Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited effective dispersal in the northeastern Pacific Ocean

Kathryn Maja Cunningham, Michael Francis Canino, Ingrid Brigette Spies, and Lorenz Hauser

Abstract: Genetic population structure of Pacific cod, *Gadus macrocephalus*, was examined across much of its northeastern Pacific range by screening variation at 11 microsatellite DNA loci. Estimates of F_{ST} (0.005 ± 0.002) and R_{ST} (0.010 ± 0.003) over all samples suggested that effective dispersal is limited among populations. Genetic divergence was highly correlated with geographic distance in an isolation-by-distance (IBD) pattern along the entire coastal continuum in the northeastern Pacific Ocean (~4000 km; r^2 = 0.83), extending from Washington State to the Aleutian Islands, and over smaller geographic distances for three locations in Alaska (~1700 km; r^2 = 0.56). Slopes of IBD regressions suggested average dispersal distance between birth and reproduction of less than 30 km. Exceptions to this pattern were found in samples taken from fjord environments in the Georgia Basin (the Strait of Georgia (Canada) and Puget Sound (USA)), where populations were differentiated from coastal cod. Our results showed population structure at spatial scales relevant to fisheries management, both caused by limited dispersal along the coast and by sharp barriers to migration isolating smaller stocks in coastal fjord environments.

Résumé : Nous avons examiné la structure génétique de la population des morues du Pacifique, *Gadus macrocephalus*, dans presque toute son aire de répartition dans le nord-est du Pacifique, par la détermination de la variation à 11 locus microsatellites d'ADN. Les valeurs estimées de F_{ST} (0,005 ± 0,002) et R_{ST} (0,010 ± 0,003) pour l'ensemble des échantillons laissent penser que la dispersion entre les populations est limitée. Il y a une forte corrélation entre la divergence génétique et la distance géographique selon un patron d'isolement par la distance (IBD) sur l'ensemble du continuum côtier dans le nord-est du Pacifique (~4000 km; r^2 = 0,83), depuis l'état de Washington aux îles Aléoutiennes, ainsi que sur des distances plus courtes dans trois sites d'Alaska (~ 700 km; r^2 = 0,56). Les pentes des régressions d'IBD indiquent que la distance moyenne de dispersion de la naissance à la reproduction est inférieure à 30 km. Des exceptions à ces patrons se retrouvent dans les échantillons provenant des environnements de fjords dans le bassin de Géorgie, soit le détroit de Géorgie (Canada) et Puget Sound (É.-U.), où les populations se différencient des morues de la côte. Nos résultats présentent une structure de population à des échelles qui sont d'intérêt pour la gestion des pêches, causée à la fois par une dispersion restreinte le long de la côte et par d'importantes barrières à la migration qui isolent les stocks plus petits dans les environnements des fjords côtiers.

[Traduit par la Rédaction]

Introduction

Although the notion that exploited species should be managed at the population level can be traced back to seminal work by Heincke (1889) and Hjort (1914), the practical implementation of such insight is still fraught with difficulties. Population boundaries are often ill defined by environmental, biological, or genetic discontinuities, especially in marine fishes (Hauser and Ward 1998). The result is that demographically independent populations may appear genetically homogenous and thus may be managed as a single stock. Most genetic approaches to population identification are further limited by their lack of sensitivity to high levels of connectivity and their dependence on the number of migrants rather than migration rates, complicating their application for the identification of independent management units (Waples and Gaggiotti 2006). This discrepancy between genetic and demographic definitions of populations

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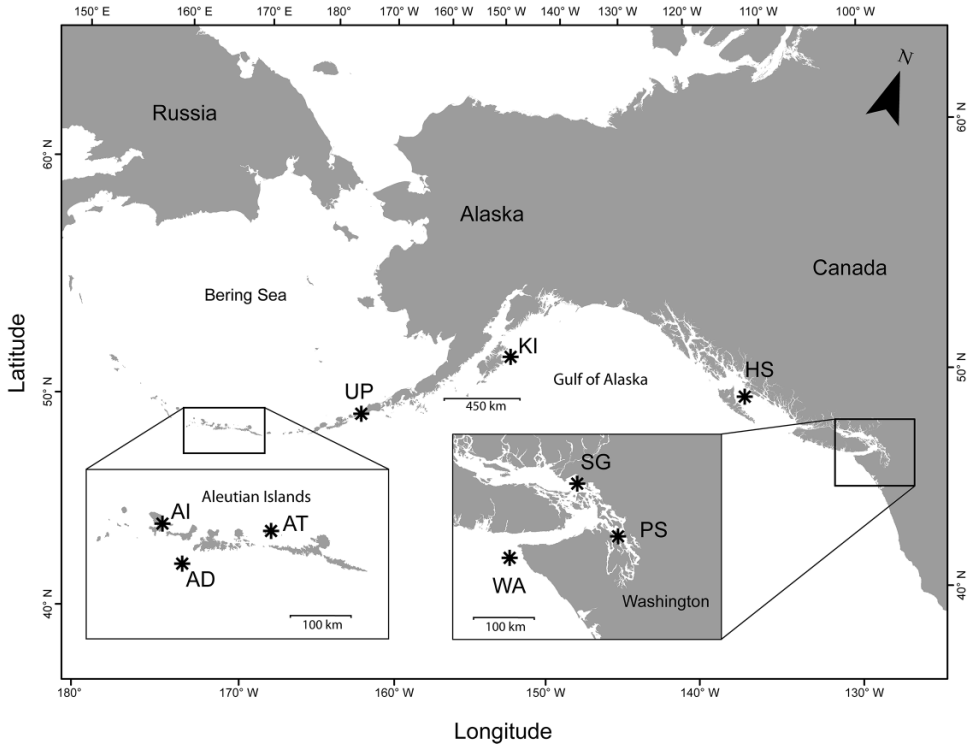
K.M. Cunningham[1] and L. Hauser. Marine Molecular Biotechnology Laboratory, School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, USA.
M.F. Canino[2] and I.B. Spies. NOAA Fisheries, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115, USA.

[1]Present address: 738 N 74th Street, Seattle WA 98103, USA.
[2]Corresponding author (e-mail: mike.canino@noaa.gov).

Fig. 1. Sample locations for Pacific cod (*Gadus macrocephalus*). Sample abbreviations are central Aleutian Islands (AI, AD, and AT), Unimak Pass (UP), Kodiak Island (KI), Hecate Strait (HS), coastal Washington State (WA), Puget Sound (PS), and Strait of Georgia (SG).



means that genetic estimates of population structure tend to be very conservative in marine species; genetic differentiation demonstrates population structure, but genetic homogeneity does not preclude the existence of demographically independent populations (Shaklee and Bentzen 1998).

Recently, the explicit inclusion of geographic information in genetic analyses has improved our ability to identify populations as management units. The search for sharp population boundaries may be futile in marine species, which often are distributed continuously and may disperse during all life history stages. Instead, the dispersal of individuals within that continuous distribution may be limited, resulting in a continuous increase of genetic differentiation with geographic distance (isolation by distance, IBD). Under certain assumptions (e.g., migration-drift equilibrium, exponential dispersal curve), the slope of the relationship can be used to estimate average dispersal distance of individuals (Rousset 1997). Although not providing stock boundaries, such estimates can be used in models to define geographic sectors of the fishery that may respond independently to exploitation.

IBD has been primarily described in demersal species of fish with pelagic larvae, e.g., species of the family Gadidae. In particular, several studies on Atlantic cod (*Gadus morhua*) showed IBD (Pogson et al. 2001; Beacham et al. 2002; O'Leary et al. 2007), whereas other studies identified patchy population structure that was not as clearly correlated with geographic distribution (e.g., Hutchinson et al. 2001; Knutsen et al. 2003). Furthermore, IBD was primarily identified when high variability markers, such as mitochondrial DNA and especially microsatellites, were applied. Pacific gadid species such as walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) are relatively understudied in this respect, despite their high economic importance (Hiatt et al. 2007), and knowledge on their stock structure lags behind that for Atlantic cod by perhaps a decade or more (Cunningham 2007).

Pacific cod has historically supported large- and small-scale commercial fisheries throughout its range. Global catch landings in 2006 were 235 296 tonnes (t), and in the United States, Pacific cod catches exceeded those of declining Atlantic cod stocks by the mid-1980s (http://www.st.nmfs.noaa.gov/st1/commercial/index.html). Although numerous putative stocks of Pacific cod have been identified using a variety of methods (see review in Gustafson et al. 2000), no general consensus has been reached on stock structure and Alaskan fisheries are managed as two separate stocks: the Gulf of Alaska (GOA) stock and the much larger Bering Sea – Aleutian Islands (BSAI) stock (Thompson et al. 2007*a*, 2007*b*). The largest Pacific cod fishery in Alaska (average catch 1990–2007 = 156 482 t) occurs in the eastern Bering Sea (Thompson et al. 2007*a*). Smaller fisheries, representing approximately 16% of the total harvest in the BSAI management area, occur in the Aleutian Islands region. Near the southern end of the species' distribution, populations have declined rapidly in the Georgia Basin, which consists of the Strait of Georgia, British Columbia, Canada, and fjord-like estuary of Puget Sound, Washington State, USA (Fig. 1). These two regions are directly connected to the North Pacific Ocean via the Johnstone Strait and the Strait of Juan de Fuca, respectively.

