An examination of spatial and temporal genetic variation in walleye pollock (*Theragra chalcogramma*) using allozyme, mitochondrial DNA, and microsatellite data*

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Abstract–We used allozyme, microsatellite, and mitochondrial DNA (mtDNA) data to test for spatial and interannual genetic diversity in walleye pollock (*Theragra chalcogramma*) from six spawning aggregations representing three geographic regions: Gulf of Alaska, eastern Bering Sea, and eastern Kamchatka. Interpopulation genetic diversity was evident primarily from the mtDNA and two allozyme loci (*SOD-2**, *MPI**). Permutation tests indicated that $\hat{F}_{ST}$ values for most allozyme and microsatellite loci were not significantly greater than zero. The microsatellite results suggested that high locus polymorphism may not be a reliable indicator of power for detecting population differentiation in walleye pollock. The fact that mtDNA revealed population structure and most nuclear loci did not suggests that the effective size of most walleye pollock populations is large (genetic drift is weak) and migration is a relatively strong homogenizing force. The allozymes and mtDNA provided mostly concordant estimates of patterns of spatial genetic variation. These data showed significant genetic variation between North American and Asian populations. In addition, two spawning aggregations in the Gulf of Alaska, in Prince William Sound, and off Middleton Island, appeared genetically distinct from walleye pollock spawning in the Shelikof Strait and may merit management as a distinct stock. Finally, we found evidence of interannual genetic variation in two of three North American spawning aggregations, similar in magnitude to the spatial variation among North American walleye pollock. We suggest that interannual genetic variation in walleye pollock may be indicative of one or more of the following factors: highly variable reproductive success, adult philopatry, source-sink metapopulation structure, and intraannual variation (days) in spawning timing among genetically distinct but spatially identical spawning aggregates.

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Detecting spatial structure in the genetic variation of some marine fishes is challenging because populations are often closely related due to high gene flow, and the relationships between populations may change over years (Hedgecock, 1994; Shaklee and Bentzen, 1998; Waples, 1998). For these species, independent population studies of genetic variation may result in conflicting evidence on the extent and complexity of population structure (McQuinn, 1997; Shaklee and Bentzen, 1998). Interpreting these results may be further complicated if the loci and classes of genetic markers differ by study and year. In addition, the discordant patterns of population structure may reflect complex population biology and ecology or merely differences in the resolving power of loci and marker classes to detect spatial structure. The walleye pollock (*Theragra chalcogramma*) is a marine fish that typifies this situation.

Walleye pollock inhabit basin, slope, and shelf waters of four major seas in the North Pacific Ocean: Sea of Japan, Sea of Okhotsk, Bering Sea, and Gulf of Alaska (Bailey et al., 1997). Their abundance is greatest in the eastern Bering Sea and the Sea of Okhotsk, but large aggregations are also found in the Sea of Japan, western Bering Sea, and the Gulf of Alaska. Smaller aggregations occur along coastal waters in bays and fjords such as in Prince William Sound, Alaska, and Puget Sound in Washington State. Population boundaries for walleye pollock may be correlated with the margins of spatially distinct spawning aggregates that occur throughout the species range in predictable locations during late winter and early spring (Bailey et al., 1999). Many of these spawning aggregates can be distinguished by spawning time and habitat, and by meristic and morphometric characters (e.g. Iwata, 1975b; Hinckley, 1987; Mulligan et al., 1989). Nevertheless, genetic support for discrete populations is equivocal, and the extent of population structuring in walleye pollock remains a controversial and unresolved issue for management and conservation in U.S. and international waters (Bailey et al., 1999).

Independent attempts to define genetic population structure in walleye pollock on a regional scale have shown mixed results. Some allozyme loci show genetic differentiation between walleye pollock from contiguous sea basins (e.g. eastern Bering Sea and Gulf of Alaska; Grant and Utter, 1980), and a single locus (*SOD*) appears to reveal a major east–west division between Asian and North American populations (Iwata, 1975a, 1975b; Grant and Utter, 1980). This major division is supported by a

