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Biochemical Genetics of Sablefish

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BIOCHEMICAL GENETICS OF SABLEFISH[1/]

by

A. J. Gharrett[2/], Margery A. Thomason[2/], and
Lisa N. Wishard[3/]

Resource Assessment and Conservation Engineering Division
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

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2/ University of Alaska-Juneau, 11120 Glacier Highway, Juneau, AK 99801.

3/ University of Washington, Seattle, WA 98195

ABSTRACT

Between the summer of 1978 and the summer of 1981, 37 collections of tissue samples of sablefish (*Anoplopoma fimbria*) were collected for biochemical genetic (electrophoretic) analysis. The collections included geographical locations from southern California to the end of the Aleutian Chain. An enormous amount of polymorphism was observed both with respect to the number of variants and to the frequency of variant alleles. Log-likelihood ratio analyses indicated significant genetic heterogeneity within regional groupings of collections and even more heterogeneity among regions. In addition, more heterogeneity was observed within geographical regions in the center of the range sampled than at the extremes of the range. The data indicated that the collections were probably taken from admixtures of various stocks, mixtures created by movements of the fish. The tremendous amount of genetic variation and the genetic heterogeneity are also consistent with the existence of a number of somewhat discrete populations between which some gene flow exists.

INTRODUCTION

Sablefish (*Anoplopoma fimbria*) are distributed along the offshore waters of the eastern Pacific Ocean and Gulf of Alaska from Baja California through the Aleutian Islands as well as in the Bering Sea and along the western Pacific Ocean along the Kamchatka Peninsula through Japan. Effective management of a commercially valuable species such as the sablefish requires information regarding the stock structure in order to maximize potential production. Such information is not presently available, but it is difficult to believe that a species distributed over such a vast geographical range would not have some substructure.

One traditional means for obtaining information on stock structures is the analysis of tag and recovery data. Such data may eventually be sufficient for describing the stock structure of sablefish, but at this time these data appear adequate only for examining some migration patterns (Bracken, 1982). Another technique that may be used to examine stock structure depends on genetic differences that are often observed among genetically isolated populations as a result of genetic drift. One category of genetically determined traits that are useful for this kind of approach is enzymatic activities which display genetic variability that can be resolved through the technique of starch gel electrophoresis. Such biochemical genetic data has often been shown to be useful for stock identification and separation problems involving fish. (See e.g., Grant et al. 1980; Milner et al. 1981).

In this report an attempt is made to examine the structure of eastern Pacific Ocean sablefish stocks by using biochemical genetic data obtained from specimens collected throughout the North American range. Because most of the collections of specimens were not made from spawning populations, it is quite possible that many collections may actually represent admixtures of several stocks. This possibility is especially important to consider when examining the results.

MATERIALS AND METHODS

Between the summer of 1978 and the fall of 1980 thirty-five collections of sablefish samples were made at geographical locations along the continental shelf from southern California to near the western end of the Aleutian Chain, from five seamounts in the Gulf of Alaska, and from the Bering Sea. In addition, two collections were made from the inside waters of southeastern Alaska by The Northwest and Alaska Fisheries Center (NWAFC) and by the investigators during the summer of 1981 (Figure 1 and Table 1).

Two different laboratories, those of Gharrett and of Wishard, have been involved in examining the biochemical genetic composition of these collections. Standard techniques