Here we present an assessment of population structure in Pacific cod inferred from microsatellite DNA variation across much of its North American range. The results demonstrated a clear IBD pattern, suggesting restricted gene

Table 1. Sampling locations, abbreviations, dates, and numbers (*n*) for adult Pacific cod (*Gadus macrocephalus*).

Geographic location, state or province	Sample abbreviation	Collection agency*	Date (month/year)	Latitude	Longitude	*n*
Adak Island, Alaska	AD	NMFS	3/2006	51°40′N	176°30′W	45
Central Aleutian Islands, Alaska	AI	NMFS	2/2005	51°50′N	177°36′W	92
Atka Island, Alaska	AT	NMFS	4/2006	52°18′N	173°38′W	45
Unimak Pass, Alaska	UP05	NMFS	1/2005	54°38′N	168°10′W	87
Unimak Pass, Alaska	UP03	NMFS	1/2003	54°38′N	168°10′W	95
Kodiak Island, Alaska	KI05	UW	3/2005	57°55′N	152°18′W	106
Kodiak Island, Alaska	KI03	NMFS	3/2003	57°48′N	152°31′W	94
Hecate Strait, British Columbia, Canada	HS	DFO	3/2004	53°13′N	130°57′W	89
Coastal Washington, Washington	WA	UW	2/2005	47°55′N	125°33′W	69
Strait of Georgia, Washington	SG	WDF&W	4/2003	48°54′N	123°06′W	94
Puget Sound, Washington	PS	WDF&W	2/2004, 3/2006	47°32′N	122°30′W	18

*NMFS, National Marine Fisheries Service; UW, University of Washington, School of Aquatic and Fisheries Science; DFO, Fisheries and Oceans Canada; WDF&W, Washington Department of Fish and Wildlife.

flow, and thus a substantial amount of self-recruitment, among putative stock components at spatial scales relevant to current fisheries management and conservation practices. In particular, Pacific cod (like Atlantic cod) appear to form localized populations in fjord environments or where deep-water barriers, such as submarine canyons, may limit adult dispersal.

Materials and methods

Sample collection

Samples were collected from large spawning and pre-spawning aggregates of Pacific cod in eight locations across the northeastern Pacific Ocean from January–March (Table 1; Fig. 1). Samples were primarily taken from trawls, although those from Kodiak Island in 2005 were caught with jigging gear. Replicate samples were taken at two-year intervals at two locations (Unimak Pass (UP03 and UP05) and Kodiak Island (KI03 and KI05), Alaska) to investigate temporal stability of population structure. Two samples from the central Aleutian Islands region (AD and AT), taken during commercial fishing operations in 2006, were in relatively close proximity (180 and 275 km, respectively) to one sample collected during a trawl survey in 2005 (AI). Samples obtained from Puget Sound in 2004 and 2006 were pooled because of small sample sizes. Pectoral fin clips were preserved in 95% ethanol and stored at room temperature prior to DNA extraction.

DNA extraction and microsatellite amplification

DNA was extracted from pectoral fin tissue using Qiagen DNeasy kits (QIAGEN, Valencia, California) following the manufacturer's protocols. Twelve microsatellite markers were initially screened for variation: 10 novel loci isolated from Pacific cod (Canino et al. 2005), one (*GMO37*) isolated from Atlantic cod (Miller et al. 2000), and one (*TCH20*) from walleye pollock (O'Reilly et al. 2000). Polymerase chain reaction (PCR) amplification of loci was conducted in 10 μL reaction volumes containing 4 μL template DNA, 1 μL 10× buffer (Genechoice Inc., Fredrick, Maryland), $MgCl_2$ concentration of 1.5 mmol·L^{-1}, 0.4 mmol·L^{-1} of each dNTP, 0.5 mmol·L^{-1} labeled forward and reverse primers, and 0.5 units of *Taq* Polymerase (Genechoice Inc., Fredrick, Maryland). A single "touchdown" thermal cycle for amplifying all loci was performed in a PTC-100 thermal cycler (MJ Research Inc., Waltham, Massachusetts). This consisted of an initial denaturation step at 95 °C (2 min) followed by five cycles of 95 °C (1 min), 60 °C (1 min (–1 °C per cycle)), and 72 °C (1 min) and then by 20 cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (30 s). Microsatellite PCR amplicons were size-separated in four panels of three loci each on a MegaBACE 1000 DNA sequencer (Amersham Pharmacia Biotech, Inc., Piscataway, New Jersey) and analyzed with Genetic Profiler software (version 2.2, Amersham Pharmacia Biotech).

Data analysis

Single locus statistics for all samples (Appendix A, Table A1) were calculated using FSTAT 2.9.3.2 (Goudet 2002) and Genetix 4.05 (Belkhir 2000). Genotype frequency conformance to Hardy–Weinberg equilibrium (HWE) and genotypic linkage equilibrium were determined using exact tests implemented in GENEPOP 3.3 (Raymond and Rousset 1995) with Markov chain parameters of 5000 dememorization steps, 500 batches, and 5000 iterations per batch. Significance levels for comparisons of loci across samples were adjusted using a sequential Bonferroni correction (Rice 1989) to maintain a type 1 error rate ($\alpha = 0.05$) over multiple tests. The program MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004) was used to check for the presence of null alleles, scoring errors, stuttering, and large allele dropout.

Exact tests for genic and genotypic differentiation for each locus–sample combination were conducted in GENEPOP using the same Markov chain Monte Carlo (MCMC) parameters used to test for HWE. In addition, we tested the joint null hypothesis of no frequency differences at any locus for each sample using Fisher's combined test implemented in GENEPOP. Randomization tests permuting either alleles or genotypes among samples were conducted in FSTAT to examine differentiation between sample pairs.

Genetic differentiation (F_{ST}) over all samples and between sample pairs was determined in GENEPOP from variances in allele frequencies using the unbiased estimator θ (Weir and Cockerham 1984). The analog statistic based on allele size (R_{ST}; Slatkin 1995) was calculated over all samples and between sample pairs using SPAGeDi 2.1 (Hardy

Table 2. Results from analysis of molecular variance (AMOVA) for sample groupings containing (*n*) samples.

Grouping scheme	Groups	*n*	F_{ST}	F_{SC}	F_{CT}
All samples					
(Coastal samples) (SG,PS)	2	11	0.0116***	0.0010***	0.0094**
All samples excluding SG and PS					
(AD, AT, AI, UP03, UP05, KI03, KI05) (HS, WA)	2	9	0.0038***	0.0012**	0.0026*
(AD, AT, AI) (UP03, UP05, KI03, KI05) (HS, WA)	3	9	0.0029***	0.0005ns	0.0025***
(AD, AT, AI) (UP03, UP05) (KI03, KI05) (HS, WA)	4	9	0.0025***	0.0003ns	0.0021**
Alaska only					
(AD, AT, AI) (UP03, UP05) (KI03, KI05)	3	7	0.0010*	–0.0004ns	0.0014*
(AD, AT, AI, UP03, UP05) (KI03, KI05)	2	7	0.0012*	0.0002ns	0.0009ns
(AD, AT, AI) (UP03, UP05, KI03, KI05)	2	7	0.0015*	–0.0001ns	0.0017*
(AD, AT, AI, KI03, KI05) (UP03, UP05)	2	7	0.0008*	0.0009*	–0.0001ns

Note: Coastal samples: AD, AT, AI, UP03, UP05, KI03, KI05, HS, WA; F_{ST}, variance of groups relative to total; F_{SC}, variance among samples within groups; F_{CT}, variance of samples among groups. *P* values for variance components: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant. Sample abbreviations are as in Table 1.

and Vekemans 2002). We then tested for significant differences between the two estimators to examine the relative effects of genetic drift (F_{ST}) and mutation (R_{ST}) on genetic differentiation using 5000 permutations of allelic sizes among allelic states (Hardy et al. 2003).

Analysis of molecular variation (AMOVA) was conducted in Arlequin 3.11 (Excoffier et al. 2005) to test population structure across the entire geographic sample range and among putative regional groupings of samples. Correlations between pairwise estimates of F_{ST} and R_{ST} with the shortest geographic distance between samples along continental margins (<200 m) were assessed using Mantel's test with 1000 permutations of linearized F_{ST} and R_{ST} values and distance matrices with the Isolde subroutine implemented in GENEPOP. A decomposed regression analysis (Koizumi et al. 2006) was performed to detect potential outliers in the pattern of genetic and geographic distances. Means and 95% confidence intervals of residuals from the linear regression of F_{ST} on geographic distance were calculated for each sample, and putative outliers were identified if the confidence interval excluded zero. These were sequentially removed and the subsequent fit of the data to the regression model was evaluated using Akaike's information criterion following Koizumi et al. (2006).

Mean dispersal distance per generation, σ, was calculated from the slope of the IBD regression relationship following Buonaccorsi et al. (2005):

$$\sigma = \sqrt{(8Dm)^{-1}}$$

where D is density of fish per linear kilometre (km), and m is the slope of the least-squares regression of $F_{ST}/(1 - F_{ST})$ on geographic distance. This one-dimensional model is a reasonable choice for estimating dispersal when differentiation occurs over spatial scales greater than the habitat width (Rousset 1997) and seems generally appropriate for broadly distributed species confined to continental margins. We assumed that the dispersal pattern followed a symmetrical, exponential distribution and calculated mean dispersal distances for effective population densities ranging from 1 to 100 000 fish·linear km^{-1} of coastline.

