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Population structure of sablefish *Anoplopoma fimbria* using genetic variability and geometric morphometric analysis

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Summary

Population structure of the sablefish (*Anoplopoma fimbria*) in the northeastern Pacific Ocean was determined using three approaches: geometric morphometrics (14 landmarks), mitochondrial DNA (fragment of COI gene), and nuclear DNA (four microsatellite loci). Samples came from the Bering Sea, Gulf of Alaska, offshore Oregon, and offshore the mid-Baja California Peninsula (at San Quintin). Differences in body shape were grouped in the samples from the north (Bering Sea and Gulf of Alaska). A slight but significant population structure was also observed in allele frequencies of microsatellites, F_{ST} values, AMOVA, and Bayesian individual assignment tests; however, analyses of population structure using mtDNA did not reveal any population differentiation. Differences in population structure detected by distinct approaches, in addition to the moderately high haplotype diversity and low nucleotide diversity of the COI fragment, suggest recent and developing population differentiation in the sablefish.

Introduction

Sablefish (*Anoplopoma fimbria* (Pallas, 1814)) (Teleostei: Anoplopomatidae) is a demersal fish endemic to the North Pacific Ocean (Allen and Smith, 1988). It is found in a long arc from the Pacific coast of the Baja California Peninsula northward to Alaska and the seas adjacent to Siberia and southern Japan (Kimura et al., 1998). Southeastern limit of *A. fimbria* is near Isla de Cedros, Baja California (Mexico), about 28°N (Miller and Lea, 1972). During their life cycle, sablefish show two migratory patterns: one involves movement from the continental slope across the abyssal plains to seamounts and later returning to the continental slope; the other migration is along the continental slope from the Bering Sea to Southern California (Moser et al., 1994; Kimura et al., 1998).

Population assessments of sablefish in Alaska and the northwestern coast of the U.S. have been by using growth parameters and tag-recovery data; however, the results are contradictory in describing migratory behavior and population structure: while some studies revealed resident behavior (Beamish and McFarlane, 1988), others found large dispersal migrations within their range (Kimura et al., 1998; Morita et al., 2010). For management convenience, sablefish form two populations that are based on differences in growth rate, size at first maturity, and tagging studies: a northern population inhabits Alaska and northern British Columbia, and a southern population ranges from central British Columbia to California waters (Hanselman et al., 2008). Using $\delta^{18}C$ stable isotopes in otoliths collected along the Oregon-Washington coast, Gao et al. (2004) distinguish three populations of sablefish. Migration rates estimated from tag-recovery data suggest a significant gene flow among populations, which possibly prevents the formation of reproductively isolated populations (Kimura, 1980). The lack of studies on population structure of sablefish off northwestern Mexico meant that prior to the present study, it was unknown whether sablefish in the southern region formed a separate population from that in the north. It is important to determine the population structure of the sablefish in the southern part of the Eastern Pacific Ocean because this species is a shared resource of Mexico, the United States, and Canada.

Within a species, variability in growth, development, and maturation creates variations in body shape among populations; thus, body shape constitutes one important set of features for differentiating populations (Cadrin, 2000). Geometric morphometrics is a method to distinguish variations in body shape based on landmarks that have no restriction on the direction of variation and localization of shape changes; it is therefore an effective technique for capturing information about the shape of an organism. This approach, combined with multivariate statistical procedures, is a powerful tool for testing and displaying differences in shape (Rohlf, 1996).

Genetic approaches for identifying populations have also been widely used for commercially important marine fishes (Graves, 1998). These have successfully demonstrated genetic differentiation for some deep-water species of fish among populations on a regional or oceanic scale (Roques et al., 2002; Aboim et al., 2005; Knutsen et al., 2009; White et al., 2010). Reproductive isolation observed in these species may be a consequence of the combined effect of physical and biological factors, such as the presence of an anoxic zone, low primary productivity, sea floor structures that act as barriers for migration, the Allendorf-Phelps effect, and homing behavior (Ruzzante et al., 1998; Waples, 1998; Rogers, 2003). Currently, genetic studies of the sablefish are scarce and mainly focus on protein polymorphism, which have suggested no differentiation among populations throughout the northeastern Pacific (Tsuyuki and Roberts, 1969). In our study, we used morphometrics and genetic approaches based on four microsatellite loci and the mtDNA gene cytochrome oxidase I (COI) to test