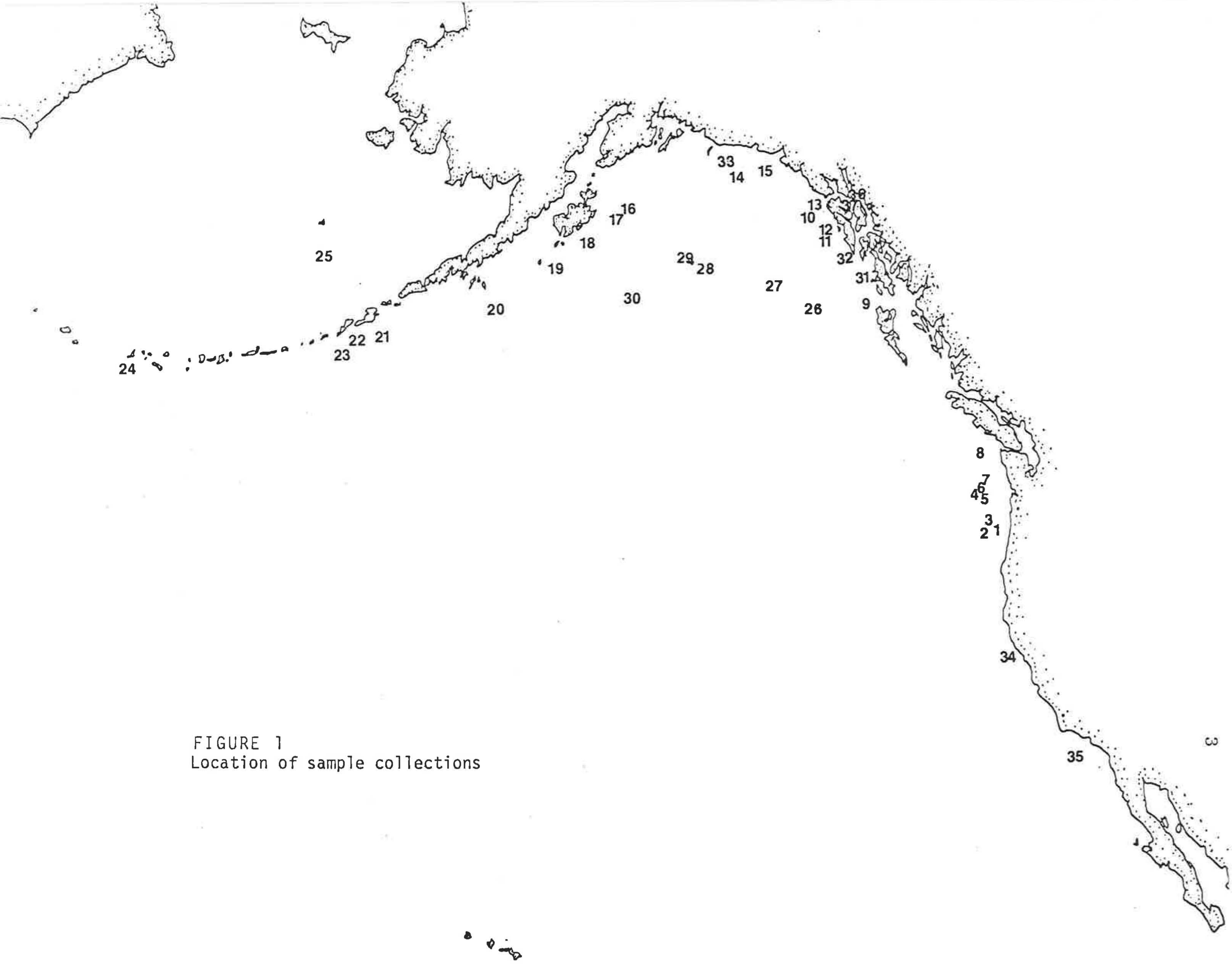

FIGURE 1
Location of sample collections

TABLE 1

Approximate Locations and Times of Collection

	Lat.	Long.	Date	Site	Grouping Designation
1.	45°18'N	124°33'W	summer 1979	Cape Lookout	Pacific Northwest
2.	45°20'N	124°46'W	summer 1979	" "	" "
3.	45°20'N	124°46'W	summer 1979	" "	" "
4.	46°46'N	124°54'W	summer 1979	Willapa Bay	" "
5.	46°45'N	124°57'W	summer 1979	" "	" "
6.	46°46'N	124°54'W	summer 1979	" "	" "
*7.	47°17'N	124°49'W	spring 1979	Point Grenville	" "
*8.	48°13'N	125°01'W	spring 1979	Cape Flattery	" "
*9.	54°31'N	133°57'W	summer 1978	Dixon Entrance	S.E. Alaska
10.	57°46'N	136°57'W	summer 1978	Cross Sound	" "
*11.	57°14'N	136°15'W	summer 1978	Kruzof Island	" "
*12.	57°14'N	136°16'W	summer 1978	" "	" "
*13.	57°55'N	136°54'W	summer 1978	Cross Sound	" "
*14.	59°15'N	141°54'W	summer 1978	Yakutat Bay	" "
*15.	59°28'N	140°27'W	summer 1978	" "	" "
*16.	57°48'N	149°37'W	summer 1978	Afognath Island	Alaska Penninsula
17.	57°35'N	149°55'W	summer 1980	" "	" "
*18.	57°02'N	152°17'W	summer 1978	Kodiak Island	" "
*19.	55°59'N	154°53'W	summer 1978	Chirikof Island	" "
*20.	54°29'N	158°43'W	summer 1978	Shumagin Islands	" "
*21.	53°28'N	165°57'W	summer 1978	Unalaska Island	Aleutian Islands
22.	52°58'N	168°00'W	summer 1980	Umnak Island	" "
23.	52°30'N	169°30'W	summer 1980	" "	" "
24.	51°46'N	177°05'E	summer 1980	Kiska Island	" "
25.	56°03'N	170°19'W	summer 1980	St. George Island	Bering Sea
26.	54°33'N	136°55'W	summer 1979	Dickins Seamount	Seamounts
27.	55°07'N	140°20'W	summer 1979	Walker Semount	"
28.	56°04'N	144°40'W	summer 1979	Surveyor Seamount	"
29.	56°17'N	145°13'W	summer 1979	Quinn Seamount	"
30.	54°38'N	150°32'W	summer 1979	Patton Seamount	"
31.	55°25'N	135°00'W	summer 1978	Dixon Entrance	Addt'l S.E. Alaska
32.	56°05'N	135°36'W	summer 1978	Cape Ommaney	" " "
33.	59°35'N	142°50'W	summer 1978	Yakutat	" " "
34.	38°11'N	123°31'W	fall 1980	Bodega Head	California
35.	32°43'N	119°38'W	fall 1980	Patton Escarpment	California
36.	58°24'N	134°38'W	summer 1981	Auke Bay	S.E. Alaska
37.	58°17'N	134°55'W	summer 1981	Funter Bay	S.E. Alaska

* Data from Wishard's Laboratory. All other from Gharrett's Laboratory.

for starch gel electrophoresis were employed (see *e.g.*, May 1980). The enzymatic activities were stained according to Harris and Hopkinson (1976). Banding patterns, which represent gene products, were designated by their relative mobilities. The most common form (allele) is usually expressed as 100. The mobilities of other alleles at a locus are expressed relative to this value. When more than one locus expressed a particular enzymatic activity, the loci were designated consecutively by Arabic numbers starting with the least anodal (Allendorf and Utter 1979). Because it was not possible to obtain breeding data to confirm our genetic interpretations of banding patterns, we adopted the following guidelines for accepting a banding system as one useful for our analysis: 1) The banding patterns of a particular enzymatic activity must be consistent with a molecular model observed for other species of fish; 2) A particular activity observed in more than one tissue of an individual must display the variants of the same mobility; 3) Data for a particular activity must not consistently show a surplus of heterozygous types in excess of Hardy Weinberg equilibrium expectations.

The data were expressed as allelic frequencies and analyzed using log-likelihood ratio analysis. Sokal and Rohlf (1969) recommend that expected frequencies less than 5 be avoided for this analysis; therefore, only loci whose most common allele was present at frequencies less than 0.95

were used in the analysis. In addition frequencies of less common alleles were pooled to avoid expected frequencies less than 5. When such pooling was done, an effort was made to maximize the number of classes for each locus.

