Genetic Population Structure of Rougheye Rockfish (Sebastes aleutianus) Inferred From Allozyme Variation

by
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INTRODUCTION

Rockfish (*Sebastes* spp.) support a valuable fishery in the northeast Pacific Ocean. Two species in particular, rougheye rockfish (*S. aleutianus*) and shortraker rockfish (*S. borealis*), are highly prized for their large size and red color. These two species are managed together as the shortraker-rougheye assemblage with commercial catch levels that averaged 2,500 metric tons (t) during 1993-97 in areas managed by the North Pacific Fishery Management Council (NPFMC). Both species are particularly sensitive to overexploitation because they are slow growing, have a high age at maturity, and are very long lived. Rougheye rockfish may take approximately 20 years to reach maturity, and individuals have been estimated to live longer than 140 years.

The annual catch quota for rockfish and most other groundfish managed by the NPFMC is partitioned into relatively large geographic regions: Eastern, Central, and Western Gulf of Alaska, Aleutian Islands, and the eastern Bering Sea (Fig. 1). These management areas were determined geographically but have little biological basis. If the population structure of a particular species has geographic boundaries different from the boundaries of the NPFMC management areas, there is risk that the fisheries might overharvest the stocks. Accordingly, members of the Auke Bay Laboratory (ABL) initiated a study in 1993 to examine the population structure of rougheye rockfish by analysis of genetic variation. Analysis of allozymes from a large number (n = 750) of rougheye rockfish sampled throughout a major portion of its range in the northeast Pacific Ocean forms the basis of the study.

METHODS

Collection - From 1993 through 1997, adult rougheye rockfish were collected with bottom trawls and longlines from the Gulf of Alaska and Aleutian Islands area (Fig. 2). Location, date, region, and sample size are reported in Table 1. Tissue samples of liver, heart, and muscle were taken from each fish, placed in freezer tubes, and temporarily stored in liquid nitrogen. Samples were shipped to the ABL and stored long term at -80°C.

Laboratory analysis - Samples were analyzed by starch gel electrophoresis. Enzymes were screened by staining all three tissue types simultaneously on six buffer systems (Table 2). Each tissue and system was stained using general staining procedures for a total of 47 enzymes, 35 of which showed activity representing 44 loci.

