

Population Identification of Silver Hake
(Merluccius bilinearis) Using Isoelectric Focusing

by

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INTRODUCTION

Many studies have been undertaken to define and separate populations of commercially important species of fish. In addition to the more common morphometric and meristic studies, various physical parameters such as scale (Ito, 1972) or otolith (Nichy, 1969) characteristics have been subjected to statistical analysis in order to discover whether sufficient discrimination could be made between different fish stocks. A problem inherent in these approaches is that these characteristics are partly, if not primarily, controlled by environmental parameters, and may not reflect the genetic makeup of the fish, while in defining fish stocks one is most interested in knowing whether the fish are reproductively isolated, which is measured by the genetic variation of the stocks.

Since the development of electrophoresis, it has been possible, when coupled with histochemical staining, to study the genetics of fish populations, and many studies have been published in which this has been done (Edmunds and Sammons, 1973; McDonald, 1975; Allendorf et al., 1977; Utter et al., 1978, for examples).

This study was aimed at the elucidation of the genetic variation between populations of the commercially important species Merluccius bilinearis Mitchell, the silver hake or whiting. This gadoid is distributed along the Eastern Seaboard from Newfoundland to S. Carolina (Domanevsky and Nozdrin, 1963; Brad Brown, pers. comm.), but the highest densities and the most important fisheries are found off the New England Coast (Fritz, 1960). Traditionally, silver hake has been managed as three stocks: one in the Gulf of Maine, one off Georges Bank, and one off the southern New England

and Mid-Atlantic coasts (Anderson, 1978). However, there is no definitive information available as to the degree of reproductive isolation of these stocks. Therefore, an extensive morphometric study was initiated by Frank Almeida (NMFS, Woods Hole) and is being supplemented by this study.

MATERIALS AND METHODS

Sampling

Samples of whole silver hake were obtained from commercial fisheries at Pt. Judith, Rhode Island, and Gloucester, Massachusetts. These two ports approximate the northern and southern extremes of the spawning grounds (Almeida, 1978). Only samples which were packed in ice and reached port within 24 hours of capture were used. Fish were either kept on ice until dissected (within 48 hours of capture) or else were frozen at -20°C until they could be processed. Sexually immature fish were discarded, and samples from several different tissues were frozen prior to being focused. The tissues which were investigated included: liver, white muscle, gonad, aqueous humor, and eye lens. Eye lenses were chosen for more thorough study because of their relatively simple patterns under general protein staining, and because they exhibited only very faint bands for the inter-loci heterotetramers of lactate dehydrogenase.

Isoelectric Focusing

Focusing was performed according to the method of Saravis (1980), using 0.8% zero electroendosmosis agarose (Marine Colloids), and 2.5% LKB Ampholine, pH range 3.5-9.0. The gels were focused at 15 W constant power from an LKB 2103 constant power power supply and were cooled to 2°C on an MRA R Model M-150 slab electrofocusing apparatus. Equilibrium was usually attained in 25 minutes.

Staining

Two enzyme systems were examined in this study: lactate dehydrogenase (LDH), and phosphohexose isomerase (PHI). Assay mixtures were prepared while the separation was progressing, and consisted of two solutions, one made of 7.0 ml. of 0.144 M TRIS pH 8.0, 0.4 ml. of 2.4 mg/ml phenazine methosulfate, and 15 mg nitroblue tetrazolium. The other contained, for LDH: 1.0 g. polyvinylpyrrolidone (PVP), 20 mg. NADP, 2.4 ml. of 0.06 M KCN, 15 ml. of 0.144 M TRIS pH 8.0 containing 5 mM $MgCl_2$, and 9.0 ml. of 0.379 M lactic acid neutralized with KOH. For PHI, the solution contained: 1.0 g. PVP, 20 mg. NADP, 2.4 ml. 0.06 M KCN, 15 ml. of 0.144 M TRIS pH 8.0, 15 IU glucose-6-phosphate dehydrogenase, and 50 mg fructose-6-phosphate. The two solutions were combined immediately prior to the end of the focusing run, and the combined solution was sufficient for two gels. The gels were sealed in plastic bags and were incubated in the dark at 37°C for 10 to 30 minutes, then dried and analyzed. These stains are a modification of the methods of Scopes (1968) and Baptist et al. (1969).

Scoring the Gels

Once dry, the gels were scored by first overlaying with very fine graph paper and marking the sites of the bands, and then arbitrarily assigning numbers to the bands. No attempt was made to quantify the activity of the various bands.

Statistical Analysis

Analysis of the data was performed using a Statistical Analysis Systems (SAS) program, the FUNCAT procedure. This analysis is based on a log distribution, and in order to avoid the possibility of log 0 being taken, all

allele pairs which appeared only once in the data set were removed. In no case did this represent more than 5.6% of the observations. Each genetic locus was analyzed separately, and since an early chi-square analysis of the data showed that there was no correlation with length, the data were modeled as functions of sex and port only.

The FUNCAT procedure uses a chi-square test comparing the logs of the probabilities of characteristics analyzed, in this case, the allele-pair frequencies.

