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ARTICLE

Landscape Genetics Reveals Population Subdivision in Bering Sea and Aleutian Islands Pacific Cod

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Abstract

Landscape genetics of Pacific cod *Gadus macrocephalus* within the Bering Sea and Aleutian Islands (BSAI) management area of Alaska was examined in samples from nine spawning locations, including one temporal replicate sample, using 17 microsatellite DNA markers. This study examined fine-scale population structure of Pacific cod in the BSAI with the goal of identifying breaks in genetic continuity associated with physical barriers to migration and larval transport. Samples were taken from spawning fish collected from the western Aleutian Islands east to Unimak Pass and as far north as the Pribilof Islands. Overall, this work confirms previous studies, which found that genetic differentiation between samples is proportional to the distance between them, and also resolves population structure on a scale not previously possible. Results indicate that dispersal is not continuous at a fine scale and several distinct genetic groups were identified that correspond to differences in the physical environment of the BSAI. The data provide evidence for limited connectivity among spawning groups; in particular, there is evidence that a barrier exists between the Aleutian Islands and the eastern Bering Sea. In addition, analysis of molecular variance and Monmonier algorithm results suggest that, within the Bering Sea, the Unimak Pass and Pribilof Islands spawning groups may be distinct from each other, and the Wombling analysis indicates that samples west of Amchitka Pass in the western Aleutians may be distinct from those in the eastern Aleutians.

Lack of migration among groups within a species can result in restricted gene flow, reproductive isolation, and complex population structure. Barriers to migration in terrestrial systems may consist of landscape features such as rivers or mountain ranges. Similar barriers exist in the ocean in the form of currents (Gaylord and Gaines 2000), bathymetry (Schüller 2011), and temperature gradients (Wyllie-Echeverria 1995). When two populations are separated for many generations, their allele frequencies at neutrally evolving DNA loci will diverge and become distinct, primarily owing to genetic drift. Over long time periods, such isolated groups may diverge into distinct species or remain as distinct evolutionary, or genetic, populations within a species (Waples and Gaggiotti 2006). Distinct genetic populations, or genetic stocks, are separated by limited or no gene flow and can be recognized by significant and temporally stable genetic differentiation if enough time has passed for genetic differences to accrue (Bentzen 1998), while a single stock consists of individuals that are characterized by connectivity, or reproductive cohesiveness.

A primary goal of marine resource management is to match management units with biological populations to avoid unintended depletion of one or more distinct stocks that may otherwise have been managed as one composite unit (Taylor 1997; Laikre et al. 2005; Sterner 2007). As defined in the National

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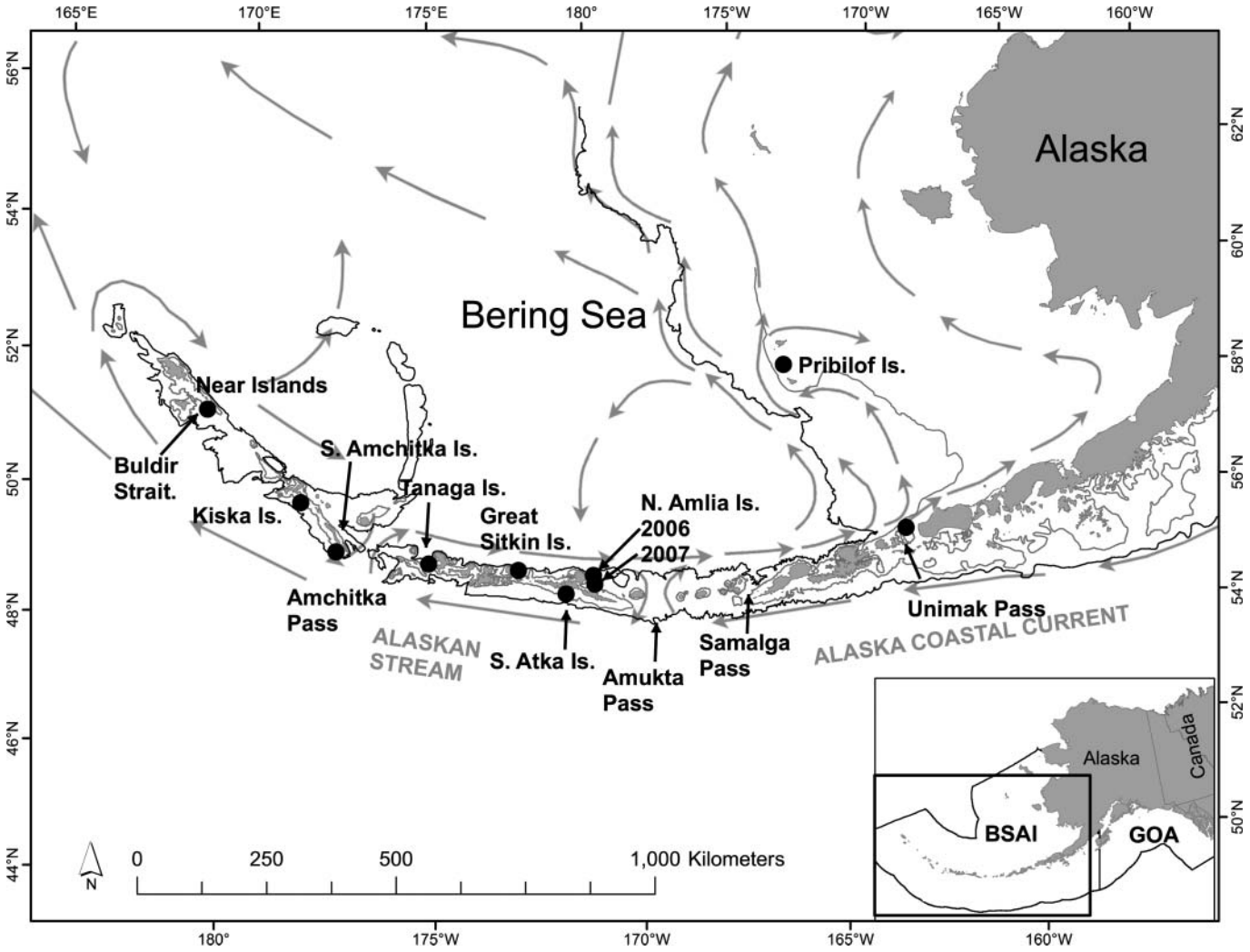

FIGURE 1. Bering Sea and Aleutian Islands (BSAI) with sample locations (black circles), major current patterns (grey arrows), 1,000-m and 100-m depth contours (dark and light bathymetry lines, respectively), and major passes. Islands west of Unimak Pass are considered the Aleutian Island chain. The eastern Bering Sea is north of Unimak Pass along the shelf, which follows the 1,000-m isobath. The BSAI and Gulf of Alaska (GOA) management areas are shown in the inset.

Standard provision to the U.S. Magnuson Stevens Act, a specified management unit is a fishery or portion of a fishery that is based on management objectives that may include biological, geographical, economic, technical, social, or ecological criteria. Although other criteria exist for management units in fisheries around the world, this definition illustrates the fact that a management unit may be established for a variety of political or administrative reasons and may not necessarily reflect population structure (Hilborn 2005; Reiss et al. 2009). Preservation of locally adapted subpopulations is important for the stability of the stock complex (Schindler 2010), and fisheries that reduce the size of a population or maintain populations at a low level for an extended period could cause a loss of genetic diversity, which can result in lowered adaptability, compromised productivity, and a higher risk of population loss (Lacy 1987; Hauser et al. 2002).

Pacific cod *Gadus macrocephalus* are important both economically and ecologically in the North Pacific Ocean. They comprise the second largest fishery in the United States next to walleye pollock *Theragra chalcogramma*, both by weight and value, and were 31st by weight among worldwide marine capture fisheries from 2004 to 2008 (www.fao.org). In the United States, all but 1% of the total catch of Pacific cod is taken off Alaska, where cod are allocated into two management units, the Bering Sea and Aleutian Islands (BSAI) and the Gulf of Alaska (GOA; Figure 1). In 2009, the Pacific cod catch in Alaska accounted for 15% of the total groundfish catch, or 228,200 metric tons (Hiatt et al. 2010), approximately 80% of which is taken from the BSAI. In this region, Pacific cod are primarily found along the Bering Sea shelf and the Aleutian Islands chain. Many discrete spawning areas, each linked to a specific geographic location, have been identified throughout this range (Shimada and

TABLE 1. Northward oceanographic flow rates and depth of selected Aleutian Islands passes (Hunt and Stabeno 2005; Mordy et al. 2005). Flow through Tanaga and Amchitka passes are reported as combined and flow rate is not known for Buldir Pass.

Pass	Buldir	Amchitka	Tanaga	Amukta	Samalga	Unimak
Flow (m^3/s)		4.4×10^6		4.0×10^6	4.0×10^6	1.0×10^5
Depth (m)	640	1,155	235	430	200	52

Kimura 1994; Logerwell and Neidetcher 2008). Pacific cod are a key species in the ecosystem, both as predators, primarily of walleye pollock and other fish, crab, and shrimp (Lang and Livingston 1996), and as prey for Pacific halibut *Hippoglossus stenolepis*, seabirds, and marine mammals, including the endangered Steller sea lion *Eumetopias jubatus* (Sinclair and Zeppelin 2002).

In the biologically similar species, Atlantic cod *Gadus morhua*, tracking data show that adults home to their natal spawning ground, a mechanism that has been used to explain localized population structure (Green and Wroblewski 2000; Robichaud and Rose 2001; Wright et al. 2006). Distinct populations of Atlantic cod have been found as close as 30 km to one another, and gene flow appears to be influenced more by dispersal of pelagic larvae than by adult movement (Knutsen et al. 2003; Stenseth et al. 2006; Jorde et al. 2007). Indirect evidence associated with tagging studies suggests that Pacific cod also exhibit site fidelity associated with spawning areas (Gustafson et al. 2000). Whether this represents fidelity to a natal spawning site is unknown; however, the presence of a temporally stable distinct population structure would require limited dispersal at all life history stages and would imply natal spawning site fidelity.