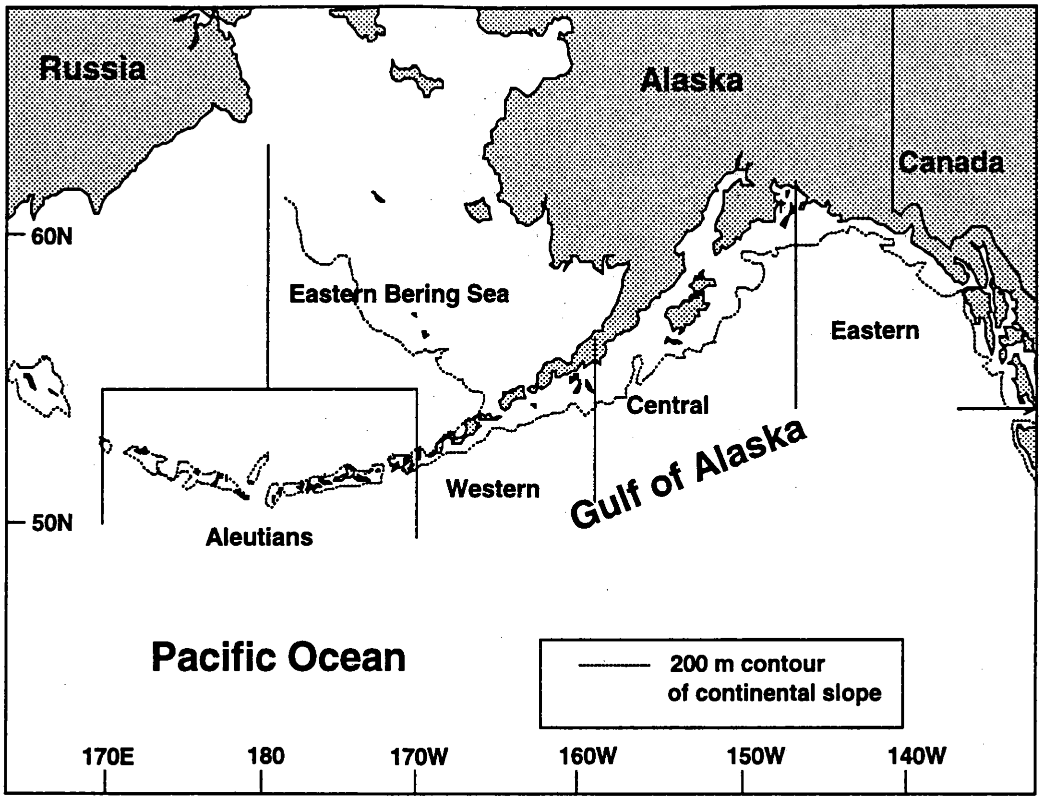

Figure 1. Northeastern Pacific Ocean showing North Pacific Fishery Management Council regulatory areas. Adult rougheye rockfish are primarily found along the continental slope at depths of 200-500 m.

Data analysis - Fish sampled from stations in close proximity were combined to form 15 regional groups (Table 1). Genotypic frequencies were calculated and tested for departure from expected Hardy-Weinberg equilibrium frequencies using a chi-square goodness of fit test corrected for small sample sizes. Heterogeneity of allele frequencies among collections was tested with the log-likelihood ratio analysis (G-statistic). A principal component analysis was used to identify important genetic loci and to display graphically the relationship between genotypic types. Average heterozygosities and linkage disequilibriums were calculated to further describe the population structure.

RESULTS

The initial suite of 47 enzymes screened produced activity at 35 enzymes. Of these, 24 enzymes representing 31 loci were resolved for all collections. Eight loci were monomorphic, 12 loci were variable where the frequency of the common allele was greater then 0.95 for all collections, 6 loci had a common allele frequency of less than 0.95 in at least one location, and 5 loci had a common allele frequency of less than 0.95 for all the collections. Loci, allele frequencies, and level of variation are listed in Table 2.

Significant departure from Hardy-Weinberg equilibrium was found in 13 out of 180 tests (0.07) for fish from Southeast Alaska, higher than would be expected by chance alone at the P=0.05 level of probability. For the Aleutian Islands locations (excluding Unalaska Island) and Prince William Sound, significant departure from Hardy-Weinberg equilibrium was found in only 5 out of 209 tests (0.02). Significant departures were found in the Kodiak Island sample (0.13) and in the Unalaska Island sample (0.10). Five loci deviated significantly from Hardy-Weinberg equilibrium in at least one-half of the Southeast Alaska