The program Barrier 2.2 (Manni et al. 2004) was used to identify genetic discontinuities within the general IBD pattern. The program uses Delaunay triangulation to calculate a geometric network of samples connected by F_{ST} values. Using a Monmonier algorithm, the program then identifies the edge with the largest F_{ST} given the geographic distance and extends that barrier to the next connection with the largest genetic distance until it hits another edge or another barrier. One such barrier was calculated for each locus. In addition, a spatial analysis of molecular variance (SAMOVA) developed by Dupanloup et al. (2002) was conducted to define groupings of samples that were maximally differentiated from each other. Samples were randomly partitioned into two to five groups for 100 simulated annealing processes. The configuration producing the largest among-group variance component (F_{CT}) was chosen as the best spatial pattern defining the population structure.

Results

Sample sizes for single locus statistics ranged from 11 to 106 individuals per locus (Appendix A, Table A1). Observed heterozygosities ranged from 0.235 (*GMA108*) to 1.000 (*TCH20*). Locus *GMA107* did not meet Hardy–Weinberg equilibrium (HWE) expectations because of significant homozygote excesses in most samples. Results from MICRO-CHECKER inferred evidence for null alleles and this locus was excluded in subsequent analyses.

Twelve of 121 sample–locus combinations showed significant deviations from HWE expectations, with no apparent trends within loci or samples, and only one locus (*GMA106*) in the Aleutian Islands (AI) sample remained significant after Bonferroni correction (Appendix A, Table A1). Linkage disequilibrium was detected between two loci (*GMA101* and *GMO37*) in one sample from the central Aleutian Islands (AD) but was not significant in pairwise tests in any other sample or over all samples.

Hierarchical AMOVA was used to explore several potential geographic subdivisions by partitioning samples into two to four groups (Table 2). The highest F_{ST} values were found when the samples were partitioned into coastal North Pacific and Georgia Basin groups, but this also resulted in a highly significant within-group variance component (F_{SC}). Partitioning the coastal samples into three or four groups eliminated significant F_{SC} results and produced approximately

Table 3. Probability (*P*) values from exact tests of genic (above diagonal) and genotypic (below diagonal) differentiation between sample pairs.

	AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS
AD	—	0.7128	0.6908	0.0976	0.6367	0.0150*	0.0186*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
AI	0.6186	—	0.7261	0.0120*	0.1470	0.0455*	0.0603	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
AT	0.6367	0.7442	—	0.5001	0.5291	0.0640	0.1453	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
UP05	0.3139	0.0555	0.8279	—	0.5889	0.3385	0.4042	**<0.0001**	**0.0014**	**<0.0001**	**<0.0001**
UP03	0.4532	0.1742	0.4378	0.4470	—	0.6444	0.1207	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**
KI05	0.0288*	0.1514	0.0958	0.3460	0.5492	—	0.7159	**<0.0001**	**0.0009**	**<0.0001**	**<0.0001**
KI03	0.0137*	0.1681	0.2375	0.4605	0.1746	0.7669	—	**0.0011**	0.0101*	**<0.0001**	0.0060*
HS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0042*	—	0.0104*	**<0.0001**	0.0121*
WA	**<0.0001**	**<0.0001**	**<0.0001**	0.0041*	**<0.0001**		0.0259*	0.0215*	—	**<0.0001**	0.0180*
SG	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	—	0.3778
PS	0.0002*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0039*	0.0080*	0.0083*	0.2758	—

Note: Sample abbreviations are as in Table 1. Values in bold indicate sample pairs significantly differentiated following sequential Bonferroni correction for 55 multiple tests (initial $\alpha = 0.00091$). An asterisk (*) denotes significant prior to sequential correction.

Table 4. Estimates of F_{ST} (above diagonal) and R_{ST} (below diagonal) between sample pairs.

	AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS
AD	—	–0.0012	0.0000	0.0017	–0.0004	0.0026	0.0018	**0.0060**	**0.0061**	**0.0142**	0.0215
AI	–0.0040	—	0.0002	0.0024	0.0016	0.0022	0.0018	**0.0065**	**0.0062**	**0.0163**	0.0247*
AT	–0.0067	–0.0058	—	–0.0004	0.0001	0.0018*	0.0019	**0.0037**	**0.0063**	**0.0105**	0.0183*
UP05	0.0011	–0.0012	–0.0031	—	0.0001	0.0004	0.0004	**0.0036**	0.0031*	**0.0109**	0.0152*
UP03	–0.0003	–0.0017	–0.0028	–0.0017	—	0.0000	0.0001	0.0036*	**0.0045**	**0.0120**	0.0179*
KI05	–0.0036	–0.0008	–0.0056	–0.0007	0.0014	—	–0.0006	**0.0026**	**0.0029**	**0.0101**	0.0157*
KI03	–0.0057	–0.0012	–0.0062	0.0030	0.0020	–0.0025	—	0.0027*	0.0028*	**0.0114**	0.0142*
HS	0.0093	0.0024	0.0033	0.0037	0.0083	0.0071	0.0070	—	0.0030*	**0.0058**	**0.0112**
WA	–0.0028	–0.0016	–0.0036	0.0058	0.0029	0.0031	–0.0018	0.0008	—	**0.0072**	0.0097
SG	0.0195	0.0232	0.0179	0.0271	0.0331	0.0217	0.0190	0.0105	0.0103	—	0.0024
PS	0.0370	0.0251	0.0299	0.0342	0.0383	0.0364	0.0307	0.0031	0.0049	–0.0071	—

Note: Sample abbreviations are as in Table 1. F_{ST} values in bold are significant following sequential Bonferroni correction for 55 multiple tests (initial $\alpha = 0.00091$); asterisk (*) denotes significant prior to sequential correction.

Table 5. Isolation-by-distance (IBD) regression statistics for estimates of F_{ST} with geographic distance among groupings of North American samples of Pacific cod (*Gadus macrocephalus*).

Samples excluded	*n*	Slope	r^2	*P*	AIC_C	ΔAIC_C
None	11	3.10×10^{-6}	0.47	9.4×10^{-9}	–90.83	15.86
PS	10	2.31×10^{-6}	0.53	1.6×10^{-8}	–92.87	13.82
PS, SG	9	1.57×10^{-6}	0.83	1.2×10^{-14}	–106.69	0.00
PG, SG, WA, HS (Alaska only)	7	1.38×10^{-6}	0.56	9.6×10^{-5}	–87.14	19.55

Note: Sample abbreviations as in Table 2. Akaike information criterion values corrected for small sample sizes (AIC_C) and difference from the best model (ΔAIC_C) were calculated from decomposed pairwise regression analysis.

Fig. 2. Linearized F_{ST} versus geographic distance for Pacific cod (*Gadus macrocephalus*). Regression line is fitted to data from coastal samples in North America (solid circles). Open diamonds and solid squares represent data from the Strait of Georgia and Puget Sound, respectively.

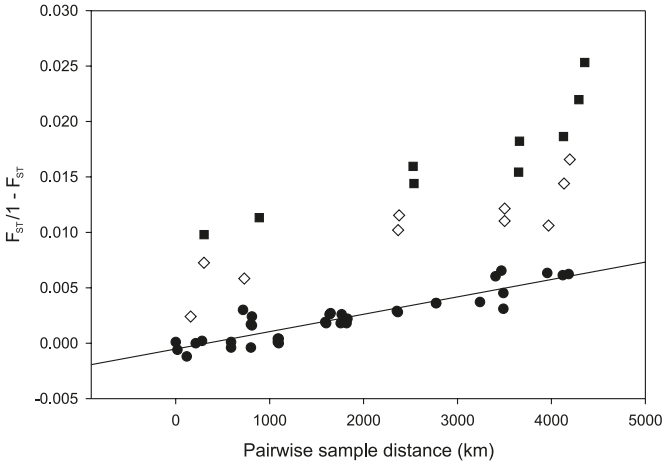


Fig. 3. Means and 95% confidence intervals of individual sample residuals from the regression of linearized F_{ST} on geographic distance over all other samples. Sample abbreviations as in Table 1.

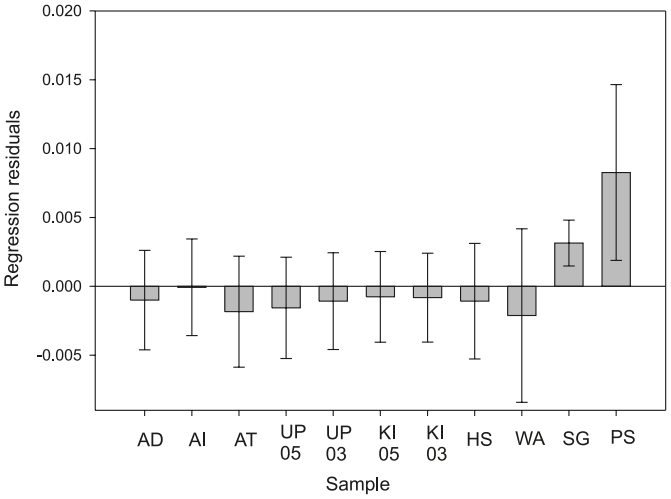


Table 6. Estimated mean dispersal distances for Pacific cod (*Gadus macrocephalus*) based on isolation-by-distance regression over a range of effective adult densities.