Although mechanisms for natal spawning site fidelity are not known in Pacific cod, an overview of their life history from egg to adult provides insight into the potential for gene flow in this species. Demersal Pacific cod eggs hatch between 15 and 45 d after spawning, depending on temperature, and at approximately 20 d in the Gulf of Alaska (Rugen and Matarese 1988; Laurel et al. 2008). Pacific cod larvae are pelagic until 3–4 months after hatching, after which they settle to depths of 30–50 m (Narimatsu et al. 2007); thus, larval dispersal may be influenced by current patterns. However, their larvae have been observed to move into the upper water column where they are transported towards coastal nurseries (Rugen and Matarese 1988; Hurst et al. 2009). Juvenile Pacific cod use inshore eelgrass in fjord-like habitats such as Prince William and Puget sounds before migrating to deeper water as they mature (Gustafson et al. 2000). The maximum age observed for a Pacific cod is 25 years, and the age at which 50% of the population reaches maturity in the BSAI is 4.9 years (Munk 2001; Stark 2007). Adult Pacific cod spawn in specific areas from January through March. Tagging studies in the Gulf of Alaska and the BSAI found that fish tagged in the summer and recaptured during spawning season migrated farther than fish recovered in other seasons, suggesting that cod migrate to feeding areas prior to returning to spawning areas (Shimada and Kimura 1994; D. Urban, National Marine Fisheries Service, Alaska Fisheries Science Center, personal communication).

Adult Pacific cod prefer depths less than 260 m (Logerwell et al. 2005); therefore, deep passes could deter adult movement and provide an additional mechanism for reduced gene flow. Adults and juveniles reside near, but not on the bottom, generally from 80 to 260 m and are not found deeper than about 500 m (Bakkala et al. 1984; Nichol et al. 2007). Bentzen et al. (1996) found significant differentiation among three northwestern Atlantic cod populations (Flemish Cap, Scotian Shelf, and northern cod), each separated by submarine trenches that were up to 1,000 m deep. There are several major passes within the U.S. region of the Aleutian Chain that exceed 260 m depth (Hunt and Stabeno 2005), including Buldir Strait and Amukta and Amchitka passes (Figure 1; Table 1). In addition, the Aleutian passes define species distributions for 63 of 245 identified fish species, suggesting that the passes themselves act as physical barriers to gene flow in this region (Logerwell et al. 2005).

Oceanographic flow may provide a third mechanism for constraining dispersal and gene flow (Gaylord and Gaines 2000); complex oceanography within the BSAI could form barriers to migration both to adult and larval Pacific cod (Figure 1). The physical environment of the Bering Sea is extremely different from the Aleutian Islands; the eastern Bering Sea is characterized by a single continental shelf adjacent to a deep sea basin while the Aleutian Islands form a chain of volcanic mountaintops separated by passes that allow transfer of water between the North Pacific Ocean and the Bering Sea (Hunt and Stabeno 2005). Along the Aleutian Islands chain, physical and biological differences demarcate ecological shifts, most abruptly at Samalga Pass (169°W), an area that divides waters of the Alaska Coastal Current to the east and the Alaskan Stream to the west (Hunt and Stabeno 2005). To the east of Samalga Pass, the Alaska Coastal Current is composed of warmer, nutrient-poor coastal water, while water to the west is oceanic, colder, and rich in nutrients (Hunt and Stabeno 2005; Figure 1). Those species with pelagic larvae are probably strongly influenced by oceanic currents and water mass origin (Hunt and Stabeno 2005; Logerwell et al. 2005); in addition, clockwise circulation patterns in the Aleutians Islands have been identified that could entrain larvae (Ladd et al. 2005).

A broad-scale examination of the genetic population structure of Pacific cod found a sample from the Aleutians Islands (Adak Island) to be distinct from both a Bering Sea (Unimak Pass) and a Gulf of Alaska (Kodiak Island) sample (Cunningham et al. 2009). This result led to the hypothesis that deep

TABLE 2. Sample locations, date, and number of samples at each location. Sample "group" refers to whether the sample is from the Aleutian Islands (AI) or the eastern Bering Sea (EBS).

Location	Group	Abbreviation	Number	Latitude	Longitude	Date (month/year)
Near Islands	AI	NR	192	52.56°N	174.29°E	2/2005
Kiska Island	AI	SK	96	51.80°N	177.79°E	3/2005
Amchitka Island	AI	AM	50	51.20°N	179.80°W	2/2005
Tanaga Island	AI	TN	96	51.67°N	178.27°W	3/2005
Great Sitkin Island	AI	GS	118	52.10°N	175.86°W	3/2005
South Atka Island	AI	AT	96	51.97°N	174.38°W	3/2007
North Amlia Island	AI	A6	96	52.42°N	173.80°W	2/2006
North Amlia Island	AI	A7	96	52.28°N	173.69°W	2/2007
Unimak Pass	EBS	UP	96	54.50°N	165.30°W	3/2005
Pribilof Islands	EBS	PR	96	56.83°N	170.00°W	2/2004

passes and current patterns may limit Pacific cod movement at all life history stages and create complex population structure (Cunningham et al. 2009). The sampling scheme in the present study was designed to address this question by investigating genetic structuring of Pacific cod in the BSAI on a fine scale. Samples were taken during spawning season from the western Aleutian Islands, spanning all the major North American passes of the Aleutian Archipelago, east to Unimak Pass and as far north as the Pribilof Islands. Several studies have found a lack of homogeneity and limited lifetime dispersal in other fish species that span the Aleutian Islands and Bering Sea, including Pacific ocean perch *Sebastes alutus* and northern rockfish *S. polyspinis* (Palof et al. 2011; Gharrett et al. 2012). Therefore, we hypothesized that multiple populations of Pacific cod exist in the BSAI and both depth and current patterns define stock boundaries.

METHODS

Samples, which consisted of fin clips collected at a particular location, span the Aleutian Island chain and a portion of the Bering shelf, from the Near Islands in the west, Unimak Pass in the east, and the Pribilof Islands in the north (Table 2; Figure 1). Whether the sample originated from the Aleutian Islands or the eastern Bering Sea is specified in Table 2. All samples were stored in nondenatured ethanol and were collected during the spawning season (February–March), when the majority of commercial cod catches occur (Thompson et al. 2010). Most samples were taken from commercial fishing trawlers, with the exception of the Unimak Pass sample, which was obtained during a research survey with pot gear. The Pribilof Islands sample was frozen until ethanol became available. Two samples were collected north of Amlia Island, in 2006 and 2007, in order to investigate temporal stability. No samples were analyzed in previous studies. Because sampling was performed opportunistically, it was not feasible to record the spawning condition of all fish collected. The Unimak Pass samples were exclusively spawning fish, and maturity state was recorded for the samples from Near, Kiska, and Amchitka islands. Spawning state was not recorded for the remaining samples. For the Near Island and the Amchitka Island samples, sufficient numbers of both spawning (Near: $n = 136$, Amchitka: $n = 30$) and immature fish (Near: $n = 16$, Amchitka: $n = 20$) were genotyped to assess genetic differentiation between them, while the Kiska Island sample consisted almost exclusively of spawning fish. The DNA was extracted using Qiagen 96 Blood and Tissue kits (QIAGEN Inc., Valencia, California) according to the manufacturer's instructions.

To determine how many samples and how many microsatellite loci are required to provide sufficient statistical power to reject the null hypothesis when it is false, a data set was simulated with similar allele frequencies to the data set of Cunningham et al. (2009) using POWSIM version 4.0 software. POWSIM estimates the statistical power associated with rejecting the null hypothesis of genetic homogeneity under varying numbers of microsatellite loci, sample sizes, alleles, and allele frequencies (Ryman and Palm 2006). The analysis indicated that 20 microsatellite loci and 100 samples per location would be sufficient to detect significant levels of allele frequency differentiation if F_{ST} was on the order of 0.001. In a previous study of Pacific cod within North America, F_{ST} values ranged from -0.0012 to 0.0163 and the smallest significant value was 0.006; thus, the slightly more conservative value of 0.001 was chosen as an estimate of expected F_{ST} values for the current study.

To attain the goal of 20 microsatellite loci, 19 walleye pollock loci (O'Reilly et al. 2000), 19 Atlantic cod loci (Miller et al. 2000; Stenvik et al. 2006; Wesmajervi et al. 2007; Skirinsdottir et al. 2008), and 11 Pacific cod loci (Cunningham et al. 2009) were screened. One additional locus (*Gma107*), which had not been used previously (Canino et al. 2005), was also evaluated. Although 59 microsatellite loci have been developed for Atlantic cod, microsatellites were limited to tetranucleotides because they tend to have less stutter than dinucleotides. Of the 38 walleye pollock and Atlantic cod loci, 14 were variable and amplified well: *GmoG13*, *GmoG16*, *Gmo8*, *GmoG18*, *Gmo19*, *GmoC82*, *GmoC83*, *GmoC88*, *GmoG5*, *Tch5*, *Tch9*, *Tch13*, *Tch17*, and *Tch18*. Further screening of 96 samples revealed that

TABLE 3. Microsatellite loci used in this study and associated information in addition to those described in Canino et al. (2005).