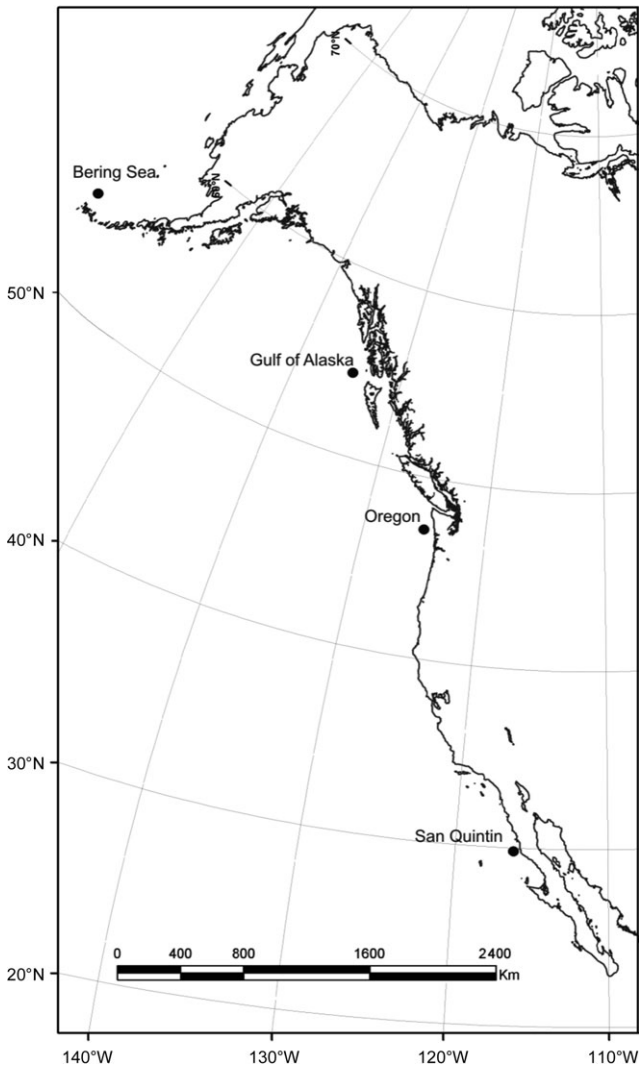

Fig. 1. Sampling locations of *Anoplopoma fimbria*

the hypothesis that there is more than one population of sablefish along the eastern Pacific Ocean.

Materials and methods

Specimen sampling

Sablefish were obtained from several areas in the eastern Pacific Ocean (Fig. 1): (1) The Bering Sea (60 fin clips and 10 complete specimens); (2) The Gulf of Alaska (60 fin clips and 10 complete specimens); (3) Off the west coast of Oregon (32 sablefish caught during the commercial fishing season); and (4) at the southern range limit of San Quintin, Mexico (32 sablefish caught by commercial fishing vessel). Specimens from the two northern locations were captured by long line at depths of 400–800 m. Sablefish off the Oregon coast were captured by trawls at 640 m; specimens off San Quintin were caught by trawls at 490 m (Table 1).

Analysis of geometric morphometrics

Of all specimens, 49 individuals were used for geometric morphometrics: Bering Sea (9), Gulf of Alaska (7), Oregon (15), and San Quintin (18). Photographs were taken from the left side of each specimen at the same distance. The x and y coordinates of 14 landmarks were digitized with software (tpsDig v2.12; Rolhf, 2008; Fig. 2). Landmark coordinates of all specimens were aligned using software CoordGen6 (Sheets, 2004) employing Procrustes-shape coordinates, which minimize least-square differences between each landmark in specimens. To evaluate variations in morphological shape of sablefish from the four areas, we made a canonical variate analysis using CVAGen6 (canonical variate analysis generating program; Sheets, 2004). An assignment of individuals to each population was made, based on Mahalanobis' distances in the space defined by the significant canonical variate analysis (CVA) axes. To corroborate these results, we made pairwise comparisons among the areas using TwoGroup6 (Sheets, 2004). In this analysis, we used Goodall's F-test between populations with 1600 bootstraps.

Genetic analysis

Four microsatellite loci, specific for sablefish, were provided by researchers at the Pacific Biological Station, Nanaimo, BC (unpublished data). These loci were amplified in 15 μl reactions (1 × PCR buffer, 0.2 mM dNTP, 0.4 μM of each primer, 2.5 mM $MgCl_2$, and 0.15 units Taq polymerase (Invitrogen). Cycling parameters were 95°C for 5 min followed by 35 steps at 95°C for 30 s each, 30 s at the specific annealing temperature (Table 2), 30 s at 72°C, and a final extension for 5 min at 72°C. PCR products were run in 6% polyacrilamide electrophoresis gels and visualized with silver staining (Benbouza et al., 2006).