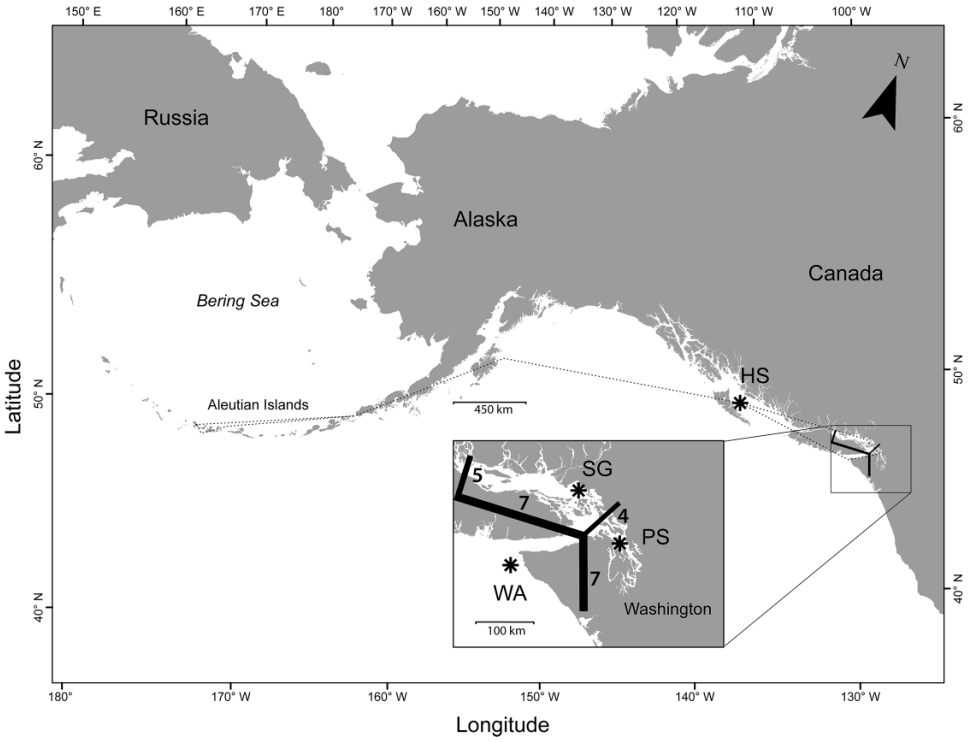
Adult density (no.·km)	Mean dispersal distance per generation (km)
1	282.2
10	89.2
100	29.2
1 000	8.9
10 000	0.9
100 000	0.3

the same levels of among-group differentiation (F_{CT}). Results for the three sample locations within Alaska provided some support for differentiation of the Aleutian Islands from the Bering Sea and the Gulf of Alaska: of all possible groupings, the only significant differentiation among groups (F_{CT}) occurred when pooled samples from the Aleutian Islands (AD, AI, and AT) were tested with samples from Unimak Pass (UP03 and UP05) and Kodiak Island (KI03 and KI05; Table 2). AMOVAs conducted on three groupings of temporal replicate samples from the Aleutian Islands (AD, AI, and AT) had F_{CT} values ranging from –0.0010 to 0.0013 that were not significant (data not shown) Likewise, grouping temporal replicates and testing pairs of geographic locations in Alaska produced no significant F_{SC} values.

There were no significant differences in allelic or genotypic frequencies between temporal samples collected at the same sites in Alaska, including the three locations in the central Aleutian Islands archipelago ranging from 75 to 275 km apart (Fig. 1). The joint null hypotheses of no significant genic or genotypic differentiation between sample pairs over all loci were rejected for most comparisons (Table 3), both before (37 of 55 tests) and after (28 of 55 tests) Bonferroni correction. Results of genotypic tests were almost identical (Table 3).

Estimates of F_{ST} and R_{ST} (± standard error, SE) over all samples were 0.005 (±0.002) and 0.010 (±0.003), respectively. No significant differentiation was found among temporal replicate samples or in pairwise geographic comparisons within Alaska (Table 4). Pooling samples from the Aleutian Islands (AD, AI, and AT), Kodiak Island (KI05 and KI03), and Unimak Pass (UP03 and UP05) resulted in significant estimates of differentiation (after sequential correction) between the first two regions and following sequential correction between the latter two regions (data not shown). Pairwise F_{ST} values were generally significant between locations in Alaska and southern coastal locations (Hecate Strait (HS), coastal Washington State (WA), and the Strait of Georgia (SG); Table 4). Pairwise estimates of F_{ST} between Puget Sound (PS) and other locations were generally not significant following sequential correction, and the small sample size (18) undoubtedly resulted in weak power in these comparisons despite relatively high F_{ST} values.

Fig. 4. Delaunay triangulation (dotted lines) with putative barriers to gene flow (solid lines) identified by BARRIER (Manni et al. 2004). Line thickness is proportional to the given number of loci supporting the barrier. Sample abbreviations as in Table 1.



Eight of 11 loci showed significant regression relationships of F_{ST} with geographic distances (data not shown). A Mantel test of linearized multilocus F_{ST} values with geographic distances was highly significant ($P < 0.0001$) and showed clear IBD patterns over the entire sample range in North America (Fig. 2). Over shorter geographic scales, the IBD relationship for samples from Alaska remained highly significant (albeit with lower r^2), with a slope comparable with that over all North American coastal samples combined (Table 5). Samples within the Georgia Basin (SG and PS) consistently yielded higher pairwise estimates of F_{ST} in comparisons with samples obtained from coastal sites than those between coastal sites (Table 4). When these two samples were removed from the regression, the correlation coefficient improved considerably ($r^2 = 0.83$; Table 5). Decomposed pairwise regression analysis showed that mean regression residuals for these two samples were significantly larger than zero (Fig. 3), and Akaike information criterion (AIC) values indicated a best fit when both Georgia Basin samples were excluded (Table 5). Pairwise R_{ST} values also exhibited a significant IBD pattern (Mantel test; $P < 0.005$), although the overall relationship was weaker than for F_{ST} ($r^2 = 0.31$), and again the Strait of Georgia (SG) and Puget Sound (PS) samples appeared to be outliers to the general trend for coastal populations. Estimates of R_{ST} were significantly higher than F_{ST} at three individual loci (*GMA101*, *GMA102*, and *GMA104*) and over all loci ($P = 0.0152$) when the Georgia Basin (PS and SG) samples were included, but not among coastal samples.

Mean dispersal distances estimated from the slope of the IBD regression of F_{ST} values across coastal North America (1.57×10^{-6}) ranged from 0 to 282 km·generation^{-1} over five orders of magnitude in adult densities (Table 6). Excluding an extreme value for a density of 1 individual·linear km^{-1}, mean dispersal estimates for cod were less than 100 km·generation^{-1}.

The BARRIER analysis identified one primary genetic discontinuity supported by seven of 11 loci between the Strait of Georgia (SG) and Puget Sound (PS) from coastal populations (Fig. 4). Other barriers between Puget Sound (PS) and the Strait of Georgia (SG) and the latter location and Hecate Strait (HS) involved four and five loci, respectively. Results from SAMOVA showed that the largest F_{CT} value (0.0269, $P = 0.0215$) was produced by grouping samples from the Georgia Basin (PS and SG) compared with all coastal samples combined, supporting results obtained from BARRIER. Partitioning the samples into three or more groups produced smaller, although significant, F_{CT} values but also introduced a significant within-group variation component.

Discussion

Our microsatellite data revealed one of the clearest IBD patterns reported to date for a marine fish species ($r^2 = 0.83$), demonstrating a monotonic increase of genetic distance with geographic distances along nearly the entire North American range of Pacific cod. Two outlier populations were identified, which appeared to be isolated from the coastal stock complex. Although genetic differentiation was low overall ($F_{ST} = 0.005$), it appeared to be temporally stable. Such patterns suggest limited dispersal in Pacific cod, although pertinent features of species biology and the marine environment need to be considered in the interpretation of our results.

IBD in North American coastal populations

Genetic differentiation among coastal sites indicated the presence of a large stock complex along continental shelves and slopes, with gene flow sufficiently restricted to develop a significant IBD pattern. Tests of genic and genotypic heterogeneity, as well as estimates of F_{ST} and R_{ST}, consistently inferred significant genetic differentiation among populations at distances exceeding ~1700 km along this coastal continuum, a spatial scale comparable with detectable IBD in Atlantic cod (1600 km) in the western North Atlantic (Pogson et al. 2001). Samples from the coasts of Washington State and British Columbia were distinct from those in Alaska and, to a lesser degree, from each other. The IBD pattern among coastal locations across North America is one of the strongest relationships for a marine fish reported to date. Only brown rockfish, *Sebastes auriculatus*, along the California coast showed genetic differentiation that was more closely explained by geographic distance ($r^2 = 0.90$; Buonaccorsi et al. 2005), while other studies revealed more scatter around a general IBD relationship (e.g., Rocha-Olivares and Vetter 1999).