RESULTS

Lactate Dehydrogenase (LDH)

The data for lactate dehydrogenase conform to the model of Shaklee et al. (1973), that is, that the enzyme is a tetramer having three genetic loci. Even the third locus (the so-called eye band) was readily apparent in all the tissues examined with the exception of the gonadal tissue. This is in contrast with the work of Utter (1979) on the Pacific hake, in which the eye band was present only in eye tissue. All three sites were polymorphic, and the distribution of the heterotetramers along the pH gradient was linear, indicating that the size and charge distribution of the subunits was similar. This enzyme system proved to be an excellent discriminator between the two populations sampled. Statistical analysis of the gene pair distribution for each of the three loci via the FUNCAT procedure showed a highly significant difference (p (homogeneity) <0.01) between the two sample populations which could not be attributed to sex or length (Table 1).

Phosphohexose Isomerase (PHI)

The PHI data indicate that the enzyme is a dimer having two genetic loci. To the best of my knowledge, there is no published data on this enzyme for this genus. However, this model agrees with that proposed for other gadoids (Avisé and Kitto, 1973). Because relatively little literature is available for this enzyme system, several experiments were performed in order to better characterize the protein. Electrophoresis in an SDS-polyacrylamide gel showed that the enzyme's activity was not destroyed by this detergent, and also showed that each of the dimeric forms had a different molecular weight, though no estimate of these weights was attempted. There were as many bands of activity detected by this method as by isoelectric focusing. Focusing in a gel containing 3 M urea (an agent which dissociates hydrogen bonds), however, completely destroyed PHI activity. This enzyme system was not a good discriminator between the two sample populations. Analysis of the data showed no difference between the two ports at the 95% level of significance (Table 1); PHI 1 did show a significant difference between the two ports at the 90% level.

The data collected in this study presented several interesting and surprising results. Each of the enzyme loci was highly polymorphic, with six to fourteen isozymes from each gene site. This is a much higher degree of polymorphism than is usually found amongst poikilotherms (Nevo, 1978). In particular, the number of isozymes for LDH was much greater than was reported by Markert and Faulhaber (1965), who found only one isozyme at the A locus and two at the B locus for this species. The work of Utter and Hodgins (1969) on Pacific hake (Merluccius productus) also shows much

less polymorphism, with two alleles at the A locus and one at the B locus. Since both of these studies were performed in starch gels this suggests that the separations of these enzymes in agarose gel IEF is superior to that in starch.

[ED. Note: The degree of polymorphism is subject to question; see the two alternate interpretations in the tables.]

Analysis of the data shows a substantial difference between the two enzyme systems. The LDH system shows highly significant differences between the two ports, with a less than one percent probability that the two populations are homogeneous for any of the three loci, and a high probability of homogeneity with respect to sex for each of the loci.

The PHI system is much more difficult to interpret. The PHI A locus has a 7.7% probability of homogeneity with respect to port, and a 17.2% probability with respect to sex. Using this test, this locus did not test to the model degrees of freedom for port, and it may be that there is, in fact, a greater degree of homogeneity than is accounted for statistically. The PHI B locus shows a high degree of homogeneity with respect to both port and sex ($P > 0.4$). This enzyme system is clearly not a good discriminator between the two sample populations. As is shown in Table 2, none of the gene sites examined exhibits a Hardy-Weinberg distribution. The greatest divergences from the Hardy-Weinberg are the excessive numbers of homozygous individuals expressing rare alleles. Almost all gene sites exhibited an excess of homozygous individuals. An exception was found in the LDH 3 ("eye band") locus; this is discussed below.

There are two possible sets of causes for these results: one is experimental error. The excess of homozygotes might be due to the presence of a silent gene, one which expresses no activity under the experimental conditions. Such an allele would not be scored, and the individual would be falsely assumed to be a homozygote. This mechanism is possible in the case of Phosphohexose Isomerase, where only one band would appear in the case of a heterozygote with a silent gene. The LDH loci, however, should still express four bands in this case. Another possibility is that there is some hidden heterogeneity which is not revealed in the separation technique employed. This could lead to a false high estimate of homozygotes.

A third possibility is that what is being examined is not a true genetic distribution of alleles but reflects artifacts due to handling or storage or some artifact of the separation. It is unlikely that handling introduced significant error in the technique. The samples were not homogenized or blended to extract the proteins. Rather, proteins were electrophoresed out of the tissues; this may be the way proteins separate in vivo during cell division (Woodruff and Telfer, 1980). Freezing damage may have occurred over the course of the study. The most likely form of such damage would introduce extra activity bands (Fairbairn and Roff, 1980).

Extra bands were not observed in this study. Rather, with the exception of LDH 3, the patterns of enzyme activity were always observed to follow the genetic models described above. The activity bands for LDH 3 were found at the extreme end of the gels, and did not always follow the pattern of either one or five bands. They were scored as though the end members of the groups of bands were the true alleles, but in some cases this was probably

not the case, and some of the bands may have migrated off the end of the gel. This might also explain why this gene site had such a limited variety of allele pairs as compared to the other LDH gene sites.

Inspection of the observed allele-pair distributions showed that there were several adjacent activity bands which showed no heterozygote banding patterns. Since the bands were on the order of 0.5 mm in width and were separated by approximately 2.0 mm, it was possible that some of the bands were mis-scored, creating artificial groups of homozygotes. Therefore, all adjacent activity bands which had no heterozygotes were re-classified as one allele, and were tested to see if they conformed to Hardy-Weinberg expectations (Table 3). This analysis was not performed on the LDH 3 gene site, since it was thought that there was a separation artifact operating in that system. In no case did this operation bring the data into conformity with the expectations of the Hardy-Weinberg Law, although the divergences from it were sometimes less extreme than in the earlier analysis. The pattern of divergencies remained the same, with an excess of heterozygotes in each case.