For the mitochondrial DNA (mtDNA) analysis, we used primers L6154 (5′-AYCARCAYYTRTTYTGRTTCT-3′ and H6556 (5′-TGRAARTGIGCIACWACRTA-3′ to amply a fragment of COI (Teletchea et al., 2006). PCR reactions were performed in volumes of 60 μl with 90 ng total DNA, buffer 1 ×, 2.5 mM MgCl2, 0.2 mM dNTP, 0.4 μM of each primer, and 0.75 U μl^{-1} Taq polymerase. Cycling consisted of 4 min at 95°C, followed by 40 cycles of 30 s each at 95°C, 45 s at 57°C, 60 s at 72°C, and a final extension for 10 min at 72°C. The fragment was sequenced in ABI PRISM 3730XL with BigDye TM (Macrogen, Seoul, Korea). Sequences were edited using Cromas v1.49 (Technelysium Pty., Ltd., Mt Gravatt Plaza, Queensland, Australia) and alignment was made with Clustal W (Thompson et al., 1997). For the

Table 1
Sampled areas

Location	Date	Code	Depth (m)	Latitude (N)	Longitude (W)	N
Bering Sea, USA	07/06/2007	MB	465–701	56°5′4.6″	173°2′5.13″	60 (15)
Gulf of Alaska, USA	10/08/2007	GA	441–855	54°2′9.4″	134°0′0.66″	60 (14)
Oregon, USA	02/02/2006	OR	640	45°50′08″	124°48′06″	32 (15)
San Quintín, MEX	30/09/2006	SQ	490	30°26′30″	116°25′51″	31 (13)

N = sample size. Numbers in parenthesis are sample size for mtDNA analyses.

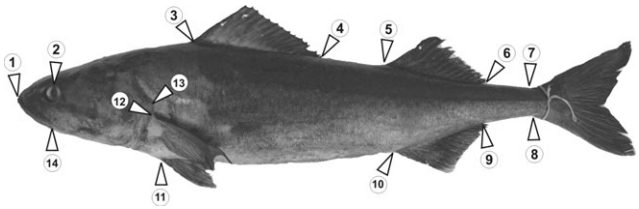

Fig. 2. Location of 14 landmarks used in the study: (1) anterior tip of the upper jaw, (2) upper middle part of the eye, (3) origin of first dorsal fin, (4) posterior insertion of first dorsal fin, (5) origin of second dorsal fin, (6) posterior insertion of second dorsal fin, (7) dorsal base of caudal fin, (8) ventral base of caudal fin, (9) insertion of anal fin, (10) origin of anal fin, (11) anterior base of pelvic fin, (12) anterior base of pectoral fin, (13) most exterior point of gill cover, (14) posterior end of jaw

alignment, default values were used with a 15-gap penalty and 6.66 extension gap. All nucleotide positions were clearly aligned.

Statistical analysis

For microsatellites analyses, the number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were calculated for each loci with GenAlEx v6.4 software (Peakall and Smouse, 2006). Genepop v4.0 software (Raymond and Rousset, 1995) was used to test deviations from the Hardy–Weinberg equilibrium for each population at each loci by calculating the fixation index F_{IS} (10 000 dememorizations and 1000 iterations) and also for testing the null hypothesis of independence between loci (linkage disequilibrium). Micro-Checker v2.2.3 software (Van Oosterhout et al., 2004) was used to determine the presence of null alleles. The frequency of these null alleles in all populations was calculated using FreeNA software (Chapuis and Estoup, 2007), following the expectation of the minimization algorithm described by Dempster et al. (1977).

Differences in allele frequencies between samples were assessed using Fisher's exact test with Genepop v4.0 software (Raymond and Rousset, 1995), with 10 000 dememorizations, 500 batches, and 5000 iterations per batch. Genetic differentiation between sample pairs was determined by calculating Wright's pairwise F_{ST} values (Weir and Cockerham, 1984) for original genotype data. Because we used only four microsatellite loci, we could not use F_{ST} values corrected for null alleles by the INA method implemented in FreeNA software; therefore, we used the genotypes corrected for null alleles from FreeNA and calculated pairwise F_{ST} values from these data. A hierarchical amova analysis assessed the component of genetic diversity attributable to variance among groups, within groups, and within populations. Two hierarchical levels were tested: (A) All samples were pooled into a single group (global amova) and (B) Samples from the Gulf of Alaska and Bering Sea (northern group) were pooled in a single northern group and compared against Oregon (middle group) and San Quintin (southern group). The amova was used on the original genotype data and for corrected null allele genotypes. These analyses were made using Arlequin v3.5 software (Excoffier et al., 2005), using 10 000 permutations in each case.