RESULTS

Genetic Variability

Of the enzymatic activities examined, thirteen proved reliable and are considered in this report. Other activities were not included 1) because interpretation or resolution of banding patterns was not possible, 2) because too little enzymatic activity was present, or 3) because the samples from which data was obtained were too few.

An extraordinary amount of polymorphism was observed (see Appendix). Eighteen different loci were resolved from the thirteen enzymatic activities and all loci displayed some degree of polymorphism. For only two loci were as few as two alleles observed while five loci had five or more alleles. The polymorphism was also reflected in the allelic frequencies. At eleven of the eighteen loci, the less common alleles comprised at least five percent of the total observed. This means that eleven loci could be analyzed statistically (Table 2).

The large number of alleles observed made interpretation

TABLE 2

Electrophoretic Loci Examined

Loci in which little detectable variability (allelic frequency of common allele ⩾.95) exists.

Enzyme	E.C. number	Designation	buffer* system	Tissue
Alphaglycerophosphate dehydrogenase	1.1.1.8	AGP(L)	2	liver
Isocitrate dehydrogenase	1.1.1.42	IDH-2	4	muscle
Lactate dehydrogenase	1.1.1.27	LDH	1	muscle
Malate dehydrogenase	1.1.1.37	MDH-1	2	muscle
Malate dehydrogenase	1.1.1.37	MDH-2	2	muscle
Phosphoglucose isomerase	5.3.1.9	PGI-1	1	muscle
Superoxide dismutase	1.15.1.1	SOD(M)	1	muscle

Loci in which variability (allelic frequency of common allele ⩽.95) exists.

Adenosine deaminase	3.5.4.4	ADA	4	muscle
Alcohol dehydrogenase	1.1.1.1	ADH	2	liver
Creatine kinase	2.7.3.2	CK	3	muscle
Glutamate Oxaloacetate transaminase	2.6.1.1	GOT-2	5	liver
Glutamate Pyruvate transaminase	2.6.1.2	GPT-2	3	liver
Phosphoglucose Isomerase	5.3.1.9	PHI-2	2	muscle
Phosphoglucomutase	2.7.5.1	PGM-1	1	muscle
6-Phosphogluconate dehydrogenase	1.1.1.44	6PG-2	both 2 & 4	muscle
Phosphomannose isomerase	3.2.1.24	PMI	3	muscle
Sorbitol dehydrogenase	1.1.1.14	SDH	1	liver
Superoxide dismutase	1.15.1.1	SOD(L)	2	liver

* 1. Ridgway et al. (1970)
2. Clayton and Tretiak (1972)
3. Markert and Faulhaber (1965)
4. Shaw and Prasad (1970) (pH 7.0 tris-citrate)
5. Clayton and Tretiak (1972) adjusted to pH 6.7

of banding patterns, enumeration and identification of particular alleles, and subsequent comparisons of results obtained by the two laboratories somewhat difficult. Samples were exchanged between our laboratories to standardize scoring practices. In addition, when frequencies of less common alleles were pooled to avoid expected frequencies less than five, an effort was made to pool frequencies of alleles possessing similar mobilities while still maximizing the number of classes at each locus (Table 3). This practice should nullify many scoring errors that may have resulted from difficulties in resolving alleles possessing slightly different mobilities as well as those that resulted from different scoring practices. An examination of heterogeneity between data observed at each of the two laboratories was made on a locus by locus basis. No significant ($P>.10$) differences were observed at ten of the eleven loci. PMI-2 showed a significant difference ($P<.01$). Because the two laboratories examined different collections not necessarily representing the same geographical locations, it was not necessary that the data be homogeneous. That they were homogeneous, however, suggests that the two laboratories were indeed interpreting the data in a uniform way.

Subsequent analyses were performed both with and without the PMI-2 data as well as for each laboratory independently.

TABLE 3: Description of pooling of alleles at each locus used for log-likelihood ratio test. Acronyms for loci are described in Table 2, numbers represent the relative mobilities of alleles.

	Pool 1	Pool 2	Pool 3	Pool 4
ADA	100	75	90, 60, 50, 150, 115, 105	-
ADH	100, 105, 120	50	30, 20, 10, 0	-
CK	100	85	-	-
GOT	100	115, 80, 70	-	-
GPT-2	100	80, 60	-	-
PGI-2	100	110, 95, 80	-	-
PGM	100	125, 140, 160	-	-
PMI-2	100	120, 90, 80	-	-
6PG-2	100	140, 120, 110, 85, 70	-	-
SDH	100	75	150, 120, 30	-
SOD(L)	100	130	140, 150	90

None of these different treatments of the data produced substantially different results; therefore, results presented below include all data from both laboratories and data from the PMI-2 locus.

Analysis of Genetic Variation

The collections were grouped and designated according to the geographical region from which they were taken. The groupings are California, the Pacific Northwest, Dixon Entrance, Southeast Alaska, the Alaskan Peninsula, Aleutian Islands, Bering Sea, and five seamounts in the middle of the Gulf of Alaska. The Dixon Entrance collections were kept separate because preliminary data analyses on incomplete data sets indicated the possibility of differences between northern and southern collections and the Dixon Entrance collections were geographically between the groups.

The Southeast Alaska data included two collections from inside waters. One of these collections was made up of adults, the other of young fish (approximately 10 inches long) which were presumably the same year class and possibly of the same brood. These collections were made to examine the possibility that stocks found in the inside waters of Southeast Alaska were genetically discrete from outside stocks. When these two collections were removed from the analysis of Southeast Alaska collections, the amount of heterogeneity among collections was decreased somewhat, but statistically significant heterogeneity did still remain. These results indicate that the collections from the inside waters are no more "unique" than is each collection from the outside waters in the region. This suggests that the genetic structure of Southeast Alaska stocks is not so simple as an "inside" and "outside" stock model.

Comparisons of the Bering Sea collection to those collected from the Aleutian Islands reveal no significant heterogeneity between those two regions. There is, therefore, no basis for assuming that more than one genetically identifiable stock exists in these regions.