Heterozygotes between adjacent bands might have appeared as an unresolved single band, and could have been scored as homozygotes of one or the other alleles. Since the enzymes examined lost their activity in two to three months, under the conditions of storage used, it was not possible to mix samples exhibiting adjacent activity bands to see if they could be resolved into the two bands.

The other set of explanations for why the data do not follow the Hardy-Weinberg distribution are based on the possibility that the populations being studied were not at equilibrium. Relatively few natural

populations fulfill the requirements for the Hardy-Weinberg equilibrium (Goodenough and Levine, 1974), either because mating is non-random, or because the genes under study are experiencing some selective pressure, or because there is some mixing between populations.

Silver hake have external fertilization and they mate in schools, so the assumption of random mating is probably valid in this species. However, some studies have indicated that the LDH enzyme system exhibits clines which are a function of latitude (Imhof, Leary, and Brook, 1980; Powers and Place, 1974). If this is true in the silver hake as well, some of the non-ideal properties of the populations may be explained. Finally, since these fish are highly migratory, it is reasonable to believe that substantial mixing between populations is occurring.

CONCLUSION

An electrophoretic study of five gene sites in the gadoid fish, Merluccius bilinearis, revealed a high degree of heterogeneity for each gene site, which was much higher than was previously reported. Statistical analysis of the data indicated that the two samples are highly distinct from each other, and that each of the two populations is not at genetic equilibrium. This disequilibrium is most likely a result of a combination of selective pressures and mixing in four of the five gene sites, while the final site was probably not accurately analyzed, due to a separation artifact.

It is not possible to infer any fine population structure from these data. Two possible situations might exist: either these two samples represent the extremes of the genetic range, and there is simple mixing between them, or there are several small stocks spawning individually along the coast.

In order to test these hypotheses, it would be necessary to collect samples in several areas along the coast and analyze and compare the gene frequencies of each of the samples. Further, the results of this study do not address the question of the dispersion of the stocks after spawning, and there may be no correlation between the areas in which the stocks spawn and the areas to which they disperse.

Table 1. Analysis of Allele Pair Distributions Using the FUNCAT Procedure.

	CHI-SQUARE	PROBABILITY OF HOMOGENEITY	DEGREES OF FREEDOM
PHI A (338 Observations)			
SEX	5.00	0.1718	3
PORT	5.13	0.0770	2
RESIDUAL	5.30	0.2508	4
PHI B (303 observations)			
SEX	5.82	0.4431	6
PORT	4.59	0.5976	6
RESIDUAL	3.45	0.7512	6
LDH A (348 observations)			
SEX	6.07	0.7331	9
PORT	23.53	0.0051	9
RESIDUAL	10.45	0.3152	9
LDH B (351 observations)			
SEX	6.28	0.6155	8
PORT	20.88	0.0075	8
RESIDUAL	6.56	0.5843	8
LDH C (371 observations)			
SEX	5.47	0.4849	6
PORT	30.23	0.0001	6
RESIDUAL	-3.76	1.0000	6

See text for discussion.

Table 2. Initial chi-square calculations.

PHI 1 Gloucester			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
3,3	1.66	10	41.9
3,4	2.16	0	2.16
3,5	29.27	16	6.02
3,7	1.35	0	1.35
3,8	0.81	1	0.04
4,4	0.7	11	151.56
4,5	18.99	1	17.04
4,7	0.87	0	0.87
5,5	128.98	143	1.52
5,7	11.87	15	0.83
7,7	0.27	0	0.27
4,8	0.52	1	0.44
5,8	7.12	7	0.00
7,8	0.33	0	0.33
8,8	0.10	0	0.10
	<u>205.0</u>	<u>205</u>	<u>224.42</u>
Point Judith			
3,3	0.05	1	18.05
3,5	0.18	0	0.18
3,5	4.18	3	0.33
3,6	0.25	0	0.25
3,7	0.11	0	0.11
3,9	0.18	0	0.18
4,4	0.18	4	81.07
4,5	8.36	1	6.48
4,6	0.51	1	4.71
4,7	0.22	0	0.22
4,9	0.36	0	0.36
5,5	96.68	106	0.90
5,6	11.71	2	8.05
5,7	5.04	3	0.83
5,9	8.36	10	0.32
6,6	0.35	5	61.78
6,7	0.31	1	1.50
6,9	0.51	0	0.51
7,7	0.18	1	3.74
7,9	0.22	0	0.22
9,9	0.18	0	0.18
			<u>185.75</u>

Table 2. Initial Chi-square Calculations (cont'd)