Structure v2.3.3 software (Pritchard et al., 2000) was used to perform a multi-loci Bayesian individual assignment test. For this, we selected the admixture model and the correlated allele frequencies between populations. The Markov chain Monte Carlo consisted of 100 000 steps with a burn-in period of 25 000 steps. We explored a range of K (inferred clusters) from 1 to 6 with 20 runs of each K value, as described by Evanno et al. (2005).

For mtDNA analyses, genetic diversity was estimated as the number of different haplotypes, haplotype diversity (h), and nucleotide diversity (π) (Nei, 1987) using Arlequin v3.5 software (Excoffier et al., 2005). Hierarchical amova of the COI gene fragment was used to estimate levels of genetic flow and distribution of the actual genetic diversity in the four regions. For this, we tested the same groups used in the amova analysis for microsatellites. The Φ_{ST} value, which is a mtDNA analogue of the F_{ST} (Excoffier et al., 1992), is generated from the frequency and similarity of haplotypes at sites using Markov chains with 1000 permutations (Raymond and Rousset, 1995) with Arlequin v3.5 software (Excoffier et al., 2005).

Results

Analysis of geometrics morphometrics

Canonical variate analysis (CVA) of the 49 individuals showed two distinct canonical variables (λ = 0.0275; P = 0.0002 for CVA1 and λ = 0.1507; P = 0.0382 for CVA2). The larger discrimination is given by the CVA1 with a clear distinction between northern samples (Bering Sea and Gulf of Alaska) and Oregon and San Quintin (Fig. 3). The Mahalonobis assignment test correctly classified 94% of the individuals from San Quintin, 87% of the individuals from Oregon, and 89% of the individuals from the Bering Sea; only 57% of the individuals from the Gulf of Alaska were correctly assigned. Goodall's F-test was calculated to test pairwise shape differences between sampled areas. All comparisons showed significant statistical differences (P < 0.05), indicating significant variations in shape; however, after a Bonferroni correction (P = 0.008), the comparison between the Gulf of Alaska and Bering Sea was not significant (P > 0.008) although the remainder of the comparisons remained significant (P < 0.008).

Table 2
Characteristics of the four microsatellite loci of sablefish, *Anoplopoma fimbria*

Locus	Primer sequence (5′, 3′)	Repeat motif	T_A	Size (bp)
Afi3	F: ACG TCT TCC CAC ATT TC R: GGG ATG CAT GTT GAT ATG	$(GGT)_{10}$	51	120–139
AfiP3A	F: CAT GTG CAC GTT CAC CTT R: CTC CTT TTA CTT TGC CCT TT	$(CT)_{18}$	57	96–170
Afi70I	F: GTC ATC AGC CTT TAC AGA R: CAC TGT CTG TAC ACA TTG A	$(CTTT)_{15}$	54	86–150
Afi32A	F: TCC ACG TGT GCG TTC AT R: TTT CGC TGT CTC CTT AC	$(CT)_{14}17x(CT)_{12}$	52.5	70–110