Locus	Repeat sequence	Primer sequence 5′–3′	Annealing temperature (°C)	Accession number.	Reference
Gmo19	(GACA)	F: CACAGTGAAGTGAACCCACTG R: GTCTTGCCTGTAAGTCAGCTTG	50	AFI159232	Miller et al. (2000)
Gmo37	(GACA)	F: GGCCAATGTTTCATAACTCT R: CGTGGGATACATGGGTACT	46	AFI159237	Miller et al. (2000)
GmoC82	$(TG)_{13}$	F: CCTGGAAGAGAACCCTTTTCA R: GTTTCTTGGGAACTCCTACATT CCTACTCTC	51	50353897	Stenvik et al. (2006)
GmoC83	$(TG)_{11}$	F: CGGTGCGTTGGATTTCAT R: GTTTCTTAACTGCTCTCCTGATT TTGTTTT	51	50353980	Stenvik et al. (2006)
GmoG5	(AG)	F: GTCTCTTGCCCTACGTTTGTTCG R: GTTTCTTTTCTGGTTGTGGTG TGCCCTGAC	65	DQ836317	Wesmajervi et al. (2007)
GmoG13	(GTCA)	F: ATGCGCTAGACACAGGGCTT GTT R: GTTTCTTGACGGACTGTGT CAGTGTCTGGTG	55	DQ648550	Wesmajervi et al. (2007)
GmoG16	(TGTC)	F: GTCTGCACATCTTGGTGCGTG ATT R: GTTTCTTTATGGTTTCAATACCG CCGGTTTC	55	DQ648552	Wesmajervi et al. (2007)
Tch13	(GT)	F: TTTCCGATGAGGTCATGG R: AATCCACTGGTGCAGACC	54	AF178503	O'Reilly et al. (2000)
Tch20	$(GA)_6$GGGAA$(GGAA)_3$ GGAT$(GGAA)_2$GGAAT $(GAAA)_{10}$GAAG$(GAAA)_5$	F: ACATTGTAAACGGCGATTC R: TGGTTAGTCTGAGACCCAG	54	AF178509	O'Reilly et al. (2000)

only *GmoG13*, *GmoG16*, *Gmo19*, *GmoC82*, *GmoC83*, *GmoG5*, and *Tch13* were in Hardy–Weinberg equilibrium (HWE) and could be scored reliably (Table 3), for a total of 19 loci screened in the present study.

Polymerase chain reactions (PCR) were performed as described in Canino et al. (2005), with 55°C used as the annealing temperature for all loci. Reruns for the Pribilof sample were performed differently since the tissue had degraded slightly before being stored in ethanol. For these PCRs, the Velocity PCR kit (Bioline, London, UK) in a 5-μL volume was used following the manufacturer's instructions, with 3% DMSO (v/v), 2 mM $MgCl_2$, 0.4 μM each primer, and 0.05 U/μL Velocity DNA polymerase.

Owing to equipment failures, two different types of genotyping platforms were used. Seven loci (*Gma100*, *Gma101*, *Gma102*, *Gma103*, *Gma106*, *Gma109*, and *Tch20*) were run on one of two MegaBACE 1000 DNA sequencers (Amersham Biosciences/GE Healthcare, Uppsala, Sweden). All other loci were run on an ABI3730xl (Applied Biosystems/Life Technologies Corporation, Carlsbad, California) via contract with Eurofins MWG Operon (Huntsville, Alabama). Cross-platform results were compared by running a set of 96 samples at each locus on each platform. MegaBACE data were scored using Genetic Profiler version 2.2 software (Amersham Pharmacia Biotech, Piscataway, New Jersey) and ABI data were scored using GeneMarker version 1.85 (SoftGenetics LLC, State College, Pennsylvania). Data quality was evaluated using a double-blind genotyping protocol, where two individuals score the gel image independently. This method was employed on all samples run on the ABI platform (12 loci), and multiple runs of the same sample were compared for reliability on samples run with the MegaBACE genotyping platform. All loci were rerun up to four times when scores were questionable to ensure scoring reliability.

Several tests were performed to ensure that data met expectations of quality and neutrality. Tests for HWE, F_{IS} calculations, and associated *P*-values were performed using GENEPOP on the web (genepop.curtin.edu.au/) with a burn-in period of 10,000 iterations, 1,000 batches, and 10,000 iterations per batch. Observed and expected heterozygosity values and pairwise tests for linkage disequilibrium were analyzed in GENETIX 4.05 (Belkhir et al. 2000), and significance for pairwise tests was determined using 1,000 permutations of the data. Locus neutrality was assessed with fdist2 (Beaumont and Nichols 1996) to

determine whether any of the loci were under selection. Fdist2 provides a comparison between observed F_{ST} values as a function of heterozygosity with those simulated with the same size and number as the actual data set, using the island model with 100 islands and an infinite alleles mutational model. This allows 0.025, 0.5, and 0.975 quantiles for a selectively neutral distribution to be estimated. Micro-Checker version 2.2.3 was used to look for the presence of null alleles, upper allele dropout, and errors owing to stutter (Van Oosterhout et al. 2004).

Tests for genetic differentiation between samples were performed using several standard methods, although samples were not assumed to represent populations. Pairwise theta (Weir and Cockerham 1984), an estimator of F_{ST}, was calculated in GENETIX, and significance was assessed with 1,000 permutations of the data. Pairwise theta and a 95% confidence interval (CI) for the combined Bering Sea samples versus the combined Aleutian Islands were calculated in FSTAT 2.9.3.2 (Goudet 2001). Exact tests for allele frequency differentiation among samples (genic tests) were performed using GENEPOP with the same burn-in, iterations, and batches as the F_{IS} calculations. Corrections for multiple tests were performed using the sequential goodness of fit metatest (SGoF) method, which increases the statistical power of the test as the number of comparisons increases, unlike the Bonferroni correction (Carvajal-Rodríguez et al. 2009). A locus-by-locus analysis of molecular variance (AMOVA) was implemented in Arlequin version 2.000 to examine divergence within and among groups of samples, with 15% missing samples allowed. Groupings were determined systematically using adjacent-sample pooling; adjacent sites were pooled to determine whether samples represented distinct populations. This method was chosen because isolation-by-distance population structure has been demonstrated in Pacific cod, implying that nearest neighbors are most likely to be similar (Cunningham et al. 2009; Canino et al. 2010). The best grouping was defined to be the one with the highest differentiation among groups (F_{CT}) and nonsignificant differentiation within groups (F_{SC}). Because theta is constrained to lower values at loci with high heterozygosity, a trend that was observed in the data, single estimates of D_{EST} were used to determine the most influential loci. A standardized estimate of differentiation is represented by D_{EST}, and both single and multilocus D_{EST} values were calculated using software for the measurement of genetic diversity (SMOGD; Jost 2008; Crawford 2010).

To compare population structure to physical oceanographic and landscape features, approaches were used that incorporate genetic and geographic information from each sample. The maximum distance Monmonier algorithm is designed to transfer data contained in a genetic distance matrix onto a geographical map to identify boundaries (Monmonier 1973; Manel et al. 2003). This algorithm, implemented in the program BARRIER version 2.2 (Manni et al. 2004), creates a connectivity network among samples given a matrix of pairwise multilocus genetic distance values. In this case, residual F_{ST} values based on the expected slope of the isolation-by-distance (IBD) relationship were used rather than F_{ST} estimates, which is recommended in the case of IBD (Manni et al. 2004). Barriers are created by extending from the edge with the largest genetic distance to the edge of the adjacent network with the largest genetic distance until the edges of the network are reached. One drawback of this program is that it does not estimate the number of barriers; the user provides the number they suspect and the program provides the location. However, the significance of each barrier can be assessed using bootstrapping; 100 resampled data sets were created by randomly resampling original sample names. Pairwise theta was calculated in the bootstrapped data sets using the R package Geneclust version 1.0.1. (Weir and Cockerham 1984; Ancelet and Guillot 2006), and significance was assessed based on the proportion of resampled data sets that identified a barrier in the same location as the true data. Because the data included a temporal sample at North Amlia Island, two data sets were constructed: one with all samples except North Amlia 2006 and another with all data except North Amlia 2007, so that the effect of the different temporal samples could be examined separately (Table 2). A second spatial analysis, Wombling (Crida and Manel 2007), takes into account both spatial information on an (*x*,*y*) grid, and multilocus genetic information in a systemic function:

$$S(x, y) = \sum_j \left\| \nabla f_j(x, y) \right\|, \quad (1)$$

where f_j is the estimation of allele frequencies *j* at each point on the (*x*,*y*) grid, $\nabla f_j(x, y)$ is the gradient of allele frequencies f_j with respect to coordinates *x* and *y*, and *j* is the summation over all alleles at all loci. Allele frequencies are estimated at each point using a local polynomial regression approximation. Calculations were performed using the R (2.12.1; R Development Core Team 2011) software package wombsoft, with settings implemented as suggested in Crida and Manel (2007). Statistical significance of boundaries was evaluated using a binomial test at a fixed *P*-value ($\alpha = 0.05$), which determines whether the gradient of allele frequencies is greater than a given percentile, P_B. Population structure was further explored using Structure 2.3.3 (Pritchard et al. 2000); given genotype data, individuals are assigned to populations such that loci are in Hardy–Weinberg and linkage equilibrium. Preliminary trials indicated that a million Markov Chain Monte Carlo (MCMC) iterations would be sufficient for convergence. Analyses, which were repeated five times, discarded the first 30,000 iterations and simulated $K = (1, 2, \ldots, 5)$ populations. Simulations were performed both with and without using sample locations as priors. A further spatial analysis, Geneland, a Bayesian model implemented with MCMC that uses individual multilocus georeferenced genotypic data to identify spatial organization of populations, was implemented with R software. Like Structure, Geneland clusters population genetics data from samples into populations such that each population is in HWE with linkage equilibrium between loci; however, it also takes into account spatial coordinates to group spatially proximate samples. Analyses were performed

using 100,000 iterations and a burn-in of 200, thinning every 100 iterations (Guillot et al. 2005). To examine whether any geographic trends were present in genetic diversity, the average estimated number of alleles at a locus was calculated for each population (Leberg 2002; Table A.1).

A Mantel test was implemented in "Isolation by distance, web service" (IBDWS) to determine whether genetic differentiation was correlated with geographic distance (Jensen et al. 2005). This program also calculated the slope of the relationship between the genetic and geographic distances using reduced major axis regression. Partial Mantel tests were implemented in the same program to determine whether a correlation was present between genetic differences and oceanographic features after controlling for geography. Linearized F_{ST}, i.e., $F_{ST}/(1-F_{ST})$, was used as input data for this test, which is standard for isolation-by-distance testing (Rousset 1997) and was used for comparison with work by Cunningham et al. (2009). Distances between points were determined using Google earth software version 5.2.1.1588 (Microsoft Corp., Seattle, Washington) and were calculated along the Aleutian Island chain rather than across the Bering Sea to account for Pacific cod depth preference of less than 500 m. Four "indicator" matrices were used in the partial Mantel tests: the depth of the deepest pass spanned between points, whether any two points spanned Amchitka Pass, whether any two points spanned Samalga and Amukta passes, and a fourth that tallied the number of passes over 260 m depth between points. Significance was determined using 1,000 permutations of the data in all cases.

