85	0.11	0.02	0.01	0.01	0.96	0.02	0.01	0.01	—	—
	6	2-12-78	35	0.77	0.16	0.07	—	—	0.97	0.01	—	0.02	—	—
Southern Stock Area	7	7-29-79	48	0.89	0.09	0.02	—	—	0.95	0.02	0.03	—	—	—
	8	7-17-79	50	0.77	0.21	0.01	—	—	0.94	0.03	0.03	—	—	—
	9	7-18-79	60	0.81	0.14	0.03	0.01	0.01	0.96	0.03	0.01	—	—	—
	10	9-14-75	50	0.87	0.11	0.02	—	—	0.94	0.02	0.03	0.01	—	—
	11	9-24-75	85	0.74	0.19	0.07	—	—	0.95	0.01	0.03	0.01	—	—
	12	8- 3-77	100	0.76	0.18	0.04	0.01	0.01	0.98	0.01	—	0.01	—	—

Table 1. Continued

	6PG				MDH-3				IDH-1			PMI	
Sample	100	90	115	95	100	115	60	125	100	80	115	100	105
1	0.96	0.04	0.01	—	1.00	—	—	—	0.95	0.03	0.02	0.99	0.01
2	0.96	0.04	—	—	0.99	0.01	—	—	0.99	—	0.01	1.00	—
3	0.88	0.10	0.02	—	0.98	0.02	—	—	0.95	0.02	0.03	1.00	—
4	0.91	0.06	0.03	—	0.98	0.01	0.01	—	1.00	—	—	1.00	—
5	0.97	0.01	0.02	—	1.00	—	—	—	1.00	—	—	1.00	—
6	0.96	0.03	0.01	—	0.97	0.01	0.02	—	0.98	0.02	—	1.00	—
7	0.92	0.06	0.02	—	0.99	—	—	0.01	0.97	0.02	0.01	0.99	0.01
8	0.95	0.03	0.02	—	0.99	—	0.01	—	0.97	0.02	0.01	0.98	0.02
9	0.90	0.07	0.02	0.01	0.99	0.01	—	—	0.98	0.01	0.01	0.99	0.01
10	0.92	0.07	0.01	—	0.99	0.01	—	—	1.00	—	—	1.00	—
11	0.99	0.01	—	—	0.98	0.01	0.01	—	1.00	—	—	1.00	—
12	0.94	0.04	0.02	—	0.96	0.02	—	0.02	1.00	—	—	1.00	—

Table 1. Continued

	ADA							PEP-2					PGI-2		
Sample	100	95	107	90	80	97	93	100	92	109	116	102	100	95	104
1	0.58	0.32	0.07	0.03	—	—	—	0.71	0.18	0.09	0.01	0.01	0.97	0.02	0.01
2	0.58	0.37	0.02	0.03	—	—	—	0.72	0.18	0.09	0.01	—	0.99	0.01	—
3	0.53	0.38	0.03	0.05	—	0.01	—	0.76	0.12	0.11	0.01	—	0.94	0.06	—
7	0.58	0.37	0.02	0.01	—	—	0.02	0.79	0.13	0.07	0.01	—	0.96	0.04	—
8	0.50	0.42	0.06	0.02	—	—	—	0.80	0.12	0.08	—	—	0.98	0.02	—
9	0.54	0.36	0.05	0.03	0.01	0.02	—	0.71	0.18	0.09	0.02	—	0.95	0.05	—

Table 2. Likelihood ratio analysis of variation at 5 codominant loci of yellowfin sole. Allelic frequencies of less than 0.04 over all samples were pooled into the next lowest frequency class.

Comparison	df	PGI-1	df	PGM	6PG	df	ADA	PEP-2
Between N-S Areas	2	2.636	1	0.893	0.123	2	0.452	1.083
Within Areas (total)	20	39.971	10	5.403	27.540*	8	5.527	5.600
Between Subareas (North)	2	11.035*	1	0.373	0.432	-	—	—
Within Subareas (total)	8	10.125	4	1.510	16.625*	-	—	—
Among Inshore Samples	4	1.433	2	0.336	7.683	4	2.659	2.410
Among offshore Samples	4	8.692	2	1.174	8.942	-	—	—
Between Subareas (South)	2	2.701	1	0.280	3.975	-	—	—
Within Subareas (total)	8	16.110	4	3.240	10.915	-	—	—
Among Inshore Samples	4	8.505	2	0.390	2.016	-	—	—
Among Offshore Samples	4	7.610	2	2.850	8.899	4	2.868	3.190
Total	22	42.607	11	6.296	27.663*	10	5.979	6.683

*$P < 0.05$

was no apparent geographic trend in the distributions of the allelic frequencies. The between offshore vs. inshore comparison was not significant.

Even though the northern subareas comparison for PGI-1 was significant, there was considerable overlap of the allelic frequencies of these two groups (0.075 - 0.80, inshore; 0.77 - 0.85, offshore). The heterogeneity, therefore, doesn't reflect true differences between the subareas, but is probably due to one unusually large sample (N = 189) having a high frequency of the common allele (0.85).

The individual tests for heterogeneity within and between stock areas at each locus are additive and these sums reflect the essential features of the stock structure of yellowfin sole. Neither of these sums were significant (Table 3). The ratio of the standardized measures (sum/df) of the between to within tests is approximately distributed as an F-ratio and is not significant (0.432). This result demonstrates that the degree of heterogeneity between the hypothesized northern and southern stock areas is not significantly different than the degree of heterogeneity within these areas.

Table 3. Likelihood ratio analysis of variation summed over five polymorphic loci, standardized measure (test criterion/d.f.), and approximate F-ratio of between area comparisons to within area comparisons.

Comparison	d.f.	Likelihood test criterion	Standardized measure	Approximate F-ratio
Between Areas	8	5.187	0.648	0.432
Within Areas	56	84.041	1.501	

DISCUSSION

Where populations have become isolated from one another because of barriers to migration or because of changes in migration patterns, the frequencies of genetically determined characters would change gradually over time because of genetic drift. In the present study the geographic distributions of genetically determined protein variants were used to test whether the two hypothesized stocks of yellowfin sole in the southeastern Bering Sea are genetically distinct. The results presented here suggest that the fish in these two areas are not genetically isolated from one another. Although tagging studies (Wakabayashi, 1974) have demonstrated that the fish in the two areas largely remain separated in their inshore-offshore annual migration, there appears to be sufficient migration between areas to prevent genetic differentiation. In addition to adult migration, the passive transport of pelagic eggs by water currents may also contribute to gene flow between the two areas. In view of the gene flow caused by adult and larval migration, and in view of the number of loci surveyed in this study (38), it seems unlikely that genetic differences, as reflected in the distributions of electrophoretic variants, exist between the stocks of yellowfin sole within the southeastern Bering Sea.

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