PHI 2 Gloucester			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
1,1	0.77	9	87.96
1,2	15.8	2	12.05
1,3	2.61	1	0.99
1,4	2.55	2	0.12
1,5	0.52	0	0.52
2,2	83.19	98	2.63
2,3	27.49	12	8.73
2,4	26.81	28	0.05
2,5	5.49	4	0.40
3,3	2.27	12	41.71
3,4	4.43	3	0.46
3,5	0.91	0	0.91
4,4	2.16	3	0.33
4,5	0.86	0	0.86
5,5	0.09	2	40.53
	<u>175.95</u>	<u>176</u>	<u>198.27</u>
Point Judith			
1,1	0.09	3	94.09
1,2	4.58	0	4.58
1,3	0.89	1	0.14
1,4	0.61	0	0.61
1,5	0.39	0	0.39
1,7	0.18	0	0.18
1,10	0.16	0	0.16
2,2	56.9	74	5.14
2,3	22.24	4	7.88
2,4	15.05	9	2.43
2,5	9.81	5	2.36
2,7	4.58	5	0.04
2,10	3.91	3	0.21
3,3	2.17	13	54.05
3,4	2.94	1	1.28
3,5	1.92	2	0.00
3,7	0.89	0	0.89
3,10	0.77	0	0.77
4,4	1.0	6	25.00
4,5	1.12	0	1.12
4,7	0.61	0	0.61
4,10	0.52	1	0.44
5,5	0.42	4	30.50
5,7	0.39	0	0.39
5,10	0.33	0	0.33
7,7	0.09	1	9.20
7,10	0.16	0	0.16
10,10	0.07	1	12.36
	<u>132.79</u>	<u>133</u>	<u>254.87</u>

Table 2. Initial Chi-square Calculations (cont'd)

LDH 1 Gloucester			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
2,2	0.18	2	18.40
2,5	0.73	1	1.00
2,7	1.22	1	0.04
2,8	9.58	7	0.70
2,9	0.85	0	0.85
2,10	0.26	0	0.26
5,5	0.73	3	7.06
5,7	2.43	4	1.01
5,8	19.12	9	5.36
5,9	1.69	1	0.28
5,10	0.59	2	3.37
7,7	2.00	19	144.5
7,8	31.70	0	31.70
7,9	2.80	0	2.80
7,10	0.84	0	0.84
8,8	124.93	159	9.29
8,9	22.10	0	22.10
8,10	6.64	2	3.24
9,9	0.98	2	1.06
9,10	0.59	1	0.28
10,10	0.09	2	40.53
	<u>230.05</u>	<u>230</u>	<u>293.79</u>

Point Judith

1,1	0.10	0	0.10
1,2	0.25	0	0.25
1,4	0.36	0	0.36
1,5	0.20	0	0.20
1,6	0.34	0	0.34
1,7	0.20	0	0.20
1,8	4.7	7	1.13
1,9	0.25	0	0.25
1,10	0.31	0	0.31
1,11	0.20	0	0.20
2,2	0.16	0	0.16
2,4	0.47	0	0.47
2,5	0.25	0	0.25
2,6	0.43	0	0.43
2,7	0.25	0	0.25
2,8	6.05	9	1.44
2,9	0.32	0	0.32
2,10	0.40	0	0.40
2,11	0.25	0	0.25

Table 2. Initial Chi-square Calculations. (cont'd)

LDH 1				
Point Judith (cont.)				
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value	
4,4	0.34	0	0.25	
4,5	0.36	0	0.36	
4,6	0.62	0	0.62	
4,7	0.36	0	0.36	
4,8	8.74	10	0.18	
4,9	0.47	2	4.98	
4,10	0.57	0	0.57	
4,11	0.36	1	1.14	
5,5	0.10	0	0.10	
5,6	0.34	0	0.34	
5,7	0.20	0	0.20	
5,8	4.7	4	0.10	
5,9	0.25	0	0.25	
5,10	0.31	2	9.21	
5,11	0.20	1	3.20	
6,6	0.29	3	25.32	
6,7	0.34	0	0.34	
6,8	8.06	0	8.06	
6,9	0.43	0	0.43	
6,10	0.53	6	56.45	
6,11	0.34	0	0.34	
7,7	0.10	0	0.10	
7,8	4.70	0	4.70	
7,9	0.25	0	0.25	
7,10	0.31	1	1.54	
7,11	0.20	0	0.20	
8,8	56.45	66	1.62	
8,9	6.05	0	6.05	
8,10	7.39	2	3.93	
8,11	4.70	4	0.10	
9,9	0.16	3	50.41	
9,10	0.40	0	0.40	
10,10	0.05	0	0.05	
10,11	0.25	0	0.25	
11,11	0.10	0	0.10	
	<u>124.51</u>	<u>125</u>	<u>276.16</u>	

Table 2. Initial Chi-square Calculations. (cont'd)

LDH 2 Gloucester			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
0,0	0.19	4	76.40
0,1	0.56	0	0.56
0,2	2.47	1	0.87
0,3	2.53	2	1.07
0,4	1.80	1	0.36
0,5	1.68	1	0.27
0,6	1.59	0	1.59
0,7	1.22	0	1.22
0,8	0.91	0	0.91
1,1	0.40	5	52.90
1,2	3.61	1	1.89
1,3	3.70	1	1.97
1,4	2.62	6	4.36
1,5	2.45	1	0.86
1,6	2.32	0	2.32
1,7	1.59	0	1.59
1,8	1.27	0	1.27
2,2	7.98	18	12.95
2,3	16.34	0	16.34
2,4	11.59	10	0.22
2,5	10.83	26	21.25
2,6	10.26	10	0.01
2,7	7.03	0	7.03
2,8	5.89	0	5.89
3,3	8.37	20	16.16
3,4	11.87	2	8.21
3,5	11.10	5	3.35
3,6	10.51	17	4.01
3,7	7.20	15	8.45
3,8	6.03	4	0.68
4,4	4.21	13	18.35
4,5	7.87	0	7.87
4,6	7.45	2	3.99
4,7	5.11	5	0.00
4,8	4.28	9	5.21
5,5	3.51	9	8.59
5,6	6.97	0	6.97
5,7	4.77	1	2.98
5,8	4.00	5	0.25
6,6	3.30	12	22.94
6,7	4.51	1	2.73
6,8	3.79	0	3.79
7,7	1.55	7	19.16
7,8	2.59	1	0.98
8,8	1.09	6	22.12
	<u>220.91</u>	<u>220</u>	<u>379.92</u>