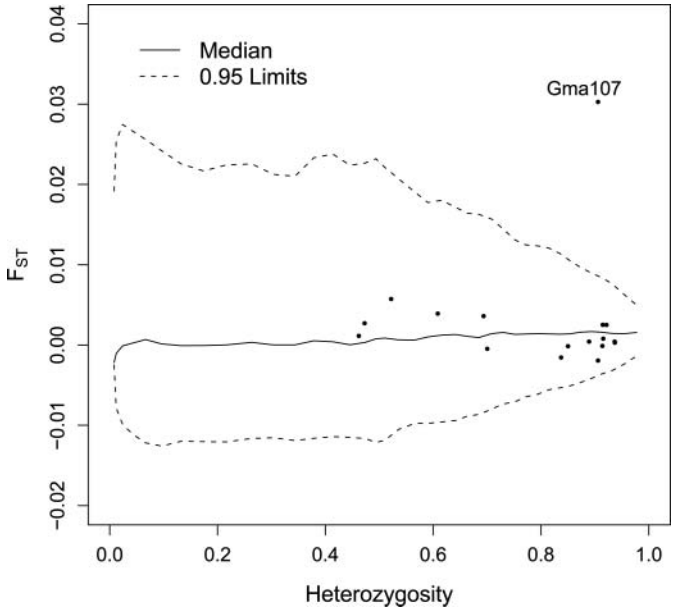

FIGURE 2. Values for F_{ST} estimated from 18 microsatellite loci are all within the 95% confidence intervals expected for neutral loci, with the exception of *Gma107*.

RESULTS

All loci and populations conformed to HWE, with two exceptions: locus *Gma106* was discarded owing to a clear excess of heterozygotes (data not shown), and locus *Gma107* was significantly out of HWE in 2 of 10 populations. The remaining 17 loci were in HWE, as were all 10 samples. Sample and locus statistics are given in Table A.1, and significant F_{IS} values after SGoF correction are shown in bold in the table. All loci conformed to the assumption of neutrality, with the exception of *Gma107* (Figure 2). The remaining 17 loci were all within the 95% quantiles expected for neutral loci. Allele frequency histograms by sample location were unremarkable at *Gma107*, discounting the possibility that this locus revealed selective differences. There was no evidence for null alleles, upper allele dropout, or error due to stutter at any locus except for *Gma107*, which contained null alleles. These 17 loci were retained for further analyses. Global F_{ST} was 0.001, and the locus significance, based on single-locus D_{EST} values, were (in order from highest to lowest D_{EST} value): *Gma100*, *Gma109*, *Tch20*, *Gmo37*, *Gmo19*, *Gma103*, *Gma101*, *Gma102*, *Gma104*, *GmoG13*, *Gma105*, *GmoG5*, *GmoC82*, *GmoG16*, *Tch13*, *GmoC83*, and *Gma108*. Of the 136 locus-by-locus comparisons for linkage disequilibrium, none were significant after SGoF correction.

Pairwise population differentiation statistics (genic tests, F_{ST} and D_{EST}) indicate differentiation among samples, both temporally and geographically, and are consistent with fine-scale population structure (Table 4). The temporal samples at Amlia Island are significantly differentiated from each other. Pairwise statistics that include the Unimak and Pribilof samples contain the most significant comparisons. When all samples from the Aleutian Islands were compared with all eastern Bering Sea samples, the F_{ST} estimate was 0.006 (95% CI: 0.005, 0.008). Mature samples were not significantly differentiated from immature ones from the Near Islands ($F_{ST} < 0$, $P = 0.62$) and Amchitka Island ($F_{ST} < 0$, $P = 0.74$), and removal of the immature samples did not change the F_{ST} value between the Near Islands and Amchitka Island samples. The AMOVA grouping pattern that included three groups (Unimak Pass, all Aleutian Island samples, and Pribilof samples; Table 5; Figure 3) resulted in significant differentiation among groups at the $\alpha = 0.05$ level ($F_{CT} = 0.0016$, $P = 0.0039$), but no additional significant differentiation within groupings ($F_{SC} = 0.0005$, $P = 0.0948$). However, the grouping that included four populations and further structuring within the Aleutian Islands appeared to be marginally better and had a slightly higher F_{CT} value ($F_{CT} = 0.0017$, $P \leq 0.05$). A break between Tanaga Pass and Great Sitkin Island, at Tanaga Pass, most fully accounted for this structure because it resulted in the highest overall F_{CT}.

With the exception of Structure and Geneland, spatial analyses were concordant with the AMOVA result; they identified the presence of a barrier between the eastern Bering Sea and the Aleutian Islands. Neither Structure nor Geneland analyses showed evidence for population structure. However, when three barriers were imposed on the Monmonier algorithm, both the North Amlia 2006 and the North Amlia 2007 data sets

TABLE 4. Multilocus pairwise population differentiation statistics with F_{ST} values above the diagonal and D_{EST} values below. Significance for both F_{ST} and pairwise genic test results are given above diagonal; values in bold italics indicate significant F_{ST} and asterisks (*) indicate comparisons with significant genic test values (*P*-values not shown). Values for F_{ST} and genic test *P*-values were corrected for multiple tests using the sequential goodness-of fit-metatest (SGoF) method (Carvajal-Rodríguez et al. 2009). Values for D_{EST} are the harmonic mean of locus-specific D_{EST} across loci.

Sample location	North Amlia 2006	North Amlia 2007	Great Sitkin	Near Island	Pribilof Islands	Amchitka Pass	South Atka	South Kiska Island	Tanaga Island	Unimak Pass
North Amlia 2006		0.0017*	−0.0007	0.0009*	***0.0023****	−0.0003*	−0.0002	0.0017*	0.0008	***0.0019****
North Amlia 2007	0.0006		0.0000	−0.0002	0.0016	−0.0001	0.0003	0.0005	0.0008	***0.0028****
Great Sitkin	0.0000	0.0000		0.0000	***0.0025****	−0.0003	−0.0003	***0.0021***	0.0010	0.0006
Near Island	0.0003	0.0000	0.0000		***0.0034****	0.0008*	0.0005	0.0011	0.0001	***0.0021***
Pribilof Islands	0.0052	0.0000	0.0047	0.0049		0.0006*	0.0016*	***0.0032****	0.0019*	***0.0023***
Amchitka Pass	0.0000	0.0000	0.0001	0.0007	0.0010		−0.0007	−0.0008	−0.0010	−0.0002
South Atka	0.0000	0.0000	0.0000	0.0002	0.0005	0.0000		0.0004	−0.0005	0.0013*
South Kiska Island	0.0002	0.0000	0.0000	0.0001	0.0003	0.0000	0.0000		0.0011	***0.0026****
Tanaga Island	0.0003	0.0005	0.0020	0.0000	0.0007	0.0000	0.0000	0.0001		***0.0022***
Unimak Pass	0.0008	0.0005	0.0000	0.0002	0.0013	0.0001	0.0002	0.0010	0.0033	

TABLE 5. Analysis of molecular variance results for all loci: fixation indices represent the average over all loci; F_{ST}, variance of samples relative to total; F_{SC}, variance among samples within groups; F_{CT}, variance of samples among groups. Sample abbreviations in groupings are defined in Table 2. Values in bold italics are significant at the $\alpha = 0.05$ level, asterisks (*) indicate that $P \leq 0.1$, and shaded rows represent the best grouping pattern.

Grouping	Groups	Fixation index		
		F_{ST}	F_{SC}	F_{CT}
(PR) (UP,A7,A6,AT,GS,TN,AM,SK,NR)	2	***0.00235***	***0.00077***	***0.00158***
(PR,UP) (A7,A6,AT,GS,TN,AM,SK,NR)	2	***0.00172***	***0.00068***	***0.00105***
(PR,UP,A6) (A7,AT,GS,TN,AM,SK,NR)	2	***0.00133***	***0.00079***	***0.00054***
(PR,UP,A7) (A6,AT,GS,TN,AM,SK,NR)	2	***0.00124***	***0.00086***	0.00038*
(PR,UP,A7,A6) (AT,GS,TN,AM,SK,NR)	2	***0.00106***	***0.00099***	0.00007
(PR,UP,A7,A6,AT) (GS,TN,AM,SK,NR)	2	***0.00101***	***0.00104***	−0.00003
(PR,UP,A7,A6,AT,GS) (TN,AM,SK,NR)	2	***0.00114***	***0.00089***	0.00025
(PR,UP,A7,A6,AT,GS,TN) (AM,SK,NR)	2	***0.00103***	***0.00102***	0.00000
(PR,UP,A7,A6,AT,GS,TN,AM) (SK,NR)	2	***0.00111***	***0.00095***	0.00016
(PR,UP,A7,A6,AT,GS,TN,AM,SK) (NR)	2	***0.00095***	***0.00106***	−0.00011
(PR) (UP) (A7,A6,AT,GS,TN,AM,SK,NR)	3	***0.00207***	0.00045*	***0.00162***
(PR) (UP,A7) (A6,AT,GS,TN,AM,SK,NR)	3	***0.00132***	***0.00077***	0.00056*
(PR) (UP,A7,A6) (AT,GS,TN,AM,SK,NR)	3	***0.00114***	***0.00089***	0.00024
(PR) (UP,A7,A6, AT) (GS,TN,AM,SK,NR)	3	***0.00110***	***0.00091***	0.00019
(PR) (UP,A7,A6, AT,GS) (TN,AM,SK,NR)	3	***0.00123***	***0.00065***	***0.00058***
(PR) (UP,A7,A6, AT,GS,TN) (AM,SK,NR)	3	***0.00117***	***0.00080***	0.00036
(PR) (UP,A7,A6, AT,GS,TN,AM) (SK,NR)	3	***0.00125***	***0.00072***	0.00052
(PR) (UP,A7,A6, AT,GS,TN,AM,SK) (NR)	3	***0.00125***	***0.00081***	0.00044
(PR) (UP) (A7) (A6,AT,GS,TN,AM,SK,NR)	4	***0.00162***	0.00046*	***0.00115***
(PR) (UP) (A7,A6) (AT,GS,TN,AM,SK,NR)	4	***0.00131***	***0.00062***	0.00068*
(PR) (UP) (A7,A6,AT) (GS,TN,AM,SK,NR)	4	***0.00123***	0.00061*	0.00062*
(PR) (UP) (A7,A6,AT,GS) (TN,AM,SK,NR)	4	***0.00182***	0.00012	***0.00171***
(PR) (UP) (A7,A6,AT,GS,TN) (AM,SK,NR)	4	***0.00124***	0.00047*	***0.00077***
(PR) (UP) (A7,A6,AT,GS,TN,AM) (SK,NR)	4	***0.00130***	0.00039	***0.00091***
(PR) (UP) (A7,A6,AT,GS,TN,AM,SK) (NR)	4	***0.00136***	0.00044	0.00092*