Patterns of genetic variation reflect both contemporary dispersal and population history (Hutchison and Templeton 1999). A critical assumption of many population genetic inferences is that populations are at migration–drift equilibrium (Slatkin 1993). This assumption may be violated in Pacific cod because of the relatively recent time since colonization of the northeastern Pacific Ocean and Bering Sea following glacial retreat in the late Pleistocene (i.e., <17 000 years for northern populations) and even more recently (ca. 6000–7000 years) for populations in the Georgia Basin (Tunnicliffe et al. 2001). However, IBD patterns may be achieved relatively soon after population separation, depending on the mode of separation (Slatkin 1993). Therefore, the slope of the IBD is often better correlated with direct estimates of dispersal than F_{ST} values themselves because overall levels of genetic differentiation take much longer to reach equilibrium (Rousset 1997). In addition, one-dimensional habitats produce higher levels of divergence and reach migration–drift equilibrium faster than two-dimensional habitats with gene flow in all directions (Crow and Aoki 1984; Pogson et al. 2001). Spawning aggregations of Pacific cod can be found primarily along the 200 m isobath (Dunn and Matarese 1987), and their distribution is thus essentially one-dimensional along continental margins.

Migration–drift equilibrium is also likely to be achieved sooner at shorter distances, resulting in a significant IBD pattern at short, but not at long, geographic distances in non-equilibrium populations (Slatkin 1993). In contrast, the IBD relationship in Pacific cod is constant over different geographic distances, as evidenced by the high r^2 and similarity of slopes along the entire coast and within just Alaska, suggesting that the IBD patterns may be close to equilibrium and thus reflect primarily contemporary dispersal rather than long-term demographic history. Alternative explanations for this IBD relationship, secondary contact among coastal subpopulations or a stepwise recolonization history following glaciation, were not evident in the data.

R_{ST} values were not higher than F_{ST} in North American coastal samples, as would be expected if subpopulations had been isolated, nor was there any geographic trend towards reduced genetic diversity at higher latitudes resulting from postglacial northward expansion of Pacific cod.

Interestingly, broad-scale circulation features of the northeastern Pacific Ocean did not appear to influence geographic patterns of genetic variation. The coastal sample from Washington State (WA) is south of the region where the North Pacific Current bifurcates, forming the northward-flowing Alaska Current and southward-flowing California Current, defining the approximate boundary of the Oregonian and Aleutian zoogeographic provinces (Cummins and Freeland 2007). This current system may be responsible for maintaining a phylogeographic discontinuity between northern and southern populations of rosethorn rockfish, *Sebastes helvomaculatus* (Rocha-Olivares and Vetter 1999). We found no evidence for such a discontinuity in our spatial analyses, suggesting that limited dispersal among subpopulations subject to genetic drift was the primary cause of the observed IBD pattern.

Estimates of dispersal depend on assumptions of average effective densities (Rousset 1997), which can be deducted from recent stock biomass surveys by National Marine Fisheries Service (NMFS). The harmonic mean of stock abundances estimated from 10 trawl surveys between 1984 and 2007 in the Gulf of Alaska was 199 million individuals (Thompson et al. 2007*b*). The coastline of the Gulf of Alaska encompasses approximately 2525 km, yielding an average estimate of 78 800 individuals·km^{-1} coastline. The mean effective density for this area, assuming a conservative ratio of effective to census population size of 0.001 (Hauser et al. 2002; Turner et al. 2002), is therefore roughly 79 individuals·km^{-1}. Realistic dispersal distances may thus not exceed 30 individuals·km^{-1}, comparable with estimates for shallow coastal rockfish species with pelagic larvae (Buonaccorsi et al. 2004, 2005; Miller and Shanks 2004).

Such very short dispersal distances infer very little larval and adult dispersal. Despite the potential for extensive adult movement, Pacific cod may not undertake directed migrations over their lifetime. Cod tagged in the eastern Bering Sea exhibited high site fidelity, with 70% of recaptures occurring within 80 km (Shi et al. 2007). Studies in the Gulf of Alaska have shown that although some fish traveled in excess of 600 km, about 75% stayed within 25 km over considerable time periods (D. Urban, NMFS, Alaska Fisheries Science Center, Kodiak, Alaska 99615, USA, personal communication, 2006). Fish captured during summer months and recaptured during the next spawning period had some of the longest recovery distances, and some tagged and recaptured over two successive spawning seasons had the shortest, suggesting that cod may make extensive annual feeding migrations prior to returning to prior spawning locations or that some stock components are nonmigratory. Archival tagging of Atlantic cod (Svedäng et al. 2007*a*) found evidence for both resident and annually migrating stock components, although whether this represents natal homing is unresolved (Bradbury and Laurel 2007; Svedäng et al. 2007*b*). Larval dispersal may also be limited: Pacific cod lay a single batch of demersal eggs (Hattori et al. 1992) in contrast to the batch-spawning Atlantic cod, which produces planktonic eggs. Larvae remain near the bottom of the water column (Dunn and Matarese 1987), thus potentially aiding larval retention.

Genetic structure in Alaska

The genetic structure of Pacific cod in Alaska is of particular interest, not only because of potential discrepancies between genetic stock structure and established fishery management units, but also because of more complex bathymetric and hydrographic features in the Gulf of Alaska and the Aleutian archipelago than the relatively homogeneous eastern Bering Sea. The IBD relationship observed for three sampled locations in Alaska was temporally stable and similar to the general coastal pattern across the northeastern Pacific Ocean. However, AMOVA results supported the grouping of samples along the contiguous Alaska peninsula (UP and KI) as distinct from those in the central AI region, a relationship that would not be predicted considering only IBD (distance AI–UP, 700–900 km; UP–KI, 1000–1050 km). This pattern of differentiation is generally concordant with meristic stock differences between the eastern Aleutian Islands – Bering Sea and the western Aleutian Islands (Wilimovsky et al. 1967).

Potential factors controlling population connectivity of Pacific cod in the Aleutian archipelago with the much larger stock biomass in the eastern Bering Sea could arise from limits to egg and larval dispersal, as well as oceanographic barriers to movements by adults. Oceanographic circulation in the Aleutian Islands is more complex than in the eastern Bering Sea, resulting in an ecological subdivision at Samalga Pass (Hunt and Stabeno 2005) characterized by differences in ecosystem productivity and species composition and abundance (Logerwell et al. 2005; Stabeno et al. 2005). Fish species with partial distributions across the Aleutian Archipelago often show distinct breaks at zoogeographic provinces separated by deep Aleutian passes (Logerwell et al. 2005), thus inferring potential barriers to population connectivity as well. Vigorous tidal transport through many of these passes creates complex circulation patterns around islands (Stabeno et al. 2005) that could result in entrainment of Pacific cod eggs and larvae at more localized scales than in more homogeneous habitats of the eastern Bering Sea and Northeast Pacific.

Water depth may be an important factor influencing movements by adult (and presumably juvenile) Pacific cod, which are rarely captured by trawl surveys deeper than 300 m (Allen and Smith 1988). Pacific cod are much more demersal in their vertical distributions than Atlantic cod and are typically found less than 10 m from the bottom (Nichol et al. 2007). These two observations suggest that passes in the Aleutian Islands, especially those exceeding 500–1000 m westward of Samalga Pass, may represent significant barriers to dispersal. Deep ocean trenches have been proposed as barriers to migration in other gadid species, contributing to genetic differentiation of Atlantic cod (Bentzen et al. 1996; Ruzzante et al. 1998; Beacham et al. 2002) and haddock (*Melanogrammus aeglefinus*) in the western Atlantic (Zwanenburg et al. 1992). The extent of adult exchange between the Bering Sea and Aleutian Islands regions has not been accurately quantified and may be influenced by suitable depth "corridors" providing habitat connectivity.

Genetic structure in Puget Sound

Pacific cod from the inland waters of the Georgia Basin were markedly differentiated from open coastal locations. Pairwise sample comparisons and spatial analyses inferred strong isolation between populations in fjord environments of the Strait of Georgia (SG) and Puget Sound (PS), a pattern consistent with previous estimates of stock structure derived from tagging studies, acquired markers, and known historical spawning locations (Westrheim 1982; Palsson 1990; Palsson et al.1997). The transboundary Strait of Georgia stock is one of four (including Hecate Strait, Queen Charlotte Sound, and the west coast of Vancouver Island) defined for management purposes in British Columbia (Fisheries and Oceans Canada 1999). Tagging studies (reviewed by Westrheim 1982) showed little movement of tagged cod among three of these regions. Three stocks have been proposed for Puget Sound based on tagging data and spawning locations (Palsson 1990). Differences in parasite frequencies also distinguished Georgia Basin populations from coastal Pacific cod (Westrheim 1987). Gao et al. (2005) reported significant differences in stable isotope ratios of cod otoliths between two sample areas near our coastal Washington State and Strait of Georgia locations.

The existence of fjord populations of Pacific cod is concordant with patterns reported in other marine fishes broadly distributed in the Northeast Pacific, including brown rockfish (Buonaccorsi et al. 2005), copper rockfish (*Sebastes caurinus*; Buonaccorsi et al. 2002), lingcod (*Ophidon elongatus*; Marko et al. 2007), Pacific hake (*Merluccius merluccius*; Iwamoto et al. 2004), and northern clingfish (*Gobbiesox maeandricus*; Hickerson and Ross 2001). Divergence from coastal populations likely arose from, and is maintained by, complex physiographic and circulatory features that restrict egg and larval dispersal. Exchange of deep water with continental shelf waters is restricted by several narrows and sills (Ebbesmeyer et al. 1984; Masson 2002). Similarly, the presence of shallow sills in Norwegian fjords appears to enhance local retention of Atlantic cod eggs (Knutsen et al. 2007), contributing to the development of localized populations (Olsen et al. 2004; Jorde et al. 2007).