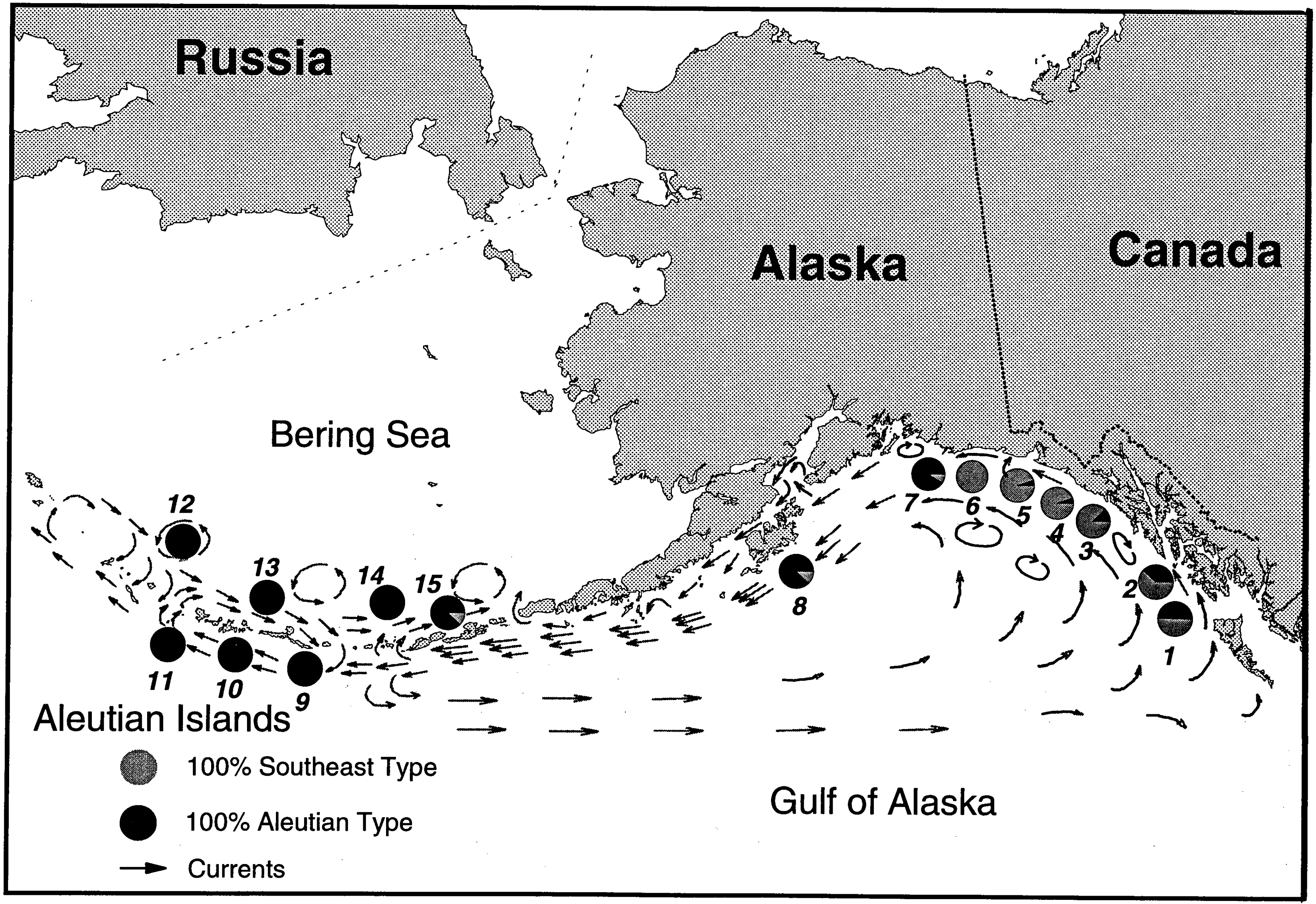

Figure 2. Map of study area showing ocean current patterns and approximate percentages of types by location. Numbers refer to locations listed in Table 1.

Table 1. Designated regional grouping, location, and sample size for rougheye rockfish sampled for genetic analysis.