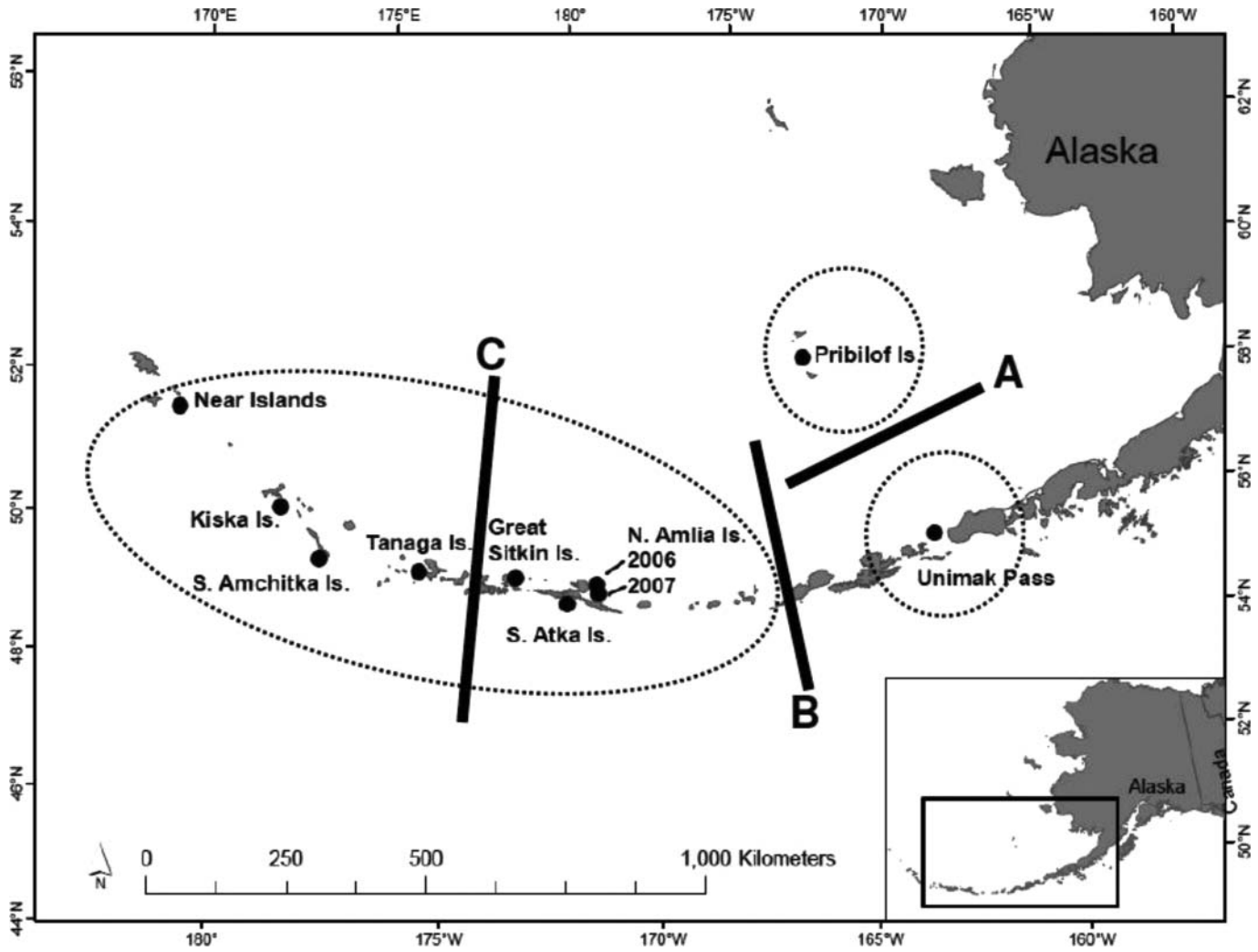

FIGURE 3. Circles encompass significant analysis of molecular variance groupings when $\alpha = 0.05$, and lines represent barrier results. Significance values (representing the probability that the null hypothesis of no barrier is correct), from the North Amlia data set followed by values from the North Amlia 2007 data set, were 0.060/0.060 at barrier A, 0.003/0.001 at barrier B, and 0.133/0.177 at barrier C.

identified barriers between Unimak Pass and Pribilof Islands, between Unimak Pass and the Aleutian Islands, and between Tanaga Pass and Great Sitkin, and (locations A, B, and C, respectively, on Figure 3). The proportion of bootstrapped data sets that randomly identified these locations as barriers, listed with values from the North Amlia 2006 data set followed by values from the North Amlia 2007 data set, were 0.060/0.060 between Pribilof Islands and Unimak Pass (barrier A), 0.003/0.001 between the Aleutian Islands and Unimak Pass, (barrier B), and 0.133/0.177 between Tanaga Pass and Great Sitkin Island (barrier C; Figure 3). Wombling results supported two barriers: one between the eastern Bering Sea and the Aleutian Islands and a second within the Aleutians west of Amchitka Pass, but provided no evidence for a barrier between the Unimak and Pribilof samples (Figure 4). A range of values for the allele frequency gradient P_B between 0.05 and 0.5 resulted in the same pattern; both samples from the Near and Kiska islands were in the area of significant change in allele frequency, and an area of strong allele frequency change appeared between the Aleutian Islands and the eastern Bering Sea. Above $P_B = 0.5$ and below $P_B = 0.05$, the signal disappears; therefore $P_B = 0.1$ was chosen because it clearly illustrates this pattern (Figure 4). In addition to identifying a barrier between the eastern Bering Sea and the Aleutian Islands, spatial analyses suggested the presence of additional barriers within the Bering Sea and within the Aleutian Islands.

Unexpectedly, genetic diversity increased proportional to the number of fin clips per sample. A simple linear regression of the effect of genetic diversity on sample size was highly significant ($P < 0.001$). However, there was no relationship between geographic location and genetic diversity; comparison of the five locations with 95 samples each (Pribilof Islands, South Atka Island, South Kiska Island, Tanaga Pass, and Unimak Pass) had similar genetic diversity values (mean = 18.98, $\sigma = 0.8$; Table A.1). The sample with the highest genetic diversity was the

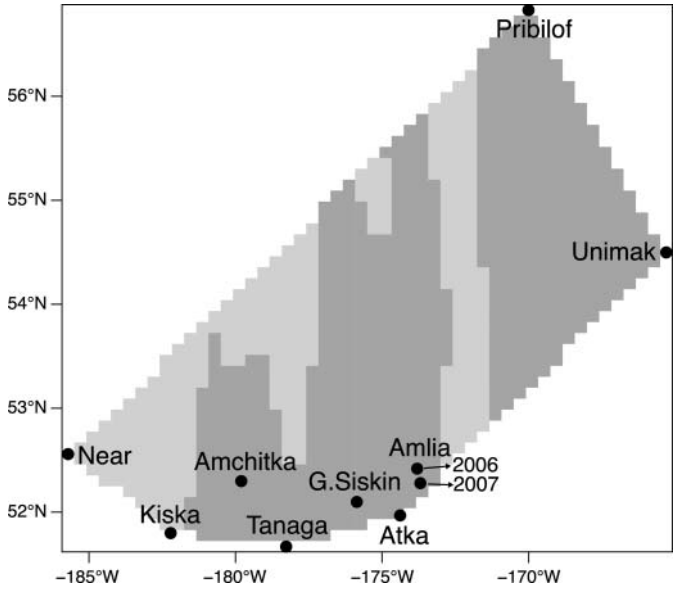

FIGURE 4. Map of boundary elements that are significant at $P_B = 0.1$ (shown in light grey). Dark grey areas represent regions of no significant change.

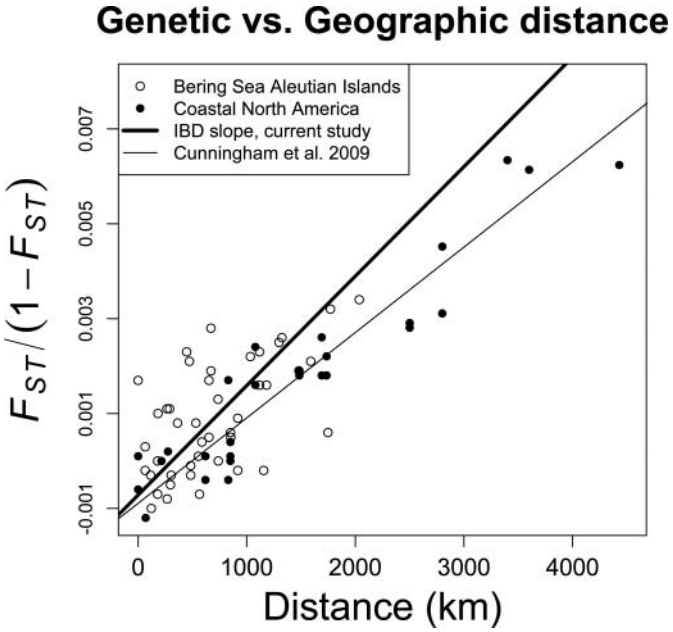

FIGURE 5. Isolation by distance (IBD), with genetic "distance" represented by $F_{ST}/(1 - F_{ST})$. The thicker solid line represents the reduced major axis regression line through pairwise samples from the present study (open circles), and the thin line corresponds to samples from the North American range of Pacific cod (solid circles; Cunningham et al. 2009).

farthest to the west from Near Island, which also had the highest number of fin clips. The lowest genetic diversity was found in the Amchitka Island sample, which was also at the western end of the range, but had the lowest number of fin clips in the sample.