Table 2. Initial Chi-square Calculations. (cont'd)

LDH 2 Point Judith			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
-1,-1	0.42	3	15.85
-1,0	0.58	0	0.58
-1,1	1.48	0	1.48
-1,2	1.85	0	1.85
-1,3	3.69	3	0.13
-1,4	1.11	0	1.11
-1,5	2.42	6	5.30
-1,6	2.48	0	2.48
-1,7	0.95	0	0.95
-1,8	0.63	1	0.22
0,0	0.20	3	39.20
0,1	1.01	0	1.01
0,2	1.27	0	1.27
0,3	2.53	1	0.93
0,4	0.76	0	0.76
0,5	1.67	0	1.67
0,6	1.70	0	1.70
0,7	0.65	0	0.65
0,8	0.43	4	29.64
1,1	1.29	5	10.67
1,2	3.23	0	3.23
1,3	6.45	3	1.85
1,4	1.93	8	19.09
1,5	4.24	5	0.14
1,6	4.33	1	2.56
1,7	1.66	1	0.26
1,8	1.11	0	1.11
2,2	2.02	7	12.28
2,3	8.07	0	8.07
2,4	2.42	3	0.14
2,5	5.30	6	0.09
2,7	2.07	2	0.00
2,8	1.38	0	1.38
3,3	8.06	12	1.93
3,4	4.84	0	4.84
3,5	10.59	5	2.95
3,6	10.82	20	7.79
3,7	4.14	12	14.92
3,8	2.77	2	0.21
4,4	7.25	3	2.49
4,5	3.18	0	3.18
4,6	3.25	1	1.56
4,7	1.24	2	0.47
4,8	0.83	1	0.03
5,5	3.48	11	16.25
5,6	7.11	0	7.11
5,7	2.73	1	1.10
5,8	1.82	1	0.37
2,6	5.41	10	3.89

Table 2. Initial Chi-square Calculations.(cont'd)

LDH 2			
Point Judith (cont.)			
Allele pair	Expected Freq.	Observed Freq.	Chi-square Value
6,6	3.63	7	3.13
6,7	2.78	0	2.78
6,8	1.86	1	0.40
7,7	0.53	0	0.53
7,8	0.71	0	0.71
8,8	0.24	1	2.41
	<u>153.19</u>	<u>152</u>	<u>250.57</u>
LDH 3			
Gloucester			
5,5	0.91	8	55.24
5,6	17.30	1	15.36
5,7	1.50	0	1.50
5,8	5.69	12	7.00
5,9	2.69	0	2.69
6,6	82.69	77	0.39
6,7	14.32	8	2.79
6,8	54.32	79	11.21
6,9	25.68	35	3.38
7,7	0.62	4	18.43
7,8	4.70	0	4.70
7,9	2.22	8	15.05
8,8	8.92	0	8.92
8,9	8.43	0	8.43
9,9	1.99	0	1.99
	<u>231.98</u>	<u>232</u>	<u>157.08</u>

Table 2. Initial Chi-square Calculations. (cont'd)

LDH 3 Point Judith			
Allele pairs	Expected Freq.	Observed Freq.	Chi-square Value
2,2	0.23	0	0.23
2,3	0.66	3	8.30
2,4	1.12	2	0.69
2,5	1.58	0	1.58
2,6	11.65	14	0.47
2,7	1.18	0	1.18
2,9	0.79	0	0.79
3,3	0.17	0	0.17
3,4	0.59	0	0.59
3,5	0.83	3	5.67
3,6	6.14	3	1.61
3,7	0.62	1	0.23
3,8	0.38	0	0.38
3,9	0.42	0	0.42
4,4	0.50	4	24.50
4,5	1.42	1	0.12
4,6	10.44	2	6.82
4,7	1.06	0	1.06
4,8	0.55	4	21.64
4,9	0.71	0	0.71
5,5	1.00	6	25.00
5,6	14.74	2	11.01
5,7	1.50	5	8.17
5,8	0.92	1	0.01
5,9	1.00	0	1.00
6,6	54.39	69	3.92
6,7	11.06	4	1.55
6,8	6.76	6	0.09
6,9	7.38	4	1.55
7,7	0.56	2	3.70
7,8	0.69	0	0.69
7,9	0.75	8	70.08
8,8	0.21	0	0.21
8,9	0.46	0	0.46
9,9	0.25	0	0.25
	<u>142.71</u>	<u>144</u>	<u>208.54</u>