The general decline of Pacific cod populations near the southern edge of its historical range in the northeastern Pacific Ocean has prompted concerns regarding their management and conservation (see review in Gustafson et al. 2000). Large declines led to restrictions on both commercial and recreational fisheries after 1987, and the stock has not shown evidence of rebuilding despite extremely low fishing levels since the 1990s. Possible reasons include a warming oceanographic trend during and following the decline (Palsson 1990; Dorn 1993), declines in prey species, and degradation of nearshore subtidal nursery areas (West 1997), or some combination of these factors (see review in Gustafson et al. 2000).

Pacific cod was one of three gadoid species in Puget Sound petitioned for listing under the US Endangered Species Act (ESA) (Wright 1999). In their status review, Gustafson et al. (2000) were unable to identify cod within Puget Sound as a distinct population segment (DPS) under the ESA definition, resulting in a finding of "not warranted" for listing, in part due to insufficient information regarding the northern boundary of a DPS that included Puget Sound (Gustafson et al. 2000). Our study clearly indicates genetic

differentiation of cod from Puget Sound and the Strait of Georgia from coastal populations, although a more detailed delineation and appropriate definition of a potential Puget Sound cod DPS requires further research with larger samples.

In summary, our results refute the paradigm of broad-scale genetic panmixia for Pacific cod in the North Pacific and Bering Sea (Grant et al. 1987) as a consequence of high migration rates (Shimada and Kimura 1994). Instead, our data suggest very limited average dispersal per generation, leading to significant genetic divergence over modest geographic scales (1500–2000 km) along contiguous coastal margins and at lesser distances (hundreds of kilometres) where circulation features and bottom topographies are more heterogeneous. Combined with tagging data, our results potentially may be used to develop a model of cod dispersal to determine the appropriate geographic scales for management and conservation. However, observed departures from IBD in localized inshore stocks suggest caution in this approach. Other isolated fjord populations may exist outside of the Georgia Basin (e.g., Prince William Sound), and more comprehensive sampling of inshore fjords and embayments in the Gulf of Alaska region and throughout the larger Bering Sea – Aleutian Islands management area is needed to more fully resolve the magnitude and geographic patterns of structure in Pacific cod.

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References

Allen, M.J., and Smith, G.B. 1988. Atlas and zoogeography of common fishes in the Bering Sea and northeastern Pacific. NOAA Tech. Rep. No. NMFS 66.

Beacham, T.D., Battey, J., Miller, K.M., Le, K.D., and Withler, R.E. 2002. Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES J. Mar. Sci. **59**: 650–665. doi:10.1006/jmsc.2002.1253.

Belkhir, K. 2000. GENETIX 4.05, logiciel sous Windows^{TM} pour la génétique des populations. Laboratoire Génomes et Populations, CNRS UPR 9060, Univeristé de Montpelier II, Montpelier, France. Available at http://www.genetix.univ-montp2.fr/genetix/genetix.htm [accessed 20 August 2008].

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**: 2706–2721. doi:10.1139/cjfas-53-12-2706.

Bradbury, I.R., and Laurel, B.J. 2007. Defining 'natal homing' in marine fish populations: comment on Svedäng et al. (2007). Mar. Ecol. Prog. Ser. **349**: 307–308. doi:10.3354/meps07281.

Buonaccorsi, V.P., Kimbrell, C.A., Lynn, E.A., and Vetter, R.D. 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Can. J. Fish. Aquat. Sci. **59**: 1374–1384. doi:10.1139/f02-101.

Buonaccorsi, V.P., Westerman, M., Stannard, J., Kimbrell, C., Lynn, E., and Vetter, R.D. 2004. Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. Mar. Biol. (Berl.), **145**: 779–788. doi:10.1007/s00227-004-1362-2.

Buonaccorsi, V.P., Kimbrell, C.A., Lynn, E.A., and Vetter, R.D. 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conserv. Genet. **6**: 697–713. doi:10.1007/s10592-005-9029-1.

Canino, M.F., Spies, I.B., and Hauser, L. 2005. Development and characterization of novel di- and tetra-nucleotide microsatellite markers in Pacific cod (*Gadus macrocephalus*). Mol. Ecol. Notes, **3**: 469–471. doi:10.1111/j.1471-8286.2005.01109.x.

Crow, J.F., and Aoki, K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci. U.S.A. **81**: 6073–6077. doi:10.1073/pnas.81.19.6073. PMID:6592602.

Cummins, P.F., and Freeland, H.J. 2007. Variability of the North Pacific Current and its bifurcation. Prog. Oceanogr. **75**: 253–265. doi:10.1016/j.pocean.2007.08.006.

Cunningham, K.M. 2007. Population genetics of Pacific cod (*Gadus macrocephalus*): evidence for large and small scale population structure. M.S. thesis, School of Fishery and Aquatic Sciences, University of Washington, Seattle, Washington.

Dorn, M.W. 1993. Pacific cod. *In* Status of living marine resources off the Pacific coast of the United States for 1993. *Edited by* L.-L. Low. NOAA Tech. Memo. No. NMFS-NWC-26. pp. 17–18.

Dunn, J.R., and Matarese, A.C. 1987. A review of the early life-history of Northeast Pacific gadoid fishes. Fish. Res. **5**: 163–184. doi:10.1016/0165-7836(87)90038-5.

Dupanloup, I., Schneider, S., and Excoffier, L. 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. **11**: 2571–2581. doi:10.1046/j.1365-294X.2002.01650.x. PMID:12453240.

Ebbesmeyer, C., Coomes, C., Cox, J., Helseth, M., Hinchey, L., Cannon, G., and Barnes, C. 1984. Synthesis of current measurements in Puget Sound, Washington. Vol. 3. Circulation in Puget Sound: an interpretation based on historical records of currents. NOAA Tech. Memo. No. NOS OMS 5.

Excoffier, L.G., Laval, G., and Schneider, S. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. Evolut. Bioinform. Online, **1**: 47–50. http://cmpg.unibe.ch/software/arlequin3/ [accessed 20 August 2008].

Fisheries and Oceans Canada. 1999. Pacific cod in Hecate Strait. Fisheries and Oceans Canada, Vancouver, BC. Science Stock Status Report No. A6-01.

Gao, Y., Bargmann, G.G., Brand, U., and Noakes, D.L.G. 2005. Stable isotopic and trace elemental composition of otoliths and the stock structure of Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fishes, **74**: 335–348. doi:10.1007/s10641-005-1332-7.

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices [online]. Ver. 2.9.3.2. Available at http://www2.unil.ch/popgen/softwares/fstat.htm [accessed 22 April 2008].

Grant, W.S., Zhang, C.I., Kobayashi, T., and Ståhl, G. 1987. Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can. J. Fish. Aquat. Sci. **44**: 490–498. doi:10.1139/f87-061.

Gustafson, R.G., Lenarz, W.H., McCain, B.B., Schmitt, C.C., Grant, W.S., Builder, T.L., and Methot, R.D. 2000. Status review of Pacific hake, Pacific cod, and walleye pollock from Puget Sound, Washington. NOAA Tech. Memo. No. NMFS-NWFSC-44.

Hardy, O.J., and Vekemans, X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes, **2**: 618–620. doi:10.1046/j.1471-8286.2002.00305.x.

Hardy, O.J., Charbonnel, N., Freville, H., and Heuertz, M. 2003. Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. Genetics, **163**(4): 1467–1482. PMID:12702690.

Hattori, T., Sakurai, Y., and Shimazaki, K. 1992. Maturation and reproductive cycle of female Pacific cod in waters adjacent to the southern coast of Hokkaido, Japan. Bull. Jpn. Soc. Sci. Fish. **58**: 2245–2252.

Hauser, L., and Ward, R.D. 1998. Population identification in pelagic fish: the limits of molecular markers. *In* Advances in molecular ecology. *Edited by* G.R. Carvahlo. IOS Press, Amsterdam, the Netherlands. pp. 191–224.

Hauser, L., Adcock, G.J., Smith, P.J., Bernal Ramirez, J.H., and Carvalho, G.R. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc. Natl. Acad. Sci. U.S.A. **99**(18): 11742–11747. doi:10.1073/pnas.172242899. PMID: 12185245.

Heincke, F.R. 1889. Naturgeschichte des Herings I. Die Lokalformen und dieWanderungen des Herings in den Europäischen Meeren. O. Salle, Berlin, Germany.

Hiatt, T., Felthoven, R., Dalton, M., Garber-Yonts, B., Haynie, A., Herrmann, K., Sepez, J., Seung, C., and Sievanene, L. 2007. Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands area: economic status of the groundfish fisheries off Alaska, 2006 [online]. Available at http://www.afsc.noaa.gov/refm/docs/2007/economic.pdf [accessed 22 April 2008].