Log-likelihood ratio analyses were made one locus at a time, first within regions and then among regions. Table 4 shows the total heterogeneity both within regions (G_W) and among regions (G_A) across all loci used. The total heterogeneity (G_T) is significant ($P<.001$) as is the heterogeneity existing both within regions ($P<.001$) and among the regions ($P<.001$) It is interesting to note that the more polymorphic loci (SOD(L), ADH and SDH) contributed most substantially to the heterogeneity (Table 5). F tests indicate that there is more heterogeneity among regions than there is on the average within regions ($P<.012$). Partitioning the data into even larger geographical regions did not successfully account for this relatively larger heterogeneity. For example there does not appear to be a systematic difference between collections

TABLE 4

Summary for log-likelihood ratio tests for all geographical areas

Degrees of freedom are in parentheses

	All data
California	7.651(15)
Pacific NW	118.727*(89)
Dixon Entrance	3.329(2)
S.E. Alaska	138.486**(94)
Sea mounts	74.654*(54)
Ak Peninsula	85.138**(49)
Aleutians	39.760(42)
Bering Sea	-

G_W 467.745***(345)

G_A 200.602***(105)

G_T 668.346***(450)

$F_{A,W}$ = 1.409(105,345) $P < .012$

* $P < .05$
** $P < .01$
*** $P < .001$

TABLE 5

Table of log-liklihood ratio tests for all loci possessing a common allele with an average frequency less than 0.95. Other alleles were pooled to create classes with frequencies greater than or equal to 0.05. Degrees of freedom are in parentheses.

	PGI-2		CK		6PG-2		PGM		ADA	
	N	G_W	N	G_W	N	G_W	N	G_W	N	G_W
California	203	.009(1)	203	.063(1)	203	.068(1)	203	1.507(1)	203	1.009(2)
Pacific NW	248	12.191(7)	248	9.601(7)	226	10.898(7)	248	3.008(7)	199	5.445(10)
Dixon Entrance	59	-	59	-	102	.017(1)	163	3.312(1)	-	-
S.E. Alaska	521	3.804(7)	516	11.941(7)	483	14.668(9)	662	14.756(9)	281	1.919(4)
Sea mounts	241	3.476(4)	217	1.211(4)	83	.346(1)	241	10.562*(4)	241	5.629(8)
Ak Peninsula	254	.266(4)	254	5.871(4)	251	.768(4)	254	5.007(4)	100	-
Aleutians	268	2.429(3)	268	.481(3)	268	.910(3)	268	8.582*(3)	208	2.876(4)
Bering Sea	102	- -	103	-	103	-	100	-	103	-
	1896		1868		1719		2139		1335	
	G_W=22.175(26		G_W=29.168(26		G_W=27.675(26)		G_W=46.734*(29)		G_W=16.877(28)	
	G_A=10.500(7)		G_A=12.097(7)		G_A=14.053(7)		G_A=14.303*(7)		G_A=19.719(12)	
	G_T=32.675(33)		G_T=41.265(33)		G_T=41.728(33)		G_T=61.037**(36)		G_T=36.596(40)	

	SOD(L)		SDH		ADH		GOT		GPT	
	N	G_W	N	G_W	N	G_W	N	G_W	N	G_W
California	202	2.938(3)	202	1.933(2)	203	.035(2)	203	.044(1)	-	-
Pacific N.W.	248	26.403(21)	241	23.923*(14)	245	25.773*(4)	49	.775(1)	-	-
Dixon Entrance	59	-	59	-	59	-	-	-	-	-
S.E. Alaska	519	28.466(21)	476	16.189(12)	491	24.538*(14)	176	7.609*(2)	144	.460(2)
Sea mounts	239	19.723(12)	240	11.537(8)	234	13.469(8)	-	-	44	3.934*(1)
Ak Peninsula	254	24.066(12)	238	13.803*(6)	254	13.748(8)	100	-	154	5.570(3)
Aleutians	268	6.963(9)	267	7.503(6)	268	6.332(6)	203	2.321(2)	60	-
Bering Sea	103	-	103	-	103	-	103	-	40	-
	1892		1826		1857		834		442	
	G_W=108.559*(78)		G_W=74.888**(48)		G_W=83.895**(52)		G_W=10.749(6)		G_W=9.964(6)	
	G_A=45.685**(21)		G_A=26.785*(14)		G_A=35.336**(14)		G_A= 9.599(5)		G_A=3.020(4)	
	G_T=154.244**(99)		G_T=101.673**(62)		G_T=119.231**(66)		G_T=20.348*(11)		G_T=12.984(10)	

TABLE 5 continued

	PMI-2	
	N	G_W
California	203	.045(1)
Pacific NW	49	.710(1)
Dixon Entrance	-	-
S.E. Alaska	520	14.136*(7)
Sea mounts	241	4.767(4)
Ak Peninsula	254	16.039**(4)
Aleutians	268	1.363(3)
Bering Sea	103	-
	1697	

G_W=37.060* (20)

G_A= 9.505(7)

G_T=46.565(27)

* P <.05

** P <.01

** P <.001

made north of Dixon Entrance and collections made south of Dixon Entrance.

It is important to realize that none of the collections were made on spawning populations. The heterogeneity observed within geographical regions is consistent with the idea that collections represent mixtures of various genetically distinct populations. Observations of more heterogeneity among geographical regions than within suggests some degree of regional integrity. Also supporting this interpretation is the observation that toward the ends of the geographical range sampled, less heterogeneity exists within each region than in the middle of the range. This kind of pattern would be expected if southern California at one end and the Aleutian Islands/Bering Sea areas at the other end were the limits of eastern Pacific stocks and the fish were relatively mobile. In this kind of model, the collections from the center of the range, i.e., Southeast Alaska and the Alaskan Peninsula, would more likely represent mixtures of a wider variety of stocks and, therefore, demonstrate more within-region heterogeneity.