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium

PHI-1

Gloucester

Allele	Frequency		
1	61		
2	320		
3	21		
4	9		
	<u>410</u>		

Allele pairs	Observed frequency	Expected frequency	Chi-square
1,1	21	4.5	59.9
1,2	16	47.5	20.9
1,3	1	3.1	1.4
1,4	2	1.3	0.3
2,2	139	124.6	1.7
2,3	18	16.4	0.2
2,4	7	7.0	0.0
3,3	1	0.5	0.4
3,4	0	0.5	0.5
4,4	0	0.1	0.1
	<u>205</u>	<u>205.5</u>	<u>85.4</u>

Point Judith

Allele	Frequency		
1	15		
2	231		
3	17		
4	13		
	<u>276</u>		

Allele pairs	Observed frequency	Expected frequency	Chi-square
1,1	5	0.4	51.7
1,2	4	12.6	5.8
1,3	1	0.9	0.0
1,4	0	0.7	0.7
2,2	106	96.7	0.9
2,3	3	14.2	8.9
2,4	12	10.9	0.1
3,3	6	0.5	57.3
3,4	1	0.8	0.0
4,4	0	0.3	0.3
	<u>138</u>	<u>138.0</u>	<u>125.8</u>

The alleles were lumped in the following manner: 1-4=1; 5=2; 6-7=3; and 8-10=4.

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium (cont'd)

PHI 2

Gloucester

Alleles	Frequency
1	23
2	242
3	40
4	39
5	8
	<u>352</u>

Allele Pairs	Observed frequency	Expected frequency	Chi-square
1,1	9	0.8	90.5
1,2	2	15.8	12.0
1,3	1	2.6	1.0
1,4	2	2.5	0.1
1,5	0	0.5	0.5
2,2	98	83.2	2.6
2,3	12	27.5	8.7
2,4	28	26.8	0.1
2,5	4	5.5	0.4
3,3	12	2.3	41.6
3,4	3	4.4	0.5
3,5	0	0.9	0.9
4,4	3	2.2	0.3
4,5	0	0.9	0.9
5,5	2	0.1	40.1
	<u>176</u>	<u>176.0</u>	<u>200.4</u>

groups lumped were: 0-1=1; 5-12=5

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium (cont'd)

PHI 2

Point Judith

Alleles	Frequency
1	7
2	174
3	34
4	23
5	28
	<u>266</u>

Allele Pairs	Observed frequency	Expected frequency	Chi-square
1,1	3	0.1	91.8
1,2	0	4.6	4.6
1,3	1	0.9	0.0
1,4	0	0.6	0.6
1,5	0	0.7	0.7
2,2	74	56.9	5.1
2,3	4	22.2	15.0
2,4	9	15.0	2.4
2,5	13	18.3	1.5
3,3	13	2.2	53.9
3,4	1	2.9	1.3
3,5	2	3.6	0.7
4,4	6	1.0	25.2
4,5	1	2.4	0.8
5,5	6	1.5	13.9
	<u>133</u>	<u>132.9</u>	<u>217.7</u>

groups lumped were: 0-1=1; 5-12=5

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium (cont'd)

LDH 1

Gloucester

Allele	Frequency
1	29
2	50
3	177
4	18
	<u>460</u>

Allele Pairs	Observed frequency	Expected frequency	Chi-square
1,1	3	1.2	2.8
1,2	8	3.5	5.7
1,3	17	26.5	3.4
1,4	2	0.6	2.8
2,2	19	2.6	102.9
2,3	3	39.3	33.5
2,4	0	1.0	1.0
3,3	173	148.0	4.2
3,4	3	7.2	2.4
4,4	3	0.1	96.3
	<u>230</u>	<u>230.0</u>	<u>255.2</u>

Groups lumped were: 1-4=1; 5-7=2; 8-9=3; 10-15=4

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium (cont'd)

LDH 1

Point Judith

Alleles	Frequency
1	29
2	26
3	177
4	<u>18</u>
	250

Allele Pair	Observed frequency	Expected frequency	Chi-square
1,1	0	1.7	1.7
1,2	0	3.0	3.0
1,3	28	20.5	2.7
1,4	1	2.1	0.6
2,2	5	1.4	9.8
2,3	4	18.4	11.3
2,4	10	1.9	35.3
3,3	69	62.7	0.6
3,4	7	12.7	2.6
4,4	<u>0</u>	<u>0.6</u>	<u>0.6</u>
	124	125.0	68.3

Groups lumped were: 1-4=1; 5-7=2; 8=9=3; 10-15=4

Table 3. Modified Calculations for Hardy-Weinberg Equilibrium (cont'd)

LDH 2

Gloucester

Allele	Frequency
1	32
2	170
3	118
4	54
5	68
	<u>442</u>

Point Judith

Allele	Frequency
1	55
2	105
3	67
4	47
5	30
	<u>304</u>

Allele Pair	Observed frequency	Expected frequency	Chi-square	Observed frequency	Expected frequency	Chi-square
1,1	9	1.2	53.1	11	5.0	7.3
1,2	5	12.3	4.3	7	19.0	7.6
1,3	9	8.5	0.0	19	12.1	3.9
1,4	0	3.9	3.9	1	8.5	6.6
1,5	0	4.9	4.9	6	5.4	0.1
2,2	38	32.7	0.9	19	18.1	0.0
2,3	43	45.4	0.1	19	23.1	0.7
2,4	27	20.8	1.9	30	16.2	11.7
2,5	19	26.2	2.0	16	10.4	3.1
3,3	22	15.8	2.5	14	7.4	5.9
3,4	2	14.4	10.7	1	10.4	8.5
3,5	20	18.2	0.2	4	6.6	1.0
4,4	12	3.3	23.0	7	3.6	3.1
4,5	1	8.3	6.4	1	4.6	2.9
5,5	15	5.2	18.2	1	1.5	0.2
	<u>221</u>	<u>221.1</u>	<u>132.1</u>	<u>152</u>	<u>151.9</u>	<u>62.5</u>

Table 4. Allele-pair Frequencies (raw data).