The Mantel test showed a significant isolation-by-distance pattern ($r = 0.4969$, $P[r \leq 0] = 0.0110$). The slope of the reduced major axis regression of genetic with respect to geographic distance was 2.307×10^{-6} (95% CI: 1.730×10^{-6}, 2.885×10^{-6}) and the intercept was -7.175×10^{-4} (95% CI: -1.219×10^{-3}, -2.158×10^{-4}; Figure 5). After accounting for geographic distances, three of the partial Mantel tests showed no significant correlation between genetic differences and any other factor. Results from the three tests were as follows: the depth of the deepest pass between samples ($r = -0.3040$, $P[r \leq 0] = 0.9200$), whether two points span Amchitka Pass ($r = -0.3667$, $P[r \leq 0] = 0.9640$), and number of deep passes between points ($r = -0.0350$, $P[r \leq 0] = 0.6330$). However, whether two samples spanned Samalga and Amukta passes was marginally significant ($r = 0.3495$, $P[r \leq 0] = 0.0813$).

DISCUSSION

Temporal Differentiation and Sampling Error

While the previous study on Pacific cod in North America found temporal stability in the two samples examined (Unimak Pass and Kodiak Island; Cunningham et al. 2009), the temporal samples at Amlia Island (2006 and 2007) were genetically different. The estimated F_{ST} value between the two samples (0.0017; Table 4) is on the order of those found between the Aleutian Islands and the eastern Bering Sea (range = 0.0006–0.0034, mean over 11 pairs = 0.0020). The fact that the two samples are significantly different from each other, as determined by the genic test (but not F_{ST} estimate), suggests that temporal fluctuations exist over time scales as short as 1 year. It was not possible to collect all fish from the same year and samples were taken from 2004 to 2007; thus, the question of temporal stability is relevant. However, the lack of stability at Amlia Island does not contradict the conclusions in this paper because the AMOVA, Barrier, and Wombling test results were robust for both years of the Amlia Island sample. Given temporal stability in two previous spawning areas (Cunningham et al. 2009), but not in the current study, the question of temporal stability in Pacific cod should be a topic for future research.

The finding that the average number of alleles per locus increases with increasing sample size is also of importance when evaluating sampling error. The highest number of alleles was found in the sample with the greatest number of fin clips (Near Islands, $n = 192$). This indicates that 100 fin clips per sample may be insufficient to thoroughly capture the pattern of allele frequencies at each sampling location and that sample size may be an additional source of variation among samples.

Genetic Differentiation between the Aleutian Islands and Eastern Bering Sea

Analyses of fine-scale population structure within the commercially important BSAI management unit consistently reject the null hypothesis of panmixia, or genetic homogeneity of Pacific cod. Higher numbers of polymorphic microsatellites typically result in higher statistical power (Ryman and Palm

2006), and the 17 microsatellites used in this study increased statistical power for detecting low levels of genetic differentiation typical of marine fish species over previous studies. With the exception of Structure and Geneland, which may have limited ability to identify small but significant levels of genetic differentiation that are often characteristic of marine fish species (Palof et al. 2011), analyses were fairly concordant with two main themes. First, significant isolation by distance was observed; genetic differences between samples are proportional to the distance that separates them, a result consistent with previous work (Cunningham et al. 2009; Figure 5). Secondly, differentiation is not continuous and is marked by several barriers to gene flow that have not been observed with previous analyses. The most notable area of limited gene flow occurs between the Aleutian Islands and the eastern Bering Sea.

Research on genetics, oceanography, and species diversity all suggest that the BSAI is a complex ecosystem. Isolation by distance demonstrated in the present study is supported by two prior genetic studies of Pacific cod (Cunningham et al. 2009; Canino et al. 2010). The slope of the isolation-by-distance line is similar to that observed with coastal North American samples (Figure 5, slope $= 2.31 \times 10^{-6}$ versus 1.57×10^{-6}; Cunningham et al. 2009). The previous analysis used this slope value to conclude that the mean dispersal per generation of Pacific cod is extremely limited, less than 100 km per generation, and the higher slope in the present study implies even more limited dispersal. All spatial analyses and F_{ST} results provide evidence for a barrier to gene flow between the eastern Bering Sea and the Aleutian Islands, an area that includes two passes, Amukta Pass and Samalga Pass. Of all the passes in the Aleutian Islands region, Samalga Pass is known as a major biophysical transition (Logerwell et al. 2005). The species assemblages change and the number of fish species decreases from east to west of Samalga Pass; 14 of 43 demersal fish species found at Samalga Pass are not found to the west at Amukta Pass (Logerwell et al. 2005). Nearly all of these species have pelagic larvae, which are affected by current patterns. Similarly, the diet composition of fish species changes at Samalga Pass. West of Samalga Pass, Pacific cod consume increasingly more Atka mackerel *Pleurogrammus monopterygius*, as well as shrimp, squid, and other fishes, whereas east of Samalga Pass these prey species are relatively rare and Pacific cod diet primarily consists of walleye pollock (Logerwell et al. 2005). Of the two passes separating the eastern Bering Sea and the Aleutian Islands, Samalga Pass is the greater transition zone and this is reflected in diet, species abundance, and biophysical factors.

In marine species, oceanographic and current patterns can act as barriers to migration both at the species and population level. Other flow-induced range boundaries include Cape Mendocino in California, where the predominantly southward California Current is diverted from shore creating a site of strong upwelling. Vermillion rockfish *S. miniatus* (Hyde and Vetter 2009), acorn barnacle *Balanus glandula* (Sotka et al. 2004), and yellowtail rockfish *S. flavidus* (Hess et al. 2011) all demonstrate limited gene flow here. As we found at Samalga Pass, differences in species assemblages as well as the ends of species' range distributions exist to the north and south of Cape Mendocino (Hess et al. 2011). A similar situation appears off the west coast of Canada where the Alaska Current and the California Current diverge. This location has been correlated with a barrier to gene flow in the rosethorn rockfish *S. helvomaculatus* (Rocha-Olivares and Vetter 1999). Although divergent current patterns may affect species differently, they have been recognized as potential zones of change, both among and within species, as they limit gene flow.

Genetic differentiation between adjacent populations could be the result of both present and historical events. A study based on mitochondrial DNA and microsatellite markers that examined current and historical gene flow in Pacific cod across its range hypothesized that glacial maxima, which reduced available habitat and lowered global sea level by as much as 120 m, forced Pacific cod to move south along the western and eastern coasts of the Pacific Ocean (Canino et al. 2010). Such glaciations probably had a significant effect on the genetic differences that have been noted between Pacific cod from the eastern versus western Pacific Ocean (Canino et al. 2010). Following the last glacial maxima 14,000–15,000 years ago, the southern refuge population in the eastern Pacific expanded northward, resulting in present day populations of Pacific cod in the Gulf of Alaska, the Bering Sea, and the Aleutian Islands. The effective population size of Pacific cod has increased from an estimated median of 1.2×10^6 before the last glacial maxima to a current estimate of 8.0×10^7 across the entire northeast Pacific (Canino et al. 2010). Considering the evolutionary history of the species, several hypotheses could explain the differentiation observed between the Aleutian Islands and eastern Bering Sea populations. First, as populations of Pacific cod expanded northward colonization could have been accompanied by founder effects. Subsequently, oceanic conditions that limited gene flow could have prevented groups from homogenizing. Alternatively, initial colonization could have represented a single panmictic group that experienced limited gene flow between the Bering Sea and the Aleutian Islands and diverged over time. The lack of a geographic trend in genetic diversity among samples is consistent with the latter hypothesis and confirms results observed with mitochondrial DNA and microsatellites (Canino et al. 2010).

Further Differentiation within the Eastern Bering Sea

Some evidence suggests that Pacific cod that spawn at Unimak Pass may be a distinct population from the Pribilof Islands spawning group. Both data sets used in the Barrier analysis indicated a barrier between the Unimak and the Pribilof samples (significance derived by bootstrap sampling of the data, $P = 0.060$). Permutation tests of F_{ST} values and the AMOVA, which also uses permutation to calculate significance, indicated that Unimak and Pribilof samples are genetically different, but genic tests and examination of allele frequency clines did not. Although permutation test results were concordant, these tests can

have lower statistical power than Fisher's exact tests (Ryman and Palm 2006). Wombling analysis, which is based on changes in allele frequencies, showed no evidence of a break between these two samples. Therefore, more samples are needed to confirm the evidence for stock structure within the eastern Bering Sea.

There is no known transition zone in species abundance or composition between Unimak Pass and the Pribilof Islands; however, physical mechanisms coupled with adult homing to natal spawning sites could account for the observed differences. Flow north of Unimak Pass diverges into an eastward component (the Bering Coastal Current) and a northward component (Aleutian North Slope Current) toward the middle and outer Bering Sea shelf (Figure 1). It has been hypothesized that larvae spawned west of Unimak Pass are potentially influenced by currents that disperse them northward while larvae spawned on the slope are transported along the eastern Bering Sea shelf towards the eastern Bering Sea via basin and shelf currents (Stabeno et al. 1999; Lanksbury et al. 2007). In addition, owing to the presence of ice along the Bering Sea shelf through spring months (Overland and Stabeno 2004), spawning at the Pribilof Islands may occur later than at Unimak Pass. Thus, both current patterns and spawn timing may prevent mixing of Pacific cod larvae spawned at Unimak Pass and those from the Pribilof Islands area, which may not be unrealistic considering that distinct populations of Atlantic cod exist on much smaller geographic scales (Knutsen et al. 2003).