DISCUSSION

The data analyzed are consistent with a model in which somewhat discrete breeding stocks of sablefish exist throughout the range from southern California to the Aleutian Islands. This model is supported by the observation that more heterogeneity exists among different geographical regions throughout this range than within these regions. Because breeding populations were not sampled, observations of greater heterogeneity toward the center of the sampled range than at the ends suggest that the collections from the center of the range represent admixtures of more breeding stocks than do collections near the ends of the range sampled. The relative similarity of allelic frequencies and the large amount of genetic variation suggests enormous effective population sizes and/or some degree of gene flow.

It is not possible in the absence of data on spawning populations to determine the numbers of stocks involved or the relative discreteness of stocks. To establish that Aleutian samples do represent one of the ends of the range, it would be necessary to examine sablefish from the Western Pacific.

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APPENDIX. Allelic frequencies of Sablefish samples, Abbreviations are explained in Tables 1 and 2

	SOD(M)			PHI-1					MDH-2				MDH-1			
	N	1.00	1.500	N	1.00	1.80	.80	.20	M	1.00	1.15	.90	N	1.00	1.10	.90
1	22	.977	.023	22	.955	.045	0	0	22	.977	0	.023	22	.955	0	.045
2	46	1.00	0	46	1.00	0	0	0	46	1.00	0	0	46	.978	0	.022
3	31	1.00	0	31	.984	0	.016	0	31	.984	0	.016	31	1.00	0	0
4	44	1.00	0	44	1.00	0	0	0	44	1.00	0	0	44	1.00	0	0
5	26	.981	.019	26	1.00	0	0	0	26	1.00	0	0	26	1.00	0	0
6	30	1.00	0	30	1.00	0	0	0	30	1.00	0	0	30	1.00	0	0
7	31	1.00	0	31	1.00	0	0	0	31	1.00	0	0	31	.983	0	.017
8	18	1.00	0	18	1.00	0	0	0	18	1.00	0	0	18	1.00	0	0
9	59	1.00	0	59	.991	.008	0	0	59	1.00	0	0	59	1.00	0	0
10	152	1.00	0	152	.997	0	.003	0	152	.997	.003	0	152	.993	.007	0
11	49	1.00	0	49	.989	.010	0	0	49	.979	0	.021	49	1.00	0	0
12	50	.990	.010	50	1.00	0	0	0	50	1.00	0	0	50	1.00	0	0
13	48	1.00	0	48	.980	.010	.010	0	48	1.00	0	0	48	1.00	0	0
14	50	.990	.010	50	1.00	0	0	0	50	.990	0	.010	50	1.00	0	0
15	45	1.00	0	45	1.00	0	0	0	45	.988	0	.012	45	1.00	0	0
16	60	.991	.009	60	1.00	0	0	0	60	.991	0	.009	60	1.00	0	0
17	100	1.00	0	100	.995	.005	0	0	100	1.00	0	0	100	.995	0	.005
18	16	1.00	0	16	1.00	0	0	0	16	1.00	0	0	16	1.00	0	0
19	50	1.00	0	50	1.00	0	0	0	50	.980	0	.020	50	1.00	0	0
20	28	1.00	0	28	1.00	0	0	0	28	.982	0	.018	28	1.00	0	0
21	60	1.00	0	60	.966	.008	.016	.008	60	.975	0	.025	60	1.00	0	0
22	40	.988	.012	40	.975	.025	0	0	40	.988	0	.012	40	1.00	0	0
23	71	1.00	0	71	.979	.007	.007	.007	71	.979	.014	.007	71	1.00	0	0
24	97	.995	.005	97	.990	.010	0	0	97	.995	.005	0	97	1.00	0	0
25	103	1.00	0	103	.985	.010	.005	0	102	.995	0	.005	102	.995	0	.005
26	44	.989	.011	44	1.00	0	0	0	44	1.00	0	0	44	1.00	0	0
27	29	1.00	0	29	1.00	0	0	0	29	1.00	0	0	29	1.00	0	0
28	67	1.00	0	67	.993	0	.007	0	67	.993	.007	0	67	1.00	0	0
29	67	.993	.007	67	1.00	0	0	0	67	1.00	0	0	67	1.00	0	0
30	34	1.00	0	34	.985	.015	0	0	33	1.00	0	0	34	1.00	0	0
31	0	—	—	0	—	—	—	—	0	—	—	—	0	—	—	—
32	0	—	—	0	—	—	—	—	0	—	—	—	0	—	—	—
33	0	—	—	0	—	—	—	—	0	—	—	—	0	—	—	—
34	97	.995	.005	97	.995	0	0	.005	97	.990	0	.010	97	.995	0	.005
35	106	1.00	0	106	.981	.014	0	.047	106	.991	0	.009	106	.981	.009	.009
36	78	1.00	0	78	1.00	0	0	0	78	1.00	0	0	78	.994	0	.006
37	50	1.00	0	50	1.00	0	0	0	50	1.00	0	0	50	1.00	0	0