PHI 1	Gloucester	Point Judith	LDH 1	Gloucester	Point Judith
1,9	1	0	1,7	1	0
2,5	1	0	1,8	4	7
3,3	10	1	2,2	2	0
3,5	15	3	2,5	1	0
4,4	11	4	2,8	3	8
4,5	0	1	3,3	1	0
4,6	1	1	3,5	2	0
4,8	1	0	3,8	2	1
5,5	139	106	4,10	2	0
5,6	3	2	4,15	0	1
5,7	15	1	4,7	4	0
5,8	1	2	4,8	7	10
5,9	3	10	4,9	1	2
5,10	3	0	5,10	0	2
6,6	1	5	5,11	0	1
6,8	0	1	5,8	3	4
7,7	0	1	6,10	0	6
	<u>206</u>	<u>138</u>	6,6	0	3
			7,10	0	1
PHI 2			7,7	19	2
0,0	1	0	8,10	1	2
0,4	1	0	8,12	1	3
1,1	8	3	8,13	0	1
1,2	2	0	8,8	159	66
1,3	1	1	9,11	1	1
1,4	1	0	9,9	14	3
11,11	0	1	10,10	2	0
12,12	2	0		<u>230</u>	<u>124</u>
2,2	98	74			
2,3	12	4			
2,4	28	9			
2,5	1	1			
2,6	3	4			
2,7	0	5			
2,10	0	3			
3,3	12	13			
3,4	3	1			
3,5	0	2			
4,4	3	6			
5,5	0	4			
7,7	0	1			
4,10	0	1			
	<u>176</u>	<u>133</u>			

Table 4. Allele-pair Frequencies (raw data) (cont'd).

LDH 2	Gloucester	Point Judith	LDH 3	Gloucester	Point Judith
-1,-1	1	3	1,3	0	2
-1,4	1	0	2,3	0	1
-1,5	1	6	2,4	0	2
-1,8	0	1	2,6	0	14
-2,2	1	0	3,5	0	3
-3,3	0	3	3,6	0	3
0,0	3	3	3,7	0	1
0,3	2	1	4,4	0	4
0,8	0	4	4,5	0	1
1,1	5	5	4,6	0	2
1,2	1	0	4,8	1	4
1,3	1	3	5,5	8	6
1,4	6	8	5,6	1	2
1,5	1	5	5,7	0	5
1,6	0	1	5,8	11	1
1,7	0	1	6,10	0	3
2,2	18	7	6,14	0	1
2,4	10	3	6,6	77	69
2,5	26	6	6,7	8	4
2,6	10	10	6,8	79	6
2,7	0	2	6,9	35	4
3,3	20	12	7,10	1	0
3,4	2	0	7,7	4	2
3,5	5	5	7,9	7	4
3,6	17	20		<u>232</u>	<u>144</u>
3,7	15	12			
3,8	4	2			
4,10	1	0			
4,4	13	3			
4,6	2	1			
4,7	5	2			
4,8	8	1			
5,5	9	11			
5,7	1	1			
5,8	4	0			
5,9	1	1			
6,6	12	7			
6,7	1	0			
6,9	0	1			
7,10	1	0			
7,7	7	0			
8,8	5	1			
9,9	1	0			
	<u>221</u>	<u>152</u>			

Table 5. Allele Frequencies

Gloucester			Point Judith		
PHI 1	Frequencies	Percent	Frequencies	Percent	
1	1	0.24	0	0	
2	1	0.24	0	0	
3	35	8.52	5	1.81	
4	24	5.84	10	3.62	
5	320	77.86	231	83.70	
6	6	1.46	14	5.07	
7	15	3.65	3	1.09	
8	2	0.49	3	1.09	
9	4	0.97	10	3.62	
10	3	0.73	0	0	
	<u>410</u>		<u>276</u>		
PHI 2					
0	3	0.85	0	0	
1	20	5.68	7	2.63	
2	242	68.75	174	65.41	
3	40	11.36	34	12.78	
4	39	11.08	23	8.65	
5	1	0.28	11	4.14	
6	3	0.85	4	1.50	
7	0	0	7	2.63	
10	0	0	4	1.50	
11	0	0	2	0.75	
12	4	1.14	0	0	
	<u>352</u>		<u>266</u>		
LDH 1					
1	5	1.09	7	2.80	
2	8	1.74	8	3.20	
3	6	1.30	1	0.40	
4	14	3.04	13	5.20	
5	6	1.30	7	2.80	
6	0	0	12	4.80	
7	43	9.35	7	2.80	
8	339	73.70	168	67.20	
9	30	6.52	9	3.60	
10	7	1.52	11	4.40	
11	1	0.22	2	0.80	
12	1	0.22	3	1.20	
13	0	0	1	0.40	
15	0	0	1	0.40	
	<u>460</u>		<u>250</u>		

Table 5. Allele Frequencies (cont'd).