Further Differentiation within the Aleutian Islands

There is evidence for some level of genetic structure within the Aleutian Islands, which corresponds with complex oceanographic forces and range limits for other demersal fish species (Logerwell et al. 2005). The range of 9 out of 28 species observed in the Aleutian Islands, including several demersal rockfish species, does not extend west of Amchitka Pass (Logerwell et al. 2005). Similarly, Amchitka Pass is the point of allopatric speciation between the Alaska skate *Bathyraja parmifera* and the congeneric leopard skate *Bathyraja panthera* (Spies et al. 2011). Taken together, flow through the central Aleutians (between Samalga and Amchitka passes) exceeds 8.8×10^6 m^3/s and includes two passes over 260 m depth (Hunt and Stabeno 2005). In the Pacific cod data examined here, a strong cline in allele frequency was observed west of Amchitka Pass, and several results indicate that a break exists between Great Sitkin and Tanaga islands, an area corresponding to Tanaga Pass. This pattern of genetic variation within the Aleutian Islands was evident with the Wombling and AMOVA analyses and appeared with the Monmonier algorithm with both data sets but was not highly significant, $P = 0.133$ (North Amlia 2006 data set), $P = 0.177$ (North Amlia 2007 data set). A partial Mantel test, which examined the effect of Amchitka Pass on gene flow after accounting for geographic distance, was not significant. Similarly, the number of passes the between samples and the depth of the deepest pass between samples had no significant effect on genetic differentiation. Because evidence was equivocal in terms of the exact location of a break, it is unclear whether isolation by distance alone or a specific barrier to gene flow is responsible for the observed patterns of differentiation within the Aleutian Islands.

Spawning State

The limited analysis of spawning versus nonspawning fish did not provide evidence for genetic differentiation between them. Many of the samples were collected without regard to spawning state, and this result seems to indicate that those samples are a sufficient representation of fish that spawn in that area. McQuinn (1997) suggests that juvenile Atlantic herring *Clupea harengus* learn migration patterns and the location of spawning grounds from mature adults. If this were the case for Pacific cod, juveniles would be expected to be related and therefore genetically similar to mature adults in spawning areas. However, the relationship between actively spawning fish and immature fish on the spawning grounds is an important biological question that deserves further analysis before broad conclusions can be drawn.

Comparative Life History

The life history of Pacific cod and the closely related walleye pollock and Atlantic cod may lend some additional insight into the mechanisms responsible for limited gene flow. Walleye pollock and Atlantic cod show very different patterns of genetic population structure. In walleye pollock, significant levels of genetic differentiation have been observed among populations from Asia compared with those from North America. However, within Alaska, tests for genetic differentiation appear inconsistent and may reflect temporal instability (Olsen et al. 2002; O'Reilly et al. 2004). In contrast, highly differentiated populations of Atlantic cod have been found at fine-scale resolution throughout their range (Bentzen et al. 1996; Knutsen et al. 2003; Pampoulie et al. 2006).

Both larval drift and adult migration are responsible for homogenization of genetic differences. Interestingly, these three species have similar periods of planktonic larval duration. Atlantic cod females produce multiple batches of pelagic eggs from February to April, which are retained in fjords, unlike the eggs of either Pacific cod or walleye pollock (Knutsen et al. 2007). Once they hatch after 2–3 weeks the larvae are planktonic for approximately 3–4 months (Lough et al. 1989). Walleye pollock spawn multiple batches of eggs in early spring, which diffuse horizontally and hatch after approximately 20 d in the Gulf of Alaska (Hinckley et al. 1991; Brodeur et al. 1996; Laurel et al. 2008). Pacific cod follow a similar pattern, except that each female deposits only a single batch of eggs, which are demersal and slightly adhesive (Sakurai and Hattori 1996; Hurst et al. 2009). After hatching at approximately 20 d in Alaska, Pacific cod larvae are transported toward coastal nurseries, whereas walleye pollock larvae are primarily pelagic and are transported considerable distances by coastal currents (Rugen and Matarese 1988; Hinckley et al. 1991; Hurst et al. 2009). Gene flow in Atlantic cod appears to be directly linked to larval drift; eggs are retained in fjords and adults have a strong homing tendency (Stenseth et al. 2006; Knutsen et al. 2007). In walleye pollock, larval drift

and migration, coupled with large effective population size, have been used to explain the lack of population structure (Olsen et al. 2002). In Pacific cod, some level of larval retention and homing to specific spawning areas could explain observed levels of genetic population structure (Shimada and Kimura 1994).

Conclusions and Management Implications

The results of population genetics studies are often difficult to translate into information that is meaningful for fisheries management. However, it is important to understand the impact of population genetics in a management context. Under Sewall Wright's infinite-island model (Wright 1931), the number of effective migrants a population receives per generation is related to F_{ST} according to the following equation:

$$F_{ST} \approx \frac{1}{4N_e m + 1}.$$

The island model assumes an infinite number of populations, each with N_e diploid individuals, m migration rate, no selection or mutation, and equilibrium between migration and genetic drift (Wright 1931). In the model, migrants leave each year, form a migrant gene pool, and are randomly assigned to a new population, and the number of individuals refers to effective rather than census numbers. Clearly, BSAI Pacific cod does not fit the island model; however, the equation relating F_{ST} and effective number of migrants can be used as a rough approximation (Neigel 2002). Given that the estimate of F_{ST} between the combined Aleutian Islands and the combined Bering Sea data is 0.006 (95% CI: 0.005, 0.008), the estimated effective number of migrants is 41 (95% CI: 31, 49). By extrapolating from estimated biomass using average fish weight, an estimate of the total number of cod in the combined Bering Sea and Aleutian Islands is 10^9, and approximately 9% of the biomass is found in the Aleutian Islands and the remainder in the eastern Bering Sea (Thompson et al. 2010). Although the ratio between effective and census size has not been determined for Pacific cod, the value is estimated at 4×10^{-5} in Atlantic cod (Hutchinson et al. 2003). Using this conversion ratio, this would imply that the effective size is on the order of 36,000 in the Aleutian Islands and 364,000 in the eastern Bering Sea. Using the more conservative value of 36,000 results in a migration rate of 0.11% (95% CI: 0.09, 0.13) per generation. Generation time in Pacific cod has been estimated at 6 years; therefore, the current data suggest demographic independence between the Aleutian Islands and the eastern Bering Sea and far less than 10% migration per year (Canino et al. 2010).

Through 2011, Pacific cod has been managed on a BSAI-wide basis, with a single total allowable catch (TAC) calculated for the entire management area (Thompson et al. 2010). Relative fishery exploitation rates in the Aleutian Islands are higher than in the eastern Bering Sea (22% versus 17%) despite the fact that exploitable biomass in the Aleutians is more than five times smaller (Thompson et al. 2010). Single and multispecies estimates of biomass in the Aleutian Islands indicate that, since the 1970s, populations may have been in decline (Kinzey and Punt 2009), and the 2010 survey estimate for Aleutian Islands Pacific cod biomass is the lowest since the time series began in 1980 (Thompson et al. 2010). Since 2006, the Scientific and Statistical Committee for the North Pacific Fishery Management Council, which manages Pacific cod in the BSAI, has recognized that a precautionary approach to management would be to apportion catch allocations separately for the eastern Bering Sea and Aleutian Islands. Efforts are ongoing to develop a protocol for such an allocation that minimizes competition among gear types and fairly disperses individual transferrable quotas while ensuring that harvest distribution remains consistent with biomass distribution and harvest strategy. In addition, starting in 2012, a separate age-structured model for Aleutian Islands cod will be prepared.

Although previous research has provided evidence that multiple populations of Pacific cod exist in the BSAI (Cunningham et al. 2009), this study provides the most comprehensive evidence to date for genetic distinctiveness and lack of gene flow between the Aleutian Islands and eastern Bering Sea. In addition, current work indicates that further population structure exists within the eastern Bering Sea and the Aleutian Islands. The hypothesis that deep passes and current patterns may act as barriers to gene flow and result in complex population structure was partially confirmed. There was evidence that Samalga Pass acts as a barrier between the eastern Bering Sea and the Aleutian Islands, but similar barriers were not identified within the Aleutian Islands. There is a strong relationship between geographic distance and genetic distinctiveness indicating low realized dispersal in this species, and the observed genetic differences are suggestive of limited gene flow both currently and historically. Evidence for limited gene flow in Pacific cod, Pacific ocean perch, and northern rockfish (Palof et al. 2011, Gharrett et al. 2012) implies that research on population structure is important for all commercial species in the BSAI, most of which are managed as a single unit, because optimal management units may be smaller than those currently implemented.

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APPENDIX: SUPPLEMENTAL GENETIC INFORMATION

TABLE A.1. Sample size (N; total sample size for site is indicated in parentheses after each sample location), number of alleles (N_A), expected and observed heterozygosities (H_e and H_o), and estimates of F_{IS} (Weir and Cockerham 1984); significant F_{IS} values after SGoF correction are indicated by bold italic font.