Data provided by K. Lynne, Pacific Biological Station, Canada

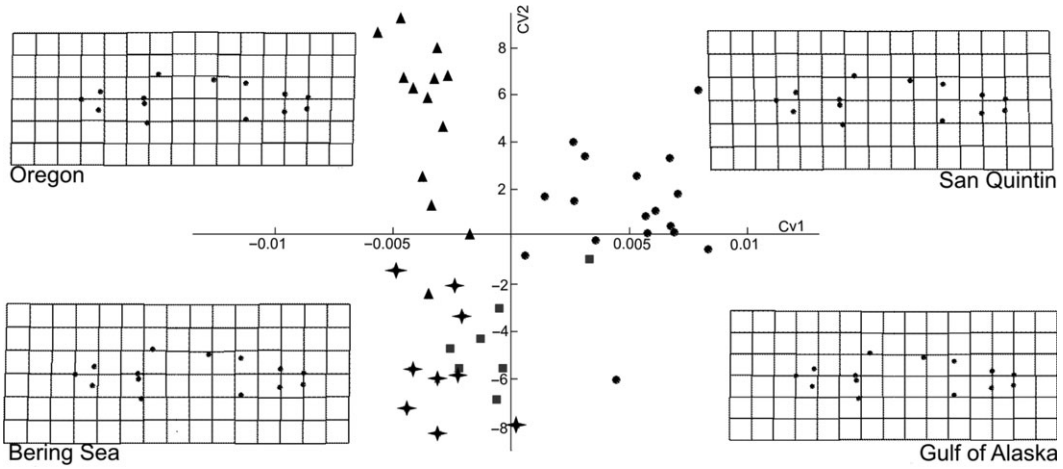

Fig. 3. Canonical variate analysis of shape variables from the sampled areas of sablefish *Anoplopoma fimbria*: (Bering Sea ▲, Gulf of Alaska ■, Oregon ✦, San Quintin ●). The transformation grids show the shape changes of each sample from the overall average.

Table 3
Summary of statistics of genetic variability for four microsatellite loci in sablefish, *Anoplopoma fimbria*

	Locus				
Sample	Afi701	Afi03	Afi32A	AfiP3A	Mean population
Bering Sea					
N	55	53	56	58	55.50
N_A	24	8	25	25	20.50
H_O	0.82	0.45	0.45	0.72	0.61
H_E	0.92	0.76	0.93	0.93	0.89
F_{IS}	0.13	**0.41**	**0.53**	**0.23**	0.32
Gulf of Alaska					
N	51	49	49	60	52.25
N_A	20	7	23	19	17.25
H_O	0.76	0.33	0.57	0.67	0.58
H_E	0.92	0.74	0.92	0.89	0.87
F_{IS}	**0.17**	**0.56**	**0.38**	**0.25**	0.34
Oregon					
N	23	29	24	31	26.75
N_A	14	7	14	20	13.75
H_O	0.87	0.34	0.54	0.74	0.62
H_E	0.90	0.72	0.88	0.89	0.85
F_{IS}	0.05	**0.53**	**0.40**	0.18	0.29
San Quintin					
N	30	29	27	25	27.75
N_A	19	9	14	19	15.25
H_O	0.87	0.72	0.78	0.76	0.78
H_E	0.90	0.76	0.87	0.90	0.86
F_{IS}	0.06	0.07	**0.13**	0.18	0.11
Mean loci					
N	39.75	40.00	39.00	43.50	40.56
N_A	19.25	7.75	19.00	20.75	16.69
H_O	0.83	0.46	0.58	0.72	0.65
H_E	0.91	0.75	0.90	0.90	0.87
F_{IS}	0.10	0.39	0.36	0.21	0.26

Sample size (N); number of alleles (N_A); observed heterozygosity (H_O); expected heterozygosity (H_e). Data in bold type are Hardy–Weinberg disequilibrium for fixation index F_{IS} ($P < 0.01$) after Bonferroni correction

Genetic analyses

Results from the genotype of the four microsatellite loci of sablefish from the four sample regions are shown in Table 3.

Table 4
Expected null allele frequencies for microsatellite loci calculated by expectation maximization algorithm method (Dempster et al., 1977) as implemented in FreeNA software

Locus / sample	Bering Sea	Gulf of Alaska	Oregon	San Quintín
*Afi*701	0.0538	0.0815	0.0099	0.0155
*Afi*03	0.1825	0.2423	0.2100	0.0064
*Afi*32A	0.2526	0.1786	0.1818	0.0662
*Afi*P3A	0.1057	0.1170	0.0611	0.0632

All four loci were polymorphic in all populations. The number of mean alleles among four loci (N_A) ranged between 20.50 and 13.75; observed heterozygosity (H_O) was between 0.78 and 0.58. F_{IS} values were highly significant ($P < 0.001$) after the Bonferroni correction (Rice, 1989) in 10 of 16 tests, indicating a deviation from the Hardy–Weinberg equilibrium to a deficit of heterozygotes. The Micro-Checker analysis detected null alleles at all locations. Frequencies of null alleles in all samples are shown in Table 4. The linkage disequilibrium test showed that no loci were in disequilibrium in any of the populations. Pairwise comparison with Fisher's exact test for genetic differentiation in allelic frequencies showed significant differences in allele frequencies between all pairs of populations ($P < 0.05$), except for the Bering Sea and Gulf of Alaska ($P = 0.10$); however, these latter two samples were not included as they would have created large differences in sample size. Pairwise F_{ST} values without the correction for null alleles showed an overall lack of genetic differentiation among samples (Table 5), but in the analysis with corrections for null alleles, the comparisons of San Quintin vs Bering Sea and San Quintin vs Gulf of Alaska had significant F_{ST} values ($P < 0.008$; Table 5) after the Bonferroni correction (Rice, 1989). AMOVA original genotype data showed no genetic differences in any of the tested structures (A and B; Table 6); however, data corrected for null alleles had significant F_{ST} values in both tested structures, suggesting a small but significant genetic differentiation among these populations (Table 6). Results of the individual assignment test made with Structure software showed the most likely K to be 2. This value was confirmed by Evanno's correction method (Evanno et al., 2005). It is important to note that the two different clusters do not correspond to an evident geographic structure,