LDH 2	Gloucester		Point Judith	
	Frequencies	Percent	Frequencies	Percent
-3	0	0	3	0.99
-2	1	0.23	0	0
-1	4	0.90	13	4.28
0	8	1.81	11	3.62
1	19	4.30	28	9.21
2	84	19.00	35	11.52
3	86	19.46	70	23.03
4	61	13.80	21	6.91
5	57	12.90	46	15.13
6	54	12.22	47	15.46
7	37	8.37	18	5.92
8	26	5.88	10	3.29
9	3	0.68	2	0.66
10	2	0.45	0	0
	<u>442</u>		<u>304</u>	
LDH 3				
1	0	0	2	0.69
2	0	0	17	5.90
3	0	0	10	3.47
4	1	0.22	17	5.90
5	28	6.03	24	8.33
6	277	59.7	177	61.46
7	24	5.17	18	6.25
8	91	19.61	11	3.82
9	42	9.05	8	2.78
10	1	0.22	3	1.04
14	0	0	1	0.35
	<u>464</u>		<u>288</u>	

These frequencies were used to construct the expected frequencies of allele pairs to be tested with a Chi-square test for homogeneity. The values with brackets were combined in order to get an allele frequency of approximately 2% or more.

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Figure 1. Gels stained for LDH activity; the C locus or "eye band" is very faint; 3 of the 14 alleles at locus 1 and 5 of the 14 alleles at locus 2 are discernible.

Figure 2. Gels stained for PHI activity; 5 of the 10 alleles at locus 1 and 4 of the 11 alleles at locus 2 are visible

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Table 5. Allele Frequencies.

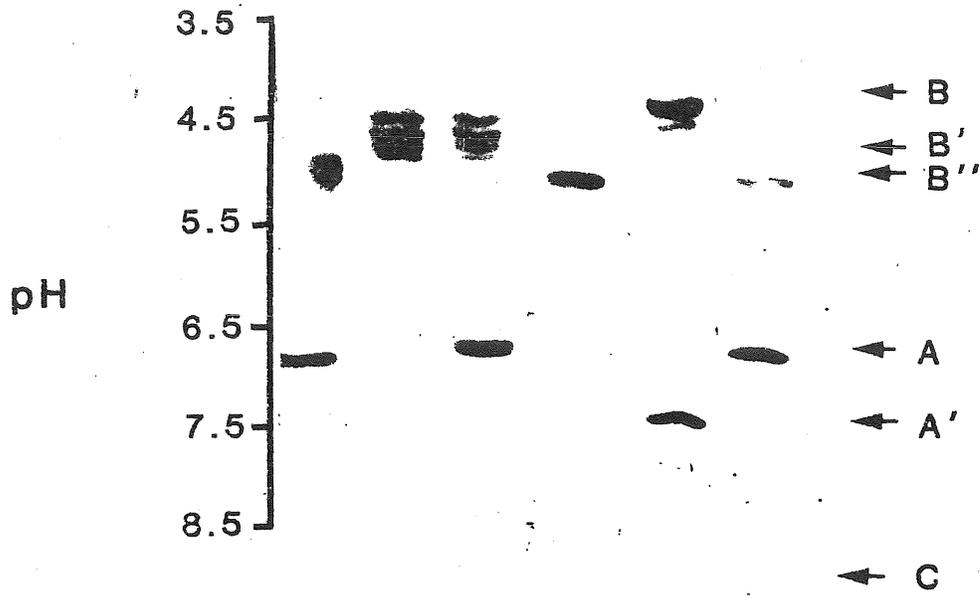


FIGURE 1

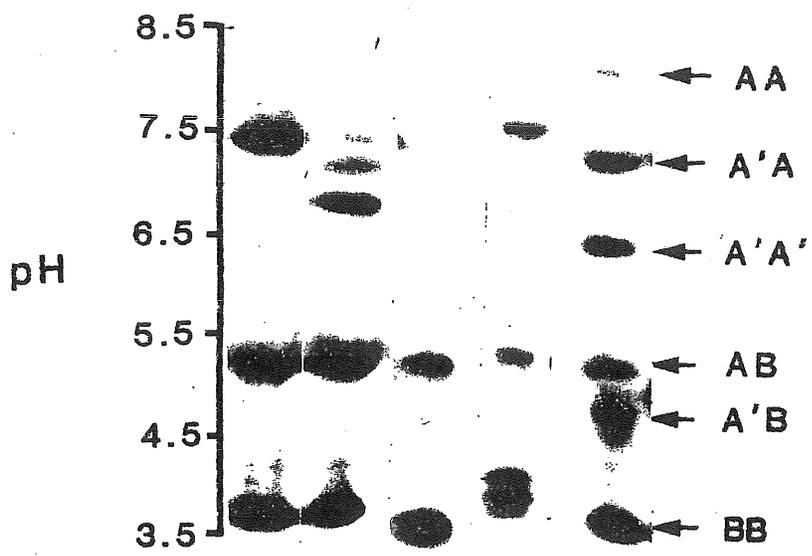


FIGURE 2