APPENDIX. Continued

	IDH-2					LDH				AGP(L)			
	N	1.00	.95	1.2	.50	N	1.00	1.20	.9	N	1.00	.80	1.1
1	22	1.00	0	0	0	22	1.00	0	0	22	1.00	0	0
2	46	1.00	0	0	0	46	.989	0	.011	46	.989	.011	0
3	31	1.00	0	0	0	31	1.00	0	0	31	1.00	0	0
4	44	.989	0	.011	0	44	.989	.011	0	44	1.00	0	0
5	26	1.00	0	0	0	26	1.00	0	0	26	1.00	0	0
6	30	1.00	0	0	0	30	1.00	0	0	30	1.00	0	0
7	31	1.00	0	0	0	31	1.00	0	0	31	1.00	0	0
8	18	.972	0	.027	0	18	1.00	0	0	18	1.00	0	0
9	59	.991	0	0	.009	59	1.00	0	0	59	1.00	0	0
10	0	—	—	—	—	152	1.00	0	0	78	1.00	0	0
11	49	1.00	0	0	0	49	.979	.021	0	49	1.00	0	0
12	50	.990	0	.010	0	50	1.00	0	0	50	.990	.010	0
13	48	1.00	0	0	0	48	1.00	0	0	48	1.00	0	0
14	50	.990	.010	0	0	50	1.00	0	0	50	.990	.010	0
15	45	.988	.012	0	0	45	1.00	0	0	45	1.00	0	0
16	60	.983	.010	0	0	60	1.00	0	0	60	.991	.009	0
17	100	.995	.005	0	0	100	1.00	0	0	100	.995	.005	0
18	16	1.00	0	0	0	16	1.00	0	0	16	1.00	0	0
19	50	.980	.020	0	0	50	1.00	0	0	50	1.00	0	0
20	28	1.00	0	0	0	28	1.00	0	0	28	1.00	0	0
21	60	.975	.008	.016	0	60	1.00	0	0	60	1.00	0	0
22	40	1.00	0	0	0	40	1.00	0	0	40	1.00	0	0
23	71	1.00	0	0	0	71	1.00	0	0	71	.993	0	.007
24	97	1.00	0	0	0	97	1.00	0	0	97	1.00	0	0
25	102	1.00	0	0	0	103	1.00	0	0	103	.990	.010	0
26	44	.989	0	.011	0	44	1.00	0	0	44	1.00	0	0
27	27	1.00	0	0	0	29	1.00	0	0	29	1.00	0	0
28	67	.992	.008	0	0	50	1.00	0	0	50	1.00	0	0
29	67	1.00	0	0	0	67	1.00	0	0	67	1.00	0	0
30	34	1.00	0	0	0	33	1.00	0	0	34	.970	.015	.015
31	0	—	—	—	—	0	—	—	—	0	—	—	—
32	0	—	—	—	—	0	—	—	—	0	—	—	—
33	0	—	—	—	—	0	—	—	—	0	—	—	—
34	97	1.00	0	0	0	97	1.00	0	0	97	1.00	0	0
35	106	1.00	0	0	0	106	.995	0	.005	106	.986	.005	.009
36	78	1.00	0	0	0	75	1.00	0	0	78	1.00	0	0
37	50	1.00	0	0	0	50	1.00	0	0	50	1.00	0	0

APPENDIX. Continued

	PGM-1					CK			ADH								
	N	1.00	1.25	1.40	1.60	N	1.00	.85	N	1.00	1.05	1.20	.50	.30	.20	.10	0
1	22	.614	.386	0	0	22	.727	.273	22	.455	.045	.045	.205	.227	.023	0	0
2	46	.717	.283	0	0	46	.652	.348	46	.326	.076	.065	.163	.304	.054	.011	0
3	31	.742	.242	.016	0	31	.581	.419	30	.417	0	.017	.250	.283	.033	0	0
4	44	.727	.273	0	0	44	.591	.409	44	.443	.068		.125	.364	0	0	0
5	26	.731	.269	0	0	26	.615	.385	26	.442	.135		.173	.250	0	0	0
6	30	.717	.283	0	0	30	.550	.450	28	.429	.071		.214	.268	.018	0	0
7	31	.666	.333	0	0	31	.759	.241	31		.387	.096	.048	.467	0	0	0
8	18	.718	.281	0	0	18	.648	.352	18		.333	0	.111	.555	0	0	0
9	59	.754	.245	0	0	59	.606	.394	59		.387	.017	.206	.370	0	.008	.008
10	168	.690	.307	.003	0	146	.692	.308	127	.437	.035	.031	.185	.276	.035	0	0
11	49	.693	.306	0	0	49	551	.449	49		.420	.040	.290	.240	0	.010	0
12	50	.673	.326	0	0	50	.610	.390	50		.370	.030	.280	.320	0	0	0
13	48	.700	.300	0	0	48	.628	.372	48		.521	.074	.202	.202	0	0	0
14	50	.600	.400	0	0	50	.643	.357	50		.447	.083	.218	.250	0	0	0
15	45	.677	.322	0	0	45	.535	.465	45		.454	.011	.181	.340	0	.011	0
16	60	.694	.305	0	0	60	.560	.440	60		.372	.067	.262	.288	0	.008	0
17	100	.770	.230	0	0	100	.630	.370	100	.375	.015	.030	.205	.370	.005	0	0
18	16	.843	.156	0	0	16	.567	.433	16		.531	0	.093	.312	0	.031	.031
19	50	.740	.240	.010	.010	50	.680	.320	50		.380	.020	.330	.270	0	0	0
20	28	.685	.314	0	0	28	.518	.482	28		.481	.037	.222	.240	.018	0	0
21	60	.728	.271	0	0	60	.632	.368	60		.450	.050	.241	.241	.008	.008	0
22	40	.638	.362	0	0	40	.662	.338	40	.312	.038	.038	.188	.412	.012	0	0
23	71	.599	.401	0	0	71	669	.331	71	.373	.063	.035	.218	.275	.028	.007	0
24	97	.732	.268	0	0	97	.665	.335	97	.356	.036	.046	.217	.345	0	0	0
25	102	.657	.343	0	0	103	.603	.397	103	340	.010	.034	.286	.311	.015	.005	0
26	44	.602	.398	0	0	44	.648	.352	44	.443	.023	.080	.227	.216	.011	0	0
27	29	.724	.276	0	0	29	.707	.293	28	.232	.036	.054	.214	.411	.054	0	0
28	67	.672	.328	0	0	46	.652	.348	61	352	.074	.041	.213	.279	.041	0	0
29	67	.582	.418	0	0	67	.687	.313	67	.299	067	.045	.239	.291	.052	.007	0
30	34	.779	.221	0	0	31	.710	.290	34	.456	.044	.029	.147	.279	.044	0	0
31	104	.659	.341	0	0	0	—	—	0	—	—	—	—	—	—	—	—
32	97	.732	.268	0	0	0	—	—	0	—	—	—	—	—	—	—	—
33	27	.592	.408	0	0	0	—	—	0	—	—	—	—	—	—	—	—
34	97	.680	.320	0	0	97	.577	.423	97	.397	.062		.258	.284	0	0	0
35	106	.736	.264	0	0	106	.590	.410	106	.392	.071		.245	.292	0	0	0
36	78	.654	.346	0	0	78	.609	.391	72	.410	.028	.056	.312	.194	0	0	0
37	50	.550	.440	.010	0	50	.590	.410	50	.410	.040	.020	.240	.290	0	0	0