Table 5
Pairwise genetic differentiation between samples. F_{ST} values calculated from microsatellite data below diagonal; upper number calculated with original genotypes, lower numbers calculated with null allele corrected genotypes. Φ_{ST} values calculated from mtDNA above diagonal

	BS	GA	OR	SQ
BS	–	−0.02	0.04	−0.03
GA	0.0001	–	0.03	−0.009
	−0.0010			
OR	−0.0055	0.0107	–	0.05
	−0.0079	0.0042		
SQ	0.0053	0.0001	−0.0015	–
	0.0141	**0.0100**	0.0021	

Numbers in bold are significant at $P < 0.008$

thus individuals from both genetic populations can be found in all four areas (Fig. 4).

A 353-bp fragment of the COI gene from 57 individuals was analyzed. We identified 12 haplotypes with 11 polymorphic sites, 8 singleton, and 3 parsimoniously-informative sites (Table 7). Nine of these haplotypes differed at only one or two transitions, and two sites showed a transversion. Haplotypes 1 and 2 were the most abundant (present in 68.4% and 10.5% of the individuals, respectively), and differed by one transversion (C – T) at position 165 (Table 7). The presence of only two high-frequency haplotypes and many rare haplotypes resulted in moderately high diversity (h from 0.371 to 0.758) and low nucleotide diversity (π from 0.0004 to 0.0007).

AMOVA analysis revealed no significant F_{ST} values (Table 6). In models A and B, more than 98% of the variations are found within populations (99.12% in model A and 98.63% in model B). Pairwise F_{ST} values were low and not significant in any comparisons (F_{ST} values from −0.03 to 0.05; $P > 0.05$; Table 5).

Discussion

For marine biological resources, one concern is reliable information to determine population structure of a species to identify management units. Despite the small sample size and artifacts present in the microsatellite markers, this study is one of the first holistic assessments of population structure of sablefish (*Anoplopoma fimbria*) in their range using morphological and genetic data.

Geometric morphometric analysis revealed statistical differences in the shape of individuals in its range. Sablefish from the north region (Bering Sea and Gulf of Alaska) are similar among them and differ from populations collected off Oregon and the Baja California Peninsula. These statistics provide evidence supporting at least two populations of sablefish for management purposes (Hanselman et al., 2008). However, as indicated in Fig. 3, the differences in shape from the four sampling areas were very subtle. This contrasts with the results in some other marine fishes, such as the Baltic Sea herring (*Clupea harengus*), which have distinct skull differences among populations, correlated with genetic differences (Jørgensen et al., 2008). Divergence in body shape observed among fish populations are related to environmental effects (Klingenberg et al., 2003; Trapani, 2003; Jørgensen et al., 2008), thus if there are environmental pressures affecting the shape of sablefish, they are very slight, affecting only northern individuals; there are not enough to produce marked differences throughout their range.

Contrary to morphometric data, microsatellite data are not exposed to selection pressure. Genetic analysis revealed a high polymorphism in the four microsatellite loci, with a mean number of alleles among samples similar to others deep-sea fishes, such as roundnose grenadier *Coryphaenoides rupestris* (N_A = 12.15–21.38; White et al., 2010) and for demersal fishes, such as the gilthead sea bream *Sparus auratus* (N_A = 13–18.25; De Innocentiis et al., 2004) and the walleye pollock *Theragra chalcogramma* (N_A = 20.4–22.4; O'Reilly et al., 2004). Levels of expected heterozygosity in sablefish (0.85–0.89) were similar to the H_E values of the three species mentioned above (*C. rupestris* = 0.74–0.79; White et al., 2010; *S. auratus* = 0.76–0.86; De Innocentiis et al., 2004; and *T. chalcograma* = 0.83–0.86; O'Reilly et al., 2004).