		Sample location (number of samples)									
Locus	Metric	Near Island (192)	Kiska Island (95)	Amchitka Island (50)	Tanaga Island (95)	Great Sitkin Island (117)	South Atka Island (95)	North Amlia Island 2006 (83)	North Amlia Island 2007 (81)	Unimak Pass (95)	Pribilof Islands (95)
Gma100	N	181	83	41	78	110	91	74	59	90	86
	N_A	70	59	44	58	61	58	58	45	57	55
	H_e	0.9749	0.9727	0.9664	0.9721	0.9721	0.9734	0.973	0.9695	0.9733	0.9712
	H_o	0.9724	0.9518	0.9512	0.9744	0.9455	0.956	0.9595	0.9661	0.9667	0.9186
	F_{IS}	0.0054	0.0275	0.0280	0.0042	0.0320	0.0233	0.0207	0.0121	0.0124	0.0600
Gma101	N	178	94	38	87	105	85	74	74	90	64
	N_A	24	22	15	23	22	21	21	22	20	17
	H_e	0.9106	0.9182	0.8979	0.9113	0.9143	0.9066	0.9145	0.9137	0.9022	0.9088
	H_o	0.9101	0.8936	0.9211	0.8851	0.8952	0.8706	0.8919	0.8784	0.8444	0.9063
	F_{IS}	0.0034	0.0322	−0.0125	0.0346	0.0256	0.0457	0.0316	0.0455	0.0695	0.0107
Gma102	N	181	94	48	89	115	90	75	81	93	95
	N_A	15	13	14	14	12	12	13	17	16	14
	H_e	0.8897	0.883	0.8904	0.8847	0.8783	0.8797	0.8875	0.8891	0.8889	0.883
	H_o	0.8564	0.9149	0.8542	0.8764	0.8870	0.9222	0.9333	0.9136	0.8817	0.8316
	F_{IS}	0.0402	−0.0307	0.0512	0.0150	−0.0055	−0.0428	−0.0450	−0.0213	0.0135	0.0635
Gma103	N	176	94	48	92	117	91	77	77	95	82
	N_A	47	38	29	37	39	32	35	29	39	39
	H_e	0.8945	0.9239	0.9269	0.9231	0.9065	0.9121	0.9132	0.8857	0.9058	0.9225
	H_o	0.9091	0.8936	0.8750	0.9130	0.9316	0.9121	0.9351	0.9481	0.8842	0.8902
	F_{IS}	−0.0135	0.0381	0.0664	0.0164	−0.0234	0.0056	−0.0174	−0.0639	0.0291	0.0411
Gma104	N	183	79	49	91	110	88	81	77	94	48
	N_A	28	21	19	25	25	23	23	22	27	16
	H_e	0.9027	0.8964	0.8955	0.8988	0.9102	0.9117	0.9096	0.9133	0.9151	0.8819
	H_o	0.8852	0.8861	0.8980	0.8571	0.9091	0.8977	0.963	0.9091	0.9149	0.8333
	F_{IS}	0.0221	0.0179	0.0075	0.0519	0.0557	0.0211	−0.0525	0.0112	0.0056	0.0656
Gma105	N	187	91	49	92	110	94	78	80	94	81
	N_A	14	9	8	8	8	10	9	9	10	10
	H_e	0.8414	0.8277	0.8286	0.8233	0.8437	0.8292	0.8328	0.8445	0.8476	0.8349
	H_o	0.8610	0.8132	0.8367	0.8043	0.8000	0.8191	0.7821	0.8375	0.7979	0.8025
	F_{IS}	−0.0205	0.0231	0.0005	0.0285	0.0564	0.0174	0.0673	0.0146	0.0639	0.0450

TABLE A.1. Continued.

Locus	Metric	Sample location (number of samples)									
		Near Island (192)	Kiska Island (95)	Amchitka Island (50)	Tanaga Island (95)	Great Sitkin Island (117)	South Atka Island (95)	North Amlia Island 2006 (83)	North Amlia Island 2007 (81)	Unimak Pass (95)	Pribilof Islands (95)
Gma107	N	165	87	49	94	106	85	75	76	93	59
	N_A	21	17	15	21	20	18	17	16	16	15
	H_e	0.9133	0.8753	0.8884	0.9003	0.9073	0.8932	0.9010	0.9029	0.9014	0.9018
	H_o	0.7636	0.9195	0.8163	0.9043	0.8491	0.7176	0.8267	0.7237	0.9570	0.9492
	F_{IS}	0.1668	−0.0447	0.0913	0.0009	0.0689	***0.2022***	0.0891	***0.2048***	−0.0563	−0.0440
Gma108	N	187	93	46	95	116	94	82	81	95	95
	N_A	11	9	10	11	14	12	10	10	10	11
	H_e	0.4349	0.4775	0.5279	0.4619	0.4242	0.4726	0.5066	0.3829	0.4892	0.4622
	H_o	0.4759	0.4946	0.5652	0.4737	0.4052	0.4787	0.5244	0.3704	0.5158	0.4947
	F_{IS}	−0.0916	−0.0304	−0.0598	−0.0201	0.0491	-0.0076	-0.0290	0.0388	−0.0491	−0.0651
Gma109	N	174	84	43	86	111	91	74	78	90	80
	N_A	29	28	20	28	28	31	23	25	27	28
	H_e	0.914	0.9285	0.8997	0.9076	0.9224	0.9374	0.9088	0.9063	0.9132	0.9125
	H_o	0.8736	0.9167	0.9103	0.8721	0.8829	0.9451	0.8784	0.8846	0.9444	0.9125
	F_{IS}	0.0471	0.0187	0.0264	0.0449	0.0473	−0.0026	0.0403	0.0304	−0.0286	0.0063
Tch20	N	177	91	48	91	112	83	79	74	93	75
	N_A	25	21	22	24	23	23	21	23	23	22
	H_e	0.9362	0.931	0.9249	0.9399	0.9305	0.9358	0.9194	0.939	0.9392	0.9335
	H_o	0.9774	0.9451	0.9167	0.9451	0.9107	0.8795	0.9494	0.9459	0.9247	0.96
	F_{IS}	−0.0412	−0.0096	0.0194	0	0.0258	0.0662	−0.0262	−0.0006	0.0208	−0.0217
Gmo37	N	188	89	49	94	115	89	78	78	94	73
	N_A	44	37	31	35	36	39	35	34	37	38
	H_e	0.9309	0.9354	0.9413	0.9217	0.9305	0.9322	0.9384	0.9303	0.9388	0.9251
	H_o	0.9468	0.8989	0.9184	0.9574	0.9304	0.9551	0.9744	0.9615	0.9149	0.9452
	F_{IS}	−0.0145	0.0446	0.0346	−0.0335	0.0044	−0.0189	−0.0318	−0.0271	0.0308	−0.0148
GmoG13	N	186	93	49	94	112	94	79	80	88	71
	N_A	12	13	11	13	14	12	12	12	13	10
	H_e	0.8471	0.8526	0.8519	0.8585	0.8374	0.8289	0.8519	0.8453	0.8539	0.8414
	H_o	0.871	0.8925	0.898	0.8936	0.8393	0.8617	0.8101	0.8125	0.8068	0.8732
	F_{IS}	−0.0255	−0.0414	−0.0437	−0.0356	0.0022	−0.0343	0.0554	0.0451	0.0608	−0.0308
GmoG16	N	190	94	49	90	111	94	79	70	90	89
	N_A	6	4	5	5	5	5	4	5	6	3
	H_e	0.4743	0.5332	0.5185	0.5635	0.5064	0.5171	0.4976	0.483	0.5826	0.4992
	H_o	0.4632	0.5426	0.5306	0.5667	0.4595	0.4681	0.443	0.4143	0.5667	0.6067
	F_{IS}	0.0262	−0.0122	−0.013	−0.0001	0.0972	0.1002	0.1159	0.1492	0.0329	−0.2099
Gmo19	N	189	90	49	93	113	90	80	79	90	70
	N_A	30	23	23	27	26	33	28	24	29	21
	H_e	0.9219	0.9119	0.893	0.8898	0.9195	0.917	0.9073	0.9222	0.9262	0.8747
	H_o	0.9206	0.9	0.8776	0.828	0.8761	0.9333	0.9	0.9367	0.9444	0.9286
	F_{IS}	0.0041	0.0186	0.0276	0.0749	0.0516	−0.0123	0.0143	−0.0094	−0.0141	−0.0544
GmoC82	N	190	93	49	94	115	93	80	79	92	80
	N_A	8	5	5	5	5	5	6	6	7	4
	H_e	0.6204	0.6087	0.6187	0.6107	0.5894	0.6089	0.5912	0.5922	0.6434	0.5588
	H_o	0.5632	0.5914	0.4898	0.5426	0.5913	0.6022	0.675	0.5823	0.6087	0.5875
	F_{IS}	0.0949	0.0339	0.2182	0.1169	0.0011	0.0165	−0.1355	0.0231	0.0594	−0.0450
GmoC83	N	190	94	49	94	111	94	81	75	92	91
	N_A	3	3	3	3	3	3	3	3	3	3
	H_e	0.4829	0.4009	0.479	0.4595	0.4972	0.4663	0.48	0.4575	0.5077	0.4731
	H_o	0.4895	0.3723	0.449	0.5426	0.5405	0.4894	0.4568	0.4667	0.5326	0.5714
	F_{IS}	−0.0109	0.0766	0.0729	−0.1755	−0.0828	−0.0441	0.0546	−0.0133	−0.0435	−0.2026
Tch13	N	184	92	49	94	116	91	80	80	94	77
	N_A	9	6	7	7	8	7	7	8	7	7
	H_e	0.7268	0.6779	0.6976	0.7052	0.7323	0.6995	0.6737	0.6697	0.7355	0.666
	H_o	0.7065	0.7826	0.7755	0.7128	0.75	0.6703	0.675	0.5625	0.7766	0.6709
	F_{IS}	0.0306	−0.1491	−0.1014	−0.0054	−0.0199	0.0472	0.0043	0.1662	−0.0505	−0.0021
GmoG5	N	190	93	49	93	116	91	77	79	94	77
	N_A	6	4	4	4	4	4	5	4	5	5
	H_e	0.6869	0.7101	0.6847	0.6909	0.6646	0.7069	0.6767	0.6787	0.6993	0.6861
	H_o	0.6737	0.7527	0.6327	0.7204	0.6724	0.6593	0.6623	0.6456	0.6915	0.7143
	F_{IS}	0.0219	−0.0545	0.0863	−0.0374	−0.0075	0.0727	0.0277	0.0551	0.0165	−0.0345
Genetic diversity		22.41	18.53	15.94	19.24	19.59	19.41	18.41	17.53	19.76	17.76