APPENDIX. Continued

	SDH						ADA								
	N	1.00	.75	.30	1.50	1.20	N	1.00	.75	.90	.60	.50	1.05	1.15	1.5
1	22	.705	.137	.068		.091	22	.682	.227	.045	.023	0	0		.023
2	42	.452	.297	.095		.155	46	.620	.272	.033	.043	0	0		.033
3	30	.583	.233	.067		.117	31	.613	.290	.048	.032	0	0		.016
4	44	.580	.273	.091		.057	44	.614	.318	.011	.034	0	0		.023
5	26	.577	.288	.077		.058	26	.731	.192	.019	.058	0	0	0	0
6	28	.750	.179	.018		.054	30	.617	.250	.067	.033	0	0		.033
7	31	.620	.310	.034	0	.034	0	-	-	-	-	-	-	-	-
8	18	.625	.218	.125	0	.034	0	-	-	-	-	-	-	-	-
9	59	.628	.280	.012	0	.024	0	-	-	-	-	-	-	-	-
10	155	.581	284	.090		045	153	.575	.327	.023	.046	0	0	.023	.007
11	49	.675	.225	.025	0	.075	0	-	-	-	-	-	-	-	-
12	50	.555	.311	.077	0	.055	0	-	-	-	-	-	-	-	-
13	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-
14	50	.622	.244	.088	0	.044	0	-	-	-	-	-	-	-	-
15	45	.639	.314	.023	.011	.011	0	-	-	-	-	-	-	-	-
16	60	.648	.287	.021	0	.042	0	-	-	-	-	-	-	-	-
17	100	.615	.300	.080		.005	100	.650	.305	.015	.020	0	0		.010
18	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-
19	50	.563	.276	.106	.010	.042	0	-	-	-	-	-	-	-	-
20	28	.795	.159	0	.022	.022	0	-	-	-	-	-	-	-	-
21	60	.654	.181	.100	0	0	0	-	-	-	-	-	-	-	-
22	40	.650	.250	.062	.031	.055	40	.600	.338	.050	.012	0	0	0	0
23	71	.641	.239	.063	.049	.007	71	.549	.338	.056	.035	0	0	.021	0
24	96	.656	266	042	.037	0	97	.572	.361	.036	.010	.005	0		.016
25	103	.616	.311	.048	.015	.010	103	.558	.311	029	078	0	0	.015	.010
26	56	.545	.304	.071	.018	.062	44	.523	.398	.023	.034	0	0		.023
27	25	.520	.340	.040	0	.100	29	.672	.276	.034	.017	0	0	0	0
28	62	.653	.258	.016	.016	.056	67	.560	.328	.052	.045	0	0		.015
29	66	.568	.258	.061	.030	.083	67	.575	.343	.052	.015	.007	0		.007
30	31	.710	.226	.016	0	.048	34	.618	.294	.029	.044	0	0		.015
31	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-
32	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-
33	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-
34	97	.650	.289	.057		.005	97	.619	.314	.041	.016	.005	0		.005
35	105	.652	.252	.057		.038	106	.627	.283	.038	.038	0	0		.014
36	77	.552	.279	.091	.013	.065	78	.583	.353	.019	.013	0	0	0	0
37	50	.560	.350	.070	.010	.010	50	.600	.310	.040	.030	0	.010	.010	0

APPENDIX. Continued

	GPT-2				GOT					SOD(L)					
	N	1.00	.80	.60	N	1.00	.80	.70	1.15	N	1.00	1.30	1.40	1.50	.90
1	0	–	–	–	0	–	–	–	–	22	.545	.114	.250	0	.091
2	0	–	–	–	0	–	–	–	–	46	.543	.152	.196	0	.109
3	0	–	–	–	0	–	–	–	–	31	.629	.161	.129	0	.081
4	0	–	–	–	0	–	–	–	–	44	.705	.159	.080	0	.057
5	0	–	–	–	0	–	–	–	–	26	.808	.058	.077	0	.058
6	0	–	–	–	0	–	–	–	–	30	.650	.067	.150	0	.133
7	0	–	–	–	31	.800	.133		.066	31	.596	.112	.193	0	.096
8	0	–	–	–	18	.722	.194		.083	18	.611	.111	.194	.027	.055
9	0	–	–	–	0	–	–	–	–	59	.601	.033	.237	0	.127
10	0	–	–	–	0	–	–	–	–	149	.617	.070	.245	0	.067
11	49	.612	.338	0	49	.898	.102		0	49	.660	.110	.140	0	.090
12	50	.607	.393	0	0	–	–	–	–	50	.570	.120	.210	0	.100
13	0	–	–	–	0	–	–	–	–	48	.574	.117	.212	0	.095
14	0	–	–	–	0	–	–	–	–	50	.590	.050	.190	0	.170
15	45	.619	.381	0	0	–	–	–	–	45	.704	.079	.170	0	.045
16	60	.724	.276	0	0	–	–	–	–	60	.569	.137	.198	0	.094
17	0	–	–	–	100	.820	.090	.005	.085	100	.640	.065	.260	0	.035
18	16	.533	.467	0	0	–	–	–	–	16	.468	.125	.343	0	.062
19	50	.616	.384	0	0	–	–	–	–	50	.590	.060	.260	.010	.080
20	28	.620	.380	0	0	–	–	–	–	28	.785	.071	.107	0	.035
21	60	.650	.350	0	0	–	–	–	–	60	.616	.108	.200	0	.075
22	0	–	–	–	36	.750	.208	.014	.028	40	.538	.088	.275	.012	.087
23	0	–	–	–	71	.817	.162	.021	0	71	.578	.106	.246	0	.070
24	0	–	–	–	96	.833	.109	.010	.047	97	.650	.057	.227	.005	.062
25	40	.550	.440	.050	103	.864	.092	.019	.024	103	.655	.078	.199	0	.068
26	0	–	–	–	0	–	–	–	–	44	.682	.159	.102	.011	.045
27	0	–	–	–	0	–	–	–	–	28	.607	.232	.107	0	.054
28	20	.525	.475	0	0	–	–	–	–	66	.621	.114	.174	0	.091
29	24	.729	.271	0	0	–	–	–	–	67	.515	.142	.239	0	.104
30	0	–	–	–	0	–	–	–	–	34	.662	.074	.162	0	.103
31	0	–	–	–	0	–	–	–	–	0	–	–	–	–	–
32	0	–	–	–	0	–	–	–	–	0	–	–	–	–	–
33	0	–	–	–	0	–	–	–	–	0	–	–	–	–	–
34	0	–	–	–	97	.866	.093		.041	97	.629	.098	.216	.005	.052
35	0	–	–	–	106	.873	.080		.047	105	.557	.124	.238	0	.081
36	0	–	–	–	78	.769	.167	.019	.045	78	.583	.096	.256	0	.064
37	0	–	–	–	49	.847	.102	.020	.031	50	.590	.140	.200	0	.070