Table 6
Analysis of molecular variance of genetic variations in sablefish, *Anoplopoma fimbria*, for microsatellite data and a fragment of the COI region

		% of variance			*F*-statistic		
Population structure tested	Variation source	MS_1	MS_2	COI	MS_1	MS_2	COI
A (GA and BS and OR and SQ)	Among populations (F_{ST})	0.15	0.30	0.88	0.001	**0.003**	0.008
	Within populations	99.85	99.70	99.12			
B (GA and BS) vs (OR) vs (SQ)	Among groups (F_{CT})	0.17	0.57	2.85	0.001	0.005	0.013
	Within groups (F_{SC})	0.03	−0.09	−1.48	0.0002	−0.0009	−0.015
	Within populations (F_{ST})	99.80	99.53	98.6	0.002	**0.004**	0.028

Microsatellite original genotypes data (MS_1), microsatellite null alleles corrected genotypes data (MS_2), mitochondrial DNA COI gene fragment (COI). Numbers in bold significant at $P < 0.05$.

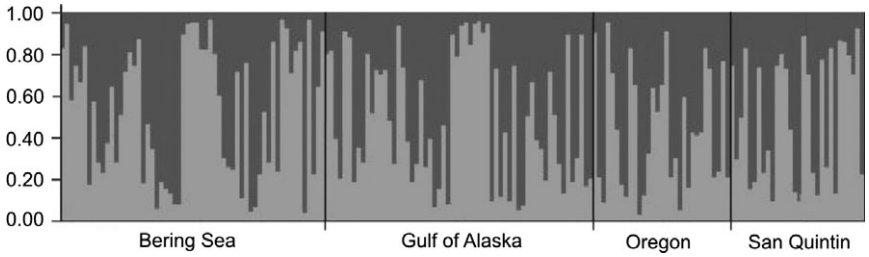

Fig. 4. Proportional membership of each individual in two clusters identified by Bayesian assignment test from STRUCTURE software. Each individual represented by a vertical line. Colored segments = estimated membership fractions of individuals in each K cluster. Black solid lines separate the four sampling areas

Table 7
Frequency distribution and variable sites for COI fragment of sablefish, *Anoplopoma fimbria*, haplotypes

	N per site					Number of sites										
Haplotypes (access number)	BS (15)	GA (14)	OR (13)	SQ (15)	Global (57)	21	45	90	165	198	204	207	213	216	228	258
h1 (HQ679946)	11	7	9	12	39	G	C	A	C	A	A	C	C	G	A	G
h2 (HQ679947)	2	2	1	1	6	.	.	.	T	.	.	.	.	.	.	.
h3 (HQ679948)	0	0	3	0	3	.	.	.	.	.	.	.	.	.	.	A
h4 (HQ679949)	1	0	0	0	1	.	.	.	.	.	.	G	.	.	.	.
h5 (HQ679950)	0	1	0	0	1	.	.	.	.	.	.	.	T	.	.	.
h6 (HQ679951)	0	1	0	0	1	.	.	.	.	.	G	.	.	.	.	.
h7 (HQ679952)	0	0	0	1	1	.	.	G	.	.	.	.	.	.	.	.
h8 (HQ679953)	0	1	0	0	1	.	G	.	.	.	.	.	.	.	.	.
h9 (HQ679954)	0	0	0	1	1	A	.	.	.	G	.	.	.	.	.	.
h10 (HQ679955)	0	1	0	0	1	.	.	.	.	.	.	.	.	A	.	.
h11 (HQ679956)	0	1	0	0	1	.	.	.	.	.	.	.	.	.	G	.
h12 (HQ679957)	1	0	0	0	1	A	.	.	.	.	.	.	.	.	.	.
H	0.467	0.758	0.5	0.371	0.524											
(SD)	(0.148)	(0.116)	(0.136)	(0.153)	(0.078)											
Π	0.00146	0.0027	0.0015	0.0015	0.00182											
(SD)	(0.00053)	(0.0006)	(0.0004)	(0.0007)	(0.00035)											

Haplotype diversity (h) and nucleotide diversity (π).

Despite these similarities, the sablefish had very low levels of observed heterozygosity (0.58–0.78; Table 3). Many of the Hardy–Weinberg tests revealed significant deviations from equilibrium by the presence of null alleles (Table 4). Null alleles have been reported in many studies using microsatellite loci to study fish populations (Jones et al., 1998; O'Connell et al., 1998; Roques et al., 1999; O'Reilly et al., 2004). Null alleles may be related to large populations with high N_E or increasing phylogenetic distance from a focal species when crossed amplification was used, which is not the case in our study (O'Reilly et al., 2004; Chapuis and Estoup, 2007).