Region	Location	N	Latitude N	Longitude W	Date
1	S.E. Alaska, Dixon Entrance	41	54.5	133.5	1993
2	S.E. Alaska, S. Baranof Is.	48	56.0	135.2	1993
3	S.E. Alaska, Cross Sound	30	58.1	136.9	1993
4	S.E. Alaska, Cape Fairweather	22	58.4	139.3	1993
5	S.E. Alaska, Yakutat	57	59.3	141.2	1993
6	S.E. Alaska, Cape Suckling	23	59.8	143.4	1993
7	South of Prince William Sound	47	58.2	148.4	1997
8	Kodiak Island, S.W.	98	56.4	151.6	1996
9	South Amlia Island				
	S. between Atka & Amlia Is.	72	51.8	173.9	1994
	South of Amlia Island	12	51.5	173.3	1996
	East of Amlia Island	4	52.2	171.7	1994
10	South Atka Pass				
	South Atka Pass	18	51.8	173.9	1994
	South of Atka Island	20	51.5	175.1	1995
	South of Adak Island	8	51.4	176.0	1995
11	South Tanaga Island				
	South of Tanaga Island	51	51.6	177.6	1994
	S.W. of Tanaga Island	19	51.6	178.3	1994
	West of Tanaga Island	12	51.5	178.1	1995
	West of Gareoli Island	12	51.5	178.6	1995
12	North of Semisopochnoi Island	28	52.5	180.0	1994
13	North Atka Pass				
	North of Adak Island	12	52.0	176.2	1995
	North of Atka Pass	12	52.1	175.4	1995
	North of Atka Island	4	52.2	174.5	1995
	North of Amlia Island	2	52.2	173.3	1995
14	N. of Islands of Four Mountains	34	53.0	170.1	1995
15	North Unalaska Island				
	North of Unalaska Island	5	53.7	167.0	1994
	North of Unalaska Island	50	53.7	167.0	1997

Table 2. Reliably scored loci with associated International Union of Biochemistry numbers (IUBNC), allele designations, tissue(s), buffer(s), and level of variability.

Enzyme	IUBNC #	Locus	Tissue	Buffer	Level Var*
Acid phosphatase	3.1.3.2	*ACP**	L	3	2/2
Aconitate hydratase	4.2.1.3	*mAH**	H	5,6	1/1
		*sAH**	L	3,4	2/2
Adenosine deaminase	3.5.4.4	*ADA**	M,H	3,6	0/0
Adenylate kinase	2.7.4.3	*AK**	M,H,L	6	0/0
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	4	1/2
Aspartate aminotransferase	2.6.1.1	*sAAT4**	L	1	1/2
		*mAAT**	M,H,L	3,4,6	1/1
beta-N-Acetylgalactosaminidase	3.2.1.53	*bGALA**	L	4	1/0
Creatine kinase	2.7.3.2	*CK-1**	H	3,6	1/1
		*CK-2**	M	6	0/0
Fumarate hydratase	4.2.1.2	*FH**	H,L	5	1/0
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M,H,L	1,3	1/1
		*GPI-B**	M,H	1,3	1/1
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	M	2	0/0
Iditol dehydrogenase	1.1.1.15	*IDDH**	L	1	2/2
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1**	H	3	1/1
		*IDHP-2**	L	3	1/1
Lactate dehydrogenase	1.1.1.27	*LDH**	M,H	3	0/0
Malate dehydrogenase	1.1.1.37	*MDH-1**	M,H	3,6	1/1
		*MDH-2**	M.H,L	3,4,6	1/1
Malic enzyme	1.1.1.40	*mMEP**	M,H	3,6	1/2
Mannose-6-phosphate isomerase	5.3.1.8	*MPi**	H	6	2/2
Dipeptidase (glycyl-leucine)	3.4.-.-	*PEPA**	M,H,L	2	2/2
Tripeptide aminopeptidase (leu-gly-gly)	3.4.-.-	*PEPB**	M,H,L	1	0/0
Phosphoglucomutase	5.4.2.2	*PGM-1**	M,H,L	1,5	1/2
		*PGM-2**	H	5	2/2
6-Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M,H,L	3	1/2
Superoxide dismutase	1.15.1.1	*SOD**	M,H,L	1,2,4,6	0/0
Triose-phosphate isomerse	5.3.1.1	*TPI**	M,H	1,3	0/0
Xanthine Oxidase		*XO**	L	2	1/22

Tissue - M=Muscle, H=Heart, L=Liver

Buffers - 1=R, 2=MF, 3=CA6.1, 4=CA6.9, 5=TC, 6=Came7.4

* First number is for Aleutian type and second is for SE Alaska type.