Hickerson, M.J., and Ross, J.R.P. 2001. Post-glacial population history and genetic structure of the northern clingfish (*Gobbiesox maeandricus*), revealed from mtDNA analysis. Mar. Biol. (Berl.), **138**: 407–419. doi:10.1007/s002270000465.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in light of biological research. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **20**: 1–228.

Hunt, G.L., and Stabeno, P.J. 2005. Oceanography and ecology of the Aleutian Archipelago: spatial and temporal variation. Fish. Oceanogr. **14**(Suppl.): 292–306. doi:10.1111/j.1365-2419.2005.00378.x.

Hutchinson, W.F., Carvalho, G.R., and Rogers, S.I. 2001. Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. Mar. Ecol. Prog. Ser. **223**: 251–260. doi:10.3354/meps223251.

Hutchison, D.W., and Templeton, A.R. 1999. Correlation of pairwise genetic and geographic measures: inferring the relative influences of gene flow and genetic drift on the distribution of genetic variability. Evolution, **53**: 1898–1914. doi:10.2307/2640449.

Iwamoto, E., Ford, M.J., and Gustafson, R.G. 2004. Genetic population structure of Pacific hake, *Merluccius productus*, in the Pacific Northwest. Environ. Biol. Fishes, **69**: 187–199. doi:10.1023/B:EBFI.0000022895.10683.c5.

Jorde, P.E., Knutsen, H., Espeland, S.H., and Stenseth, N.C. 2007. Spatial scale of genetic structuring in coastal cod *Gadus morhua* and geographic extent of local populations. Mar. Ecol. Prog. Ser. **343**: 229–237. doi:10.3354/meps06922.

Knutsen, H., Jorde, P.E., Andre, C., and Stenseth, N.C. 2003. Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol. Ecol. **12**(2): 385–394. doi:10.1046/j.1365-294X.2003.01750.x. PMID:12535089.

Knutsen, H., Olsen, E.M., Ciannelli, L., Espeland, S.H., Knutsen, J.A., Simonsen, J.H., Skreslet, S., and Stenseth, N.C. 2007. Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. Mar. Ecol. Prog. Ser. **333**: 249–255. doi:10.3354/meps333249.

Koizumi, I., Yamamoto, S., and Maekawa, K. 2006. Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. Mol. Ecol. **15**: 3175–3189. doi:10.1111/j.1365-294X.2006.03019.x. PMID:16968263.

Logerwell, E.A., Aydin, K., Barbeaux, S., Brown, E., Conners, M.E., Lowe, S., Orr, J.W., Ortiz, I., Reuter, R., and Spencer, P. 2005. Geographic patterns in the demersal ichthyofauna of the Aleutian Islands. Fish. Oceanogr. **14**(suppl): 93–112. doi:10.1111/j.1365-2419.2005.00366.x.

Manni, F., Guerard, E., and Heyer, E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum. Biol. **76**: 173–190. doi:10.1353/hub.2004.0034. PMID:15359530.

Marko, P.B., Rogers-Bennett, L., and Dennis, A.B. 2007. mtDNA population structure and gene flow in lingcod (*Ophiodon elongatus*): limited connectivity despite long-lived pelagic larvae. Mar. Biol. (Berl.), **150**: 1301–1311. doi:10.1007/s00227-006-0395-0.

Masson, D. 2002. Deep water renewal in the Strait of Georgia. Estuar. Coast. Shelf Sci. **54**: 115–126. doi:10.1006/ecss.2001.0833.

Miller, J.A., and Shanks, A.L. 2004. Evidence for limited dispersal in black rockfish (*Sebastes melanops*): implications for population structure and marine-reserve design. Can. J. Fish. Aquat. Sci. **61**: 1723–1735. doi:10.1139/f04-111.

Miller, K.M., Le, T.D., and Beacham, T.D. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Mol. Ecol. **9**: 238–239. doi:10.1046/j.1365-294x.2000.00804-2.x. PMID:10672170.

Nichol, D.G., Honkalehto, T., and Thompson, G.G. 2007. Proximity of Pacific cod to the sea floor: using archival tags to estimate fish availability to research bottom trawls. Fish. Res. **86**: 129–135. doi:10.1016/j.fishres.2007.05.009.

O'Leary, D.B., Coughlan, J., Dillane, E., McCarthy, T.V., and Cross, T.F. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. J. Fish Biol. **70**: 310–335. doi:10.1111/j.1095-8649.2007.01451.x.

O'Reilly, P.T., Canino, M.F., Bailey, K.B., and Bentzen, P. 2000. Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. J. Fish Biol. **56**: 1074–1086. doi:10.1111/j.1095-8649.2000.tb02124.x.

Olsen, E.M., Knutsen, H., Gjosaeter, J., Jorde, P.E., Knutsen, J.A., and Stenseth, N.C. 2004. Life-history variation among local populations of Atlantic cod from the Norwegian Skagerrak coast. J. Fish Biol. **64**: 1725–1730. doi:10.1111/j.0022-1112.2004.00402.x.

Palsson, W.A. 1990. Pacific cod (*Gadus macrocephalus*) in Puget

Sound and adjacent water: biology and stock assessment. Washington Department of Fisheries, Olympia, Washington. Tech. Rep. No. 112.

Palsson, W.A., Hoeman, J.C., Bargmann, G.G., and Day, D.E. 1997. 1995 status of Puget Sound bottomfish stocks (revised). Washington Department of Fish and Wildlife, Olympia, Washington. Rep. No. MRD98-03.

Pogson, G.H., Taggart, C.T., Mesa, K.A., and Boutilier, R.G. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution, **55**: 131–146. doi:10.1111/j.0014-3820.2001.tb01279.x. PMID:11263734.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 3): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Rice, W.R. 1989. Analyzing tables of statistical data. Evolution, **43**: 223–225. doi:10.2307/2409177.

Rocha-Olivares, A., and Vetter, R.D. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can. J. Fish. Aquat. Sci. **56**: 803–813. doi:10.1139/cjfas-56-5-803.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics, **145**: 1219–1228. PMID:9093870.

Ruzzante, D.E., Taggart, C.T., and Cook, D. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Mol. Ecol. **7**: 1663–1680. doi:10.1046/j.1365-294x.1998.00497.x.

Shaklee, J.B., and Bentzen, P. 1998. Genetic identification of stocks of marine fish and shellfish. Bull. Mar. Sci. **62**: 589–621.

Shi, Y., Gunderson, D., Munro, P., and Urban, J.D. 2007. Estimating movement rates of Pacific cod (*Gadus macrocephalus*) in the Bering Sea and the Gulf of Alaska using mark–recapture methods [online]. North Pacific Research Board, Anchorage, Alaska, Final Report, Project No. 620. Available at http://doc.nprb.org/web/06_prjs/NPRB.Project.620.Final.Report.doc [accessed 22 April 2008].

Shimada, A.M., and Kimura, D.K. 1994. Seasonal movements of Pacific cod, *Gadus macrocephalus*, in the eastern Bering Sea and adjacent waters based on tag–recapture data. Fish. Bull. (Washington, D.C.), **92**: 800–816.

Slatkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution, **47**: 264–279. doi:10.2307/2410134.

Slatkin, M. 1995. A measure of population subdivision based upon microsatellite allele frequencies. Genetics, **139**: 457–462. PMID:7705646.

Stabeno, P.J., Kachel, D.G., Kachel, N.B., and Sullivan, M.E. 2005. Observations from moorings in the Aleutian Passes: temperature, salinity and transport. Fish. Oceanogr. **14**: 39–54. doi:10.1111/j.1365-2419.2005.00362.x.

Svedäng, H., Righton, D., and Jonsson, P. 2007*a*. Migratory behaviour of Atlantic cod *Gadus morhua*: natal homing is the prime stock-separating mechanism. Mar. Ecol. Prog. Ser. **345**: 1–12. doi:10.3354/meps07140.

Svedäng, H., Righton, D., and Jonsson, P. 2007*b*. Defining 'natal homing' in marine fish populations: need for inference in fishery science: reply to Bradbury and Laurel (2007). Mar. Ecol. Prog. Ser. **349**: 309–310. doi:10.3354/meps07282.

Thompson, G., Ianelli, J., Dorn, M., Nichol, D., Gaichas, S., and Aydin, K. 2007*a*. Assessment of the Pacific cod stock in the eastern Bering Sea and Aleutian Islands area. North Pacific Fishery Management Council, 605 W. 4th Avenue, Anchorage, AK 99501, USA.

Thompson, G.G., Ianelli, J.N., Dorn, M.W., and Wilkins, M. 2007*b*. Assessment of the Pacific cod stock in the Gulf of Alaska. North Pacific Fishery Management Council, 605 W. 4th Avenue, Anchorage, AK 99501, USA.

Tunnicliffe, V., O'Connell, J.M., and McQuoid, M.R. 2001. A Holocene record of marine fish remains from the northeastern Pacific. Mar. Geol. **174**: 197–210. doi:10.1016/S0025-3227(00)00150-X.

Turner, T.F., Wares, J.P., and Gold, J.R. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics, **162**: 1329–1339. PMID:12454077.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**: 535–538. doi:10.1111/j.1471-8286.2004.00684.x.

Waples, R.S., and Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol. Ecol. **15**: 1419–1439. doi:10.1111/j.1365-294X.2006.02890.x. PMID:16629801.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370. doi:10.2307/2408641.