While microsatellite loci reveal high polymorphism in all samples, mtDNA results provide some information on the history of sablefish. Genetic diversity, based on COI, indicates moderately high genotype diversity (h = 0.371–0.758) and very low nucleotide diversity (π = 0.00035–0.0007). A similar pattern of genetic diversity occurs in the deep-sea fish *Helicolenus dactylopterus* (Aboim et al., 2005). Many haplotypes with low divergence are attributed to expansion after a period of low effective population. Rapid population growth enhances retention of new mutations (Grant and Bowen, 1998).

Weak, but statistically significant genetic structure was observed from F_{ST} values of microsatellite loci with null allele correction, where the population of the southern sample area showed significant values, compared with the population of the northern sample area; however, this was not the case for the population of the middle sample area. This result and two different clusters detected by Structure software suggested a subtle genetic differentiation, similar to the results of the morphometric and microsatellite analyses that are concordant with the hypothesis that there are two stocks in the northeastern Pacific (Hanselman et al., 2008). Although genetic drift becomes negligible as a cause of genetic differentiation in large populations, there are other factors that could promote reproductive isolation. Seafloor features could be effective migration barriers to adult fishes, even for a species with high dispersal capabilities (Knutsen et al., 2009). This effect has been observed in deepsea fishes, such as the deep-water redfish (*Sebastes mentella*), cusk (*Brosme brosme*), and blackbelly rosefish (*Helicolenus dactylopterus*). These three species have different populations resulting from bathymetry and oceanographic factors, with F_{ST} values similar to those of the sablefish (Roques et al., 2002; Aboim et al., 2005; Knutsen et al., 2009). Therefore, restriction of the sablefish gene flow southward is plausible. In the same direction, it is important to note that samples collected in the north have higher frequencies of null alleles at all loci (Table 4), with a direct effect on F_{IS} values. This is a larger deviation from Hardy–Weinberg equilibrium than samples from the south (Table 3). These differences may reflect population differences among samples since the presence of null alleles are a consequence of nucleotide sequence divergence (Dakin and Avise, 2004).

On the other hand, Bayesian individual assignment from Structure software analysis did not show any geographic pattern because we found individuals from both genetic clusters in the four areas. Besides, the low F_{ST} values of microsatellite data and lack of differentiation observed with mtDNA suggest that if there were a real genetic population structure, it would be very recent. Despite the uninformative results from the mtDNA data, variation of COI can reveal genetic structure with larger samples. Sablefish also display high variability in recruitment success or the Allendorf-Phelps effect (Waples, 1998; King et al., 2001; Schirripa and Colbert, 2006), which also produce variations in genetic population structure, as observed in other marine fishes (Larson and Julian, 1999). In our study, it is unknown if the samples came from the same cohort or from different cohorts with genetic dissimilarities and produced the genetic structure determined from microsatellite loci and the Structure software. Additionally, sablefish may live for more than 100 years, but reach maturity at 5 years (King et al., 2001); therefore, genetic and morphometric differences in populations would not be as evident as in species with short-lived generations (White et al., 2009).

Null alleles could also play an important role in the genetic population structure; Chapuis and Estoup (2007) observed that even when the Dempster method precisely estimates null allele frequencies when low levels of gene flow are present in the populations, the bias in the F_{ST} is larger after correction for null alleles (see Zarraonaindia et al., 2009); thus, using more microsatellite loci with a reduced number of null alleles and a large sample from each location could clarify population genetic structure of sablefish.

In summary, geometric morphometrics and genetic analyses of four populations of sablefish demonstrated that the two northern populations are similar and somewhat different than the central and southern populations. These results, in addition to the COI analyses, suggest a slight but significant genetic differentiation of sablefish in the northeastern Pacific. The genetic data suggested that the Bering Sea, Gulf of Alaska, and Oregon populations form the same stock; however, under the precautionary approach (FAO, 1996; Reiss et al., 2009), the worst-case scenario is the failure to detect population structure when this structure does exist (Type II error, Waples et al., 2008). Management strategies with this error could lead to the irrevocable loss of local genetic diversity. Since our results were different from those derived from tagging studies (Kimura et al., 1998) and otolith analysis (Gao et al., 2004), we recommend further genetic and morphological studies to gain a more realistic perspective of the population structure of sablefish and reliable information for management plans.

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