0=monomorphic, 1=frequency of common allele >.95, 2=frequency of common allele <.95 for at least one collection.

locations, Kodiak, and Unalaska Island; *PGM-2**, *XO**, *IDDH**, *MPI**, and *ACP**. Gene diversity analysis showed these loci to be the most distinquishable among collections, and any one of the five loci produced the same general relationship as that generated by the entire data set. Using this five-loci complex, individuals were visually separated by genotype into two groups. Principal component analysis (Fig. 3) confirmed this pattern. Frequencies for the five distinguishing loci by genetic types are shown in Table 3. Significant differences in allelic frequencies were detected between the two types at all five loci. Hereafter, we refer to these two types as the Southeast type and the Aleutian type.

The two types were found sympatrically in southern Southeast Alaska, with the Southeast type becoming more dominant in northern Southeast Alaska latitudes. G-test analysis showed heterogeneity ($p<0.001$) exists between Southeast Alaska fish and those from Prince William Sound, and the Kodiak Island and Aleutian Islands areas. The frequency of types abruptly changed from 100% Southeast type at Cape Suckling, to 91% Aleutian type in the region near Prince William Sound. The Aleutian type dominated the Kodiak Island (88%) and northern Unalaska Island regions (88%), with 100% Aleutian type throughout the remaining Aleutian Islands regions (Fig. 2).

G-test analysis of both the Southeast type and the Aleutian type showed no heterogeneity among collections. Average heterozygosities for each type were roughly 0.80. Linkage disequilibrium was highly significant for five loci (*ACP*, PGM-2*, XO*, IDDH*, and MPI**) when tested by sample site for Unalaska Island, Kodiak, and southern Southeast Alaska. However, when tested by type, no significant departures from equilibrium were found.

DISCUSSION

Significant departures from Hardy-Weinberg equilibrium and from linkage equilibrium are strong indicators of the Wahlund Effect, which predicts such results when there is a mixture of two or more genetically distinct populations. The principal component analysis (Fig. 3) of rougheye rockfish shows the relationship between the two types. We interpret the genetic differences between the two types as an indication of sibling speciation. Possible speciation for rougheye rockfish has been indicated in previous studies. Tsuyuki and Westrheim (1970) detected two distinct blood types, A and B, in rougheye rockfish off the coast of Vancouver Island, British Columbia. Of the 313 samples from their study, 64% were blood type A, 34% were blood type B, and 2% were the hybrid D. Our study shows a predominant Southeast type in Southeast Alaska, with an increasing proportion of Aleutian type in the southern ranges. Near Dixon Entrance, the two types were found in nearly equal proportions. We hypothesize that the Aleutian and Southeast types distinguished in our study may be the blood type A and B, respectively, found by Tsuyuki and Westrheim (1970) because of the southerly trend of increasing Aleutian type.

Seeb (1986) separated 47 rougheye rockfish morphologically and by color into two groups that were either light pink with spines under the orbit of the eye (*S. aleutianus*), or darker with considerable black on the mouth and jaw with orbital spines often lacking (*S. aleutianus unknown*). Seeb (1986) also detected significant frequency differences electrophoretically at the *PGDH**, *mMEP-1**, *MPI**, and *IDDH** loci, and fixed differences at *ACP**, *GAPDH**, and *BGALA** loci. While no fixed differences were detected in our study, significant heterogeneity was detected at *MPI**, *IDDH**, *XO**, *PGM-2**, and *ACP** (Table 3). Fixed differences were probably not detected in our study because of our larger sample size. We were unable to resolve *GAPDH**, and significant allelic frequency differences between the two types were not detected at *BGALA**, *PGDH**, or *mMEP-1**. The mobility of alleles at *IDDH** equates Seeb's *aleutianus* with our Southeast type, and *aleutianus unknown* with our Aleutian type. Seeb detected a frequency of approximately 50% of each type from a collection near Icy Bay, where we found a shift from 100% of the Southeast type to nearly 100% of the Aleutian type. All five of Seeb's samples collected from the Kodiak Island area were found to be *aleutianus unknown*, where we detected an 88% Aleutian type.