West, J.E. 1997. Protection and restoration of marine life in the inland waters of Washington State. Puget Sound/Georgia Basin Environ. Rep. Ser. No. 6.

Westrheim, S.J. 1982. Pacific cod tagging. II. Migration and stock delineation. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 1663.

Westrheim, S.J. 1987. Parabrancial X-cell lesions in trawl-caught Pacific cod (*Gadus macrocephalus*) off the west coast of Canada — incidence and effects. Can. Tech. Rep. Fish. Aquat. Sci. No. 2092.

Wilimovsky, N.J., Peden, A., and Peppar, J. 1967. Systematics of six demersal fishes of the North Pacific Ocean. Fish. Res. Board Can. Tech. Rep. No. 34.

Wright, S. 1999. Petition to the Secretary of Commerce to list as threatened or endangered 18 "species/populations" of "Puget Sound" marine fishes and to designate critical habitat. Petition to the US National Marine Fisheries Service, February 1999. More information available at http://www.epa.gov/EPA-SPECIES/2000/November/Day-24/e30028.htm.

Zwanenburg, K.C.T., Bentzen, P., and Wright, J.M. 1992. Mitochondrial DNA differentiation in western North Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. **49**: 2527–2537. doi:10.1139/f92-279.

Appendix A

Table A1 follows.

Table A1. Sample size (N), number of alleles (N_A), allelic richness (N_S) corrected to the smallest sample size (n = 18), expected (H_e) and observed (H_o) heterozygosities, and estimates of F_{IS} for 11 microsatellite loci in Pacific cod (*Gadus macrocephalus*).

		Sample											
Locus		AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS	ALL
Gma100	N	45	92	45	87	87	106	94	85	69	94	14	818
	N_A	45	68	44	56	60	62	58	56	50	52	14	101
	N_S	18.05	18.12	18.02	17.66	18.16	17.92	17.77	12.26	17.10	16.71	12.54	17.78
	H_e	0.9694	0.9748	0.9694	0.972	0.9753	0.9745	0.973	0.9685	0.9659	0.9642	0.9056	0.978
	H_o	0.9778	0.9674	0.9333	0.908	0.9655	0.9717	0.9787	0.9294	0.9565	0.9255	0.8571	0.950
	F_{is}	0.003	0.013	0.048	0.072	0.016	0.008	–0.001	0.046	0.017	0.045	0.090	0.029
Gma101	N	45	92	45	87	94	106	92	84	69	94	11	819
	N_A	16	21	16	23	23	25	23	22	19	21	12	38
	N_S	10.81	11.17	10.89	11.52	11.33	11.85	11.98	11.24	10.66	10.59	12.00	11.43
	H_e	0.9099	0.9143	0.9086	0.918	0.9179	0.9188	0.9208	0.9138	0.9041	0.9035	0.8678	0.920
	H_o	0.8667	0.8913	0.9556	0.9425	0.9255	0.8868	0.9022	0.9405	0.8696	0.8723	0.9091	0.905
	F_{is}	0.059	0.031	–0.040	–0.021	–0.003	0.040	0.026	–0.023	0.046	0.040	0.000	0.017
Gma102	N	45	92	45	87	95	106	93	89	69	94	16	831
	N_A	14	15	13	16	13	17	14	14	13	14	11	19
	N_S	9.28	9.62	9.68	9.62	9.03	9.89	9.51	9.43	9.04	9.08	9.82	9.47
	H_e	0.8686	0.8898	0.8825	0.8859	0.8811	0.8991	0.8922	0.8887	0.8766	0.8793	0.8516	0.892
	H_o	0.9111	0.9239	0.9333	0.9655	0.8737	0.9245	0.9032	0.8539	0.8841	0.8511	0.75	0.898
	F_{is}	–0.038	–0.033	–0.046	–0.084	0.014	–0.024	–0.007	0.045	–0.001	0.037	0.151	–0.006
Gma103	N	43	92	44	87	94	106	94	88	69	94	17	828
	N_A	30	37	32	36	38	38	39	39	37	41	20	59
	N_S	13.15	13.38	14.26	11.78	12.70	12.09	11.85	13.53	13.76	14.10	15.02	13.18
	H_e	0.9143	0.9301	0.9243	0.8986	0.9091	0.8991	0.8917	0.9245	0.9239	0.9374	0.9143	0.923
	H_o	0.9302	0.8913	0.9318	0.908	0.9574	0.9245	0.8617	0.9545	0.9275	0.9362	0.9302	0.920
	F_{is}	–0.006	0.047	0.003	–0.005	–0.048	–0.024	0.039	–0.027	0.003	0.007	0.070	0.003
Gma106	N	45	90	45	87	95	106	94	89	69	94	16	830
	N_A	8	12	10	12	13	10	13	10	11	12	8	17
	N_S	5.70	6.43	6.52	6.89	7.81	7.38	7.37	6.57	6.63	6.73	6.66	6.94
	H_e	0.7353	0.7404	0.7968	0.8063	0.8333	0.8257	0.8153	0.8034	0.8024	0.8061	0.7539	0.812
	H_o	0.7111	0.7556	0.8667	0.7931	0.8316	0.8585	0.8191	0.8315	0.7246	0.8191	0.875	0.807
	F_{is}	0.044	–0.015*	–0.077	0.022	0.007	–0.035	0.001	–0.029	0.104	–0.011	–0.129	0.006
Gma108	N	45	92	45	87	94	106	94	89	69	92	17	830
	N_A	8	11	10	10	14	12	11	11	9	9	5	20
	N_S	4.25	4.59	5.11	4.56	5.16	5.15	4.74	5.15	3.59	4.66	3.82	4.75
	H_e	0.4247	0.4637	0.5504	0.4345	0.5171	0.4717	0.4388	0.5463	0.2871	0.5178	0.2664	0.469
	H_o	0.4000	0.5000	0.5778	0.4138	0.5532	0.4151	0.4894	0.5618	0.2464	0.5109	0.2353	0.465
	F_{is}	0.069	–0.073	–0.039	0.054	–0.064	0.125	–0.11	–0.023	0.149	0.019	0.147	0.009
Gma109	N	44	91	44	87	94	106	94	85	69	94	11	819
	N_A	21	29	22	29	26	28	26	26	24	24	14	35
	N_S	11.58	11.72	12.29	12.71	11.99	12.13	11.55	12.55	12.63	12.13	14.00	12.37
	H_e	0.9021	0.9108	0.9179	0.9278	0.9215	0.9235	0.9082	0.9285	0.9288	0.9243	0.8802	0.929

Table A1 (*concluded*).

Locus		Sample AD	AI	AT	UP05	UP03	KI05	KI03	HS	WA	SG	PS	ALL
	H_o	0.9091	0.9011	0.8864	0.9195	0.9149	0.8774	0.8723	0.9176	0.8841	0.9362	0.8182	0.901
	F_{is}	0.004	0.016	0.046	0.015	0.012	0.055	0.045	0.018	0.055	–0.008	0.118	0.030
Gmo37	N	45	92	45	87	95	106	93	89	69	94	16	831
	N_A	26	36	25	32	36	36	34	31	27	32	12	65
	N_S	13.33	13.63	12.97	12.88	13.68	13.15	13.27	12.26	11.84	12.59	9.76	12.98
	H_e	0.9289	0.933	0.919	0.9242	0.9368	0.9247	0.9307	0.9051	0.9038	0.9165	0.8184	0.928
	H_o	0.9556	0.8913	0.9333	0.9310	0.9579	0.8962	0.9140	0.9101	0.9565	0.9468	0.7500	0.923
	F_{is}	–0.017	0.050	–0.004	–0.002	–0.017	0.036	0.023	0.000	–0.051	–0.028	0.115	0.006
Tch20	N	45	92	45	87	94	106	93	89	69	93	16	829
	N_A	20	21	23	22	22	24	22	21	22	21	13	27
	N_S	12.24	12.35	13.87	12.62	12.16	12.63	12.58	11.60	12.73	11.89	11.37	12.38
	H_e	0.9222	0.9311	0.9407	0.9322	0.9284	0.9343	0.9343	0.9213	0.9329	0.9262	0.8984	0.936
	H_o	1.0000	0.9130	0.9778	0.9540	0.9362	0.9057	0.9462	0.9326	0.9710	0.9892	0.9375	0.947
	F_{is}	–0.073	0.025	–0.028	–0.018	–0.003	0.035	–0.007	–0.007	–0.034	–0.063	–0.011	–0.012
All loci	N	44.67	91.67	44.78	87.00	93.56	106.00	93.44	87.44	69.00	93.67	14.89	
	N_A	220	282	225	268	285	283	276	263	242	255	125	
	N_S	10.74	10.83	11.10	10.78	11.06	10.89	10.86	10.65	10.55	10.32	9.97	
	H_e	0.8576	0.8624	0.8772	0.8651	0.8764	0.8684	0.8651	0.8709	0.8482	0.8610	0.8292	
	H_o	0.8521	0.8490	0.8926	0.8566	0.8790	0.8542	0.8593	0.8734	0.8340	0.8490	0.7874	
	F_{is}	0.006	0.015	–0.018	0.010	–0.003	0.016	0.007	–0.003	0.017	0.014	0.005	

Note: Sample abbreviations as in Table 1. *, $P \leq 0.05$.

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