APPENDIX. Continued.

	PGI-2					PMI-2					6 PG-2						
	N	1.00	.95	.80	1.10	N	1.00	.90	1.20	.80	N	1.00	1.10	1.20	.85	.70	1.40
1	22	.477	.523	0	0	0	-	-	-	-	22	.818	.159	0	.023	0	0
2	46	.500	.500	0	0	0	-	-	-	-	46	.935	.022	.022	.022	0	0
3	31	.548	.452	0	0	0	-	-	-	-	31	.887	.081	0	.032	0	0
4	44	.602	.398	0	0	0	-	-	-	-	44	.898	.080	.011	.011	0	0
5	26	.442	.558	0	0	0	-	-	-	-	23	.848	.065	.044	.044	0	0
6	30	.600	.400	0	0	0	-	-	-	-	11	1.00	0	0	0	0	0
7	31	.518	.482	0	0	31	.883	.117	0	0	31	.854	.096	.016	.016	.016	0
8	18	.312	.625	.062	0	18	.821	.179	0	0	18	.882	.058	.029	.029	0	0
9	59	.465	.535	0	0	59	.833	.156	0	.009	59	.949	0	.033	.016	0	0
10	151	.460	.536	.003	0	151	.917	.056	.026	0	50	.900	.060	0	.030	.010	0
11	49	.449	.541	.010	0	49	.937	.063	0	0	49	.887	0	.102	.010	0	0
12	50	.410	.580	.010	0	50	.850	.150	0	0	50	.950	0	.020	.020	0	.010
13	48	.521	.468	.011	0	48	.893	.095	.010	0	48	.925	0	.042	.031	0	0
14	50	.440	.560	0	0	50	.898	.102	0	0	50	.898	.010	.051	.010	0	0
15	45	.444	.556	0	0	45	.811	.189	0	0	45	.922	0	.066	.011	0	0
16	60	.508	.492	0	0	60	.810	.190	0	0	60	.941	0	.050	.008	0	0
17	100	.500	.490	.010	0	100	.930	.050	0	.020	97	.948	.036	.016	0	0	0
18	16	.469	.531	0	0	16	.968	.032	0	0	16	.968	0	.031	0	0	0
19	50	.510	.480	0	.010	50	.890	.100	.010	0	50	.940	0	.050	.010	0	0
20	28	.482	.518	0	0	28	.780	.200	0	.020	28	.928	0	.035	.035	0	0
21	60	.458	.542	0	0	60	.886	.114	0	0	60	.891	0	.091	.016	0	0
22	40	.525	.475	0	0	40	.912	.088	0	0	40	.925	.050	.012	.012	0	0
23	71	.465	.528	.007	0	71	.887	.092	.021	0	71	.916	.049	.035	0	0	0
24	97	.423	.572	.005	0	97	.918	.072	.005	.005	97	.918	.062	.021	0	0	0
25	102	.481	.515	.005	0	103	.908	.068	.010	.015	103	.918	.063	.019	0	0	0
26	44	.364	.613	.023	0	44	.909	.080	.011	0	0	-	-	-	-	-	-
27	29	.483	.517	0	0	29	.879	.121	0	0	0	-	-	-	-	-	-
28	67	.470	.515	.015	0	67	.851	.060	.090	0	51	.931	.049	0	.020	0	0
29	67	.448	.545	.007	0	67	.910	.082	.007	0	32	.953	.047	0	0	0	0
30	34	.485	.515	0		34	.824	.162	.015	0	0	-	-	-	-	-	-
31	0	-	-	-	-	0	-	-	-	-	43	.953	.035	0	.012	0	0
32	0	-	-	-	-	0	-	-	-	-	36	.931	.028	0	.042	0	0
33	0	-	-	-	-	0	-	-	-	-	27	1.00	0	0	0	0	0
34	97	.443	.552	.005	0	97	.907	.088	.005	0	97	.918	.031	.041	.010	0	0
35	106	.439	.561	0	0	106	.901	.094	.005	0	106	.924	.057	.019	0	0	0
36	78	.455	.538	.007	0	77	.935	.052	.006	.006	78	.891	.083	0	.019	.006	0
37	50	.400	.600	0	0	50	.910	.082	.010	0	50	.930	.070	0	0	0	0
38																	
39																	
40																	