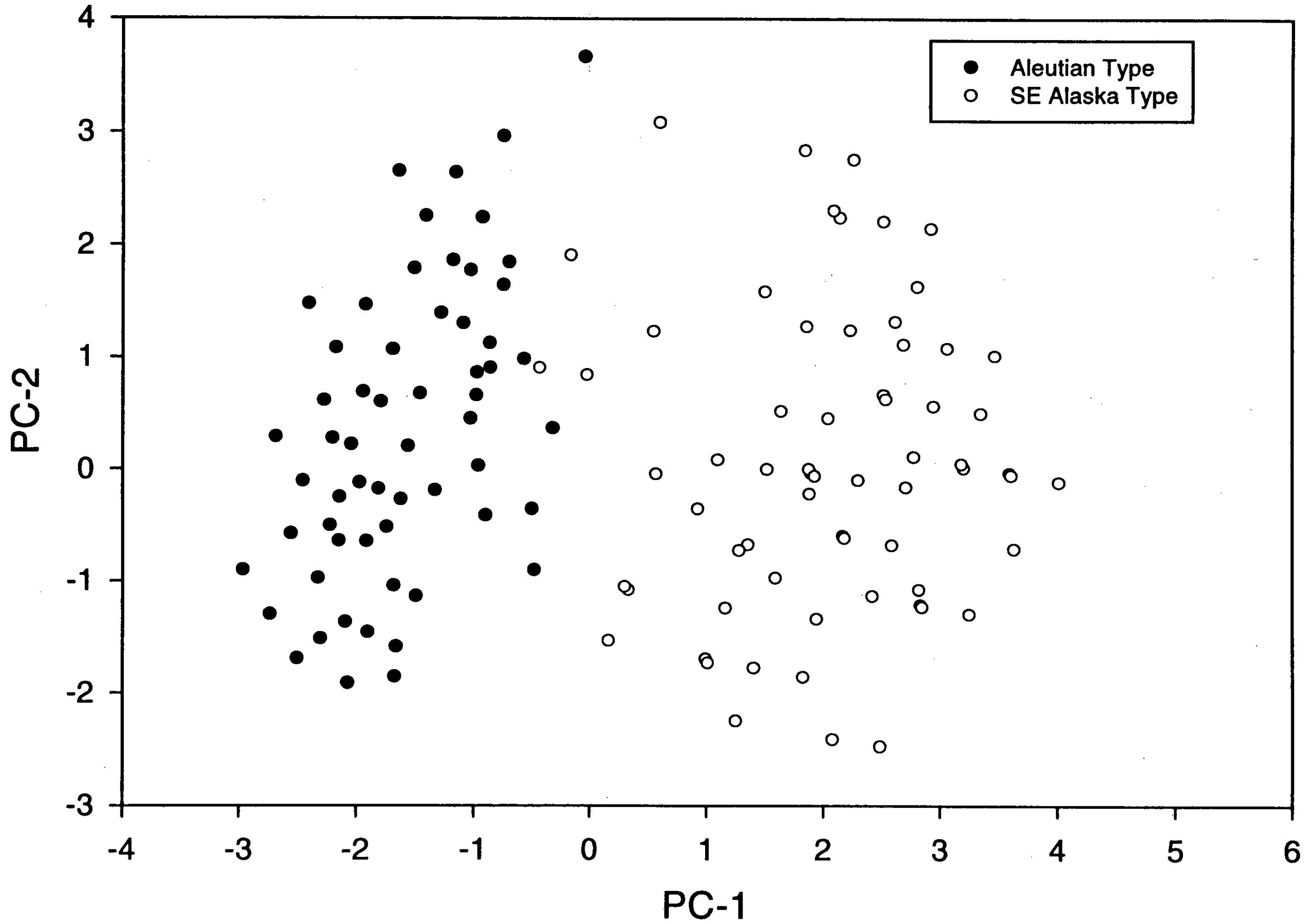

Figure 3. Principal component (PC) analysis showing the relationship between the two types of rougheye rockfish.

Table 3. Allelic frequencies for five diagnostic loci for Aleutain and SE Alaska type rougheye rockfish. A = Aleutian type and S = SE Alaska type.

	*ACP**				*PGM-2**				
Location (Region)	N	*100	*35	*83	N	*100	*83	*74	*63
SE Alaska									
Dixon Entrance (1)									
A	20	0.050	0.950	0	19	0.000	0.421	0.526	0.053
S	11	0.909	0.091	0	20	0.725	0.275	0.000	0.000
S. Baranof Island (2)									
A	14	0.000	1.000	0	16	0.000	0.281	0.594	0.125
S	25	0.820	0.180	0	32	0.781	0.219	0.000	0.000
Cross Sound (3)									
A	4	0.000	1.000	0	4	0.000	0.500	0.500	0.000
S	20	0.750	0.225	0.025	27	0.722	0.259	0.019	0.000
Cape Fairweather (4)									
A	1	0.000	1.000	0.000	1	0.000	0.500	0.500	0.000
S	15	0.900	0.067	0.033	20	0.725	0.250	0.025	0.000
Yakutat (5)									
A	2	0.000	1.000	0.000	3	0.000	0.167	0.833	0.000
S	47	0.915	0.021	0.064	53	0.774	0.217	0.009	0.000
Cape Suckling (6)									
S	19	0.921	0.079	0.000	19	0.868	0.132	0.000	0.000
Prince William Sound (7)									
A	13	0.154	0.846	0.000	42	0.000	0.405	0.440	0.155
S	1	0.000	1.000	0.000	4	0.750	0.250	0.000	0.000
Kodiak (8)									
A	75	0.047	0.947	0.006	86	0.006	0.378	0.488	0.128
S	7	1.000	0	0.000	12	0.917	0.083	0.000	0.000
Aleutian Islands									
S. Amlia Island (9)									
A	75	0.007	0.933	0.000	79	0.000	0.291	0.519	0.019
S. Atka Pass (10)									
A	32	0.125	0.875	0.000	44	0.011	0.364	0.455	0.170
S. Tanaga Island (11)									
A	54	0.000	1.000	0.000	90	0.000	0.317	0.544	0.139
N. Semisopochnoi Is. (12)									
A	20	0.025	0.975	0.000	26	0.019	0.269	0.462	0.250
N. Atka Pass (13)									
A	27	0.000	1.000	0.000	28	0.000	0.196	0.643	0.161
Is. Of Four Mountains (14)									
A	23	0.174	0.826	0.000	33	0.000	0.318	0.591	0.091
N. Unalaska Island (15)									
A	30	0.067	0.933	0.000	44	0.000	0.432	0.432	0.136
S	6	1.000	0	0.000	6	0.750	0.250	0.000	0.000

Table 3. Continued.

Location (Region)	*IDDH** N	*100	*500	*750	*MPI** N	*100	*129	*XO** N	*100	*109
SE Alaska										
Dixon Entrance (1)										
A	20	0.025	0.550	0.425	21	0.833	0.167	20	0.000	1.000
S	19	0.842	0.158	0.000	17	0.382	0.618	20	0.750	0.250
S. Baranof Island (2)										
A	15	0.000	0.533	0.467	16	0.688	0.312	15	0.000	1.000
S	32	0.875	0.125	0.000	32	0.313	0.687	30	0.867	0.133
Cross Sound (3)										
A	4	0.000	0.500	0.500	4	0.750	0.250	4	0.000	1.000
S	26	0.731	0.269	0.000	27	0.444	0.556	26	0.904	0.096
Cape Fairweather (4)										
A	1	0.000	0.500	0.500	1	0.000	1.000	1	0.000	1.000
S	18	0.611	0.361	0.028	20	0.400	0.600	20	0.950	0.050
Yakutat (5)										
A	3	0.167	0.500	0.333	3	1.000	0.000	2	0.000	1.000
S	54	0.695	0.296	0.009	53	0.377	0.623	54	0.861	0.139
Cape Suckling (6)										
S	22	0.773	0.227	0.000	19	0.316	0.684	22	0.795	0.205
Prince William Sound (7)										
A	43	0.035	0.442	0.523	43	0.814	0.186	42	0.000	1.000
S	1	0.500	0.500	0.000	4	0.500	0.500	4	0.750	0.250
Kodiak (8)										
A	86	0.012	0.523	0.465	85	0.759	0.241	86	0.023	0.977
S	12	0.750	0.250	0.000	12	0.333	0.667	12	0.917	0.083
Aleutian Islands										
S. Amlia Island (9)										
A	78	0.032	0.526	0.442	72	0.729	0.271	83	0.000	1.000
S. Atka Pass (10)										
A	42	0.036	0.559	0.405	43	0.698	0.302	42	0.012	0.988
S. Tanaga Island (11)										
A	83	0.042	0.476	0.482	87	0.787	0.213	93	0.022	0.978
N. Semisopochnoi Is. (12)										
A	27	0.055	0.426	0.519	28	0.839	0.161	27	0.037	0.963
N. Atka Pass (13)										
A	28	0.071	0.482	0.447	25	0.700	0.300	28	0.000	1.000
Is. Of Four Mountains (14)										
A	34	0.058	0.623	0.309	26	0.788	0.212	34	0.000	1.000
N. Unalaska Island (15)										
A	44	0.045	0.432	0.523	44	0.807	0.193	43	0.023	0.977
S	6	0.667	0.333	0.000	6	0.500	0.500	6	1.000	0.000

From the oceanographic surface current patterns and frequency of genetic types, we hypothesize that larval or juvenile rougheye rockfish are being carried in the Gulf of Alaska Gyre (Fig. 2). A dramatic shift from the dominance of one type to the other occurs between Cape Suckling and Prince William Sound. While the Aleutian type is the dominant type found in the central Gulf of Alaska and the Aleutian Islands region, the small percentages of Southeast type found near Prince William Sound and as far west as Unalaska Island were perhaps transported from northern Southeast Alaska as larvae or juveniles. Currents in this area range from 20 to 100 cm/sec, and larval rockfish are presumed to remain in the planktonic phase for 6 months to 1 year, thus making this hypothesis feasible. Just west of Unalaska Island, the ocean current bifurcates, and the North Pacific current travels back to Southeast Alaska at 5-15 cm/sec, reaching the continent somewhere near Vancouver Island. Aleutian type juveniles are perhaps being transported into the southern Southeast Alaska region in this current, explaining the 50% Aleutian type we found near Dixon Entrance. G-test analysis did not detect heterogeneity among each type throughout the entire region. This suggests a single panmictic group of each type with gulf-wide gene flow, possibly by way of juvenile or larval drift.

CONCLUSION

Identical mobilities at the majority of the 31 loci point to a close relationship of the two types, perhaps once existing as a single type at an earlier geologic time. Future research should include genetic analysis of rougheye rockfish off the coast of British Columbia and Washington-Oregon. Knowledge of the Aleutian/Southeast type composition in the waters off British Columbia and Washington-Oregon could help to 1) substantiate how our study corresponds to Tsuyuki and Westrheim's 1970 study, 2) determine if the Southeast type is an isolated pocket found only in Southeast Alaska and northern British Columbia, 3) contribute to an understanding about the evolution of the species, and 4) provide a greater biological basis for management of the species.

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