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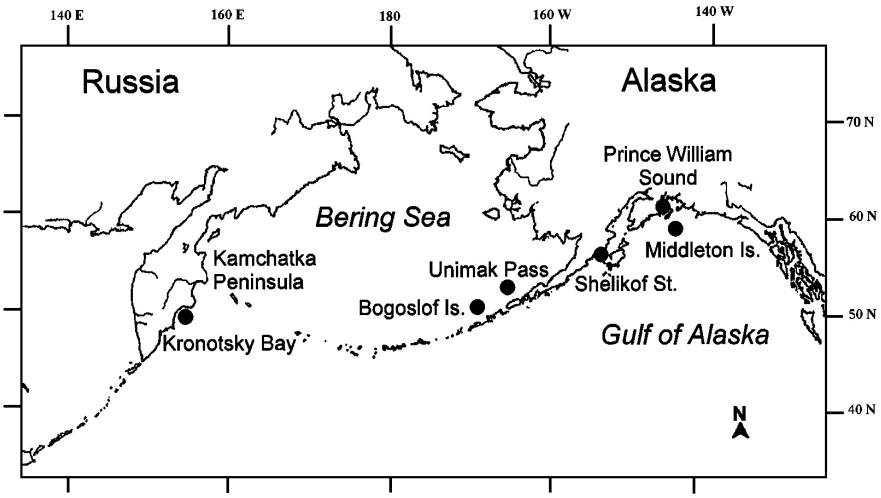

Figure 1

Map of the Gulf of Alaska and Bering Sea showing the location of walleye pollock spawning aggregations sampled for this study.

single study of mitochondrial DNA (mtDNA) sequence variation (Shields and Gust, 1995), but it is not evident in an unpublished study of microsatellite variation at two loci (reviewed in Bailey et al., 1999). Conversely, genetic differentiation among eastern Bering Sea and Gulf of Alaska populations is supported by the microsatellite study but is not supported by a study of mtDNA-RFLP (restriction fragment length polymorphism) variation (Mulligan et al., 1992). These discordant results may reflect attributes of walleye pollock population biology, the unique evolutionary properties of each marker class, or both. Nevertheless, an interpretation of the collective data is not possible because the studies were conducted as much as 22 years apart, and many samples were not from spawning aggregations.

Nonrepresentative sampling may bias attempts to define genetic population structure in walleye pollock, particularly on a fine spatial scale (within sea basins). For example, some studies using allozymes (Grant and Utter, 1980) and mtDNA (Shields and Gust, 1995) have included population samples taken during summer and fall, even though walleye pollock aggregate for spawning in late winter and early spring. These samples may obscure genetic differentiation because walleye pollock populations are believed to mingle in summer and fall during feeding migrations (Bailey et al., 1999). Also, the sample size used in some studies may be inadequate to detect population structure. The sample size in walleye pollock studies thus far has been typically less than 50, and the minimum sample size has been 22 (e.g. Grant and Utter, 1980; Mulligan et al., 1992). Sample sizes in this range (22–50) are generally insufficient to detect statistically significant differences in allele frequencies between populations that exhibit weak population structure (e.g. F_{ST} <0.02; Goudet et al., 1996; Ruzzante, 1998; Waples, 1998). These studies suggest that a sample size of 50 should be considered an absolute minimum for high gene flow species and that sample sizes of 100 or greater may be necessary when allele frequencies differ by 5% or less.

In our empirical study, we accounted for the sources of sample bias described above and addressed two important questions: 1) Do allozymes, mtDNA, and microsatellites provide concordant estimates of inter- and intraregional population structure in walleye pollock? 2) Is the genetic variation in walleye pollock populations stable from year to year? We tested for spatial patterns of genetic variation among six population samples from three regions: Gulf of Alaska, eastern Bering Sea, and eastern Kamchatka. We also tested for interannual stability of the genetic signal in replicate samples from three of the North American populations. Our results showed that two of 24 polymorphic allozyme loci (*SOD-2**, *MPI**) reveal significant spatial heterogeneity at the inter- and intraregional level. In general, the mtDNA data were concordant with these two allozymes, revealing significant genetic variation between North American and Asian (eastern Kamchatka) populations, as well as evidence of intraregional genetic variation, particularly among the Gulf of Alaska populations. The microsatellites, although highly polymorphic, showed little spatial variation. The allozyme and mtDNA data provided evidence of interannual genetic variation in two North American populations. $\hat{F}_{ST}$ values showed this interannual variation is of similar magnitude to the spatial variation in these populations.

Materials and methods

Sample collection

Tissue samples for allozyme and DNA analysis were obtained from six spawning aggregations of walleye pollock representing three major geographic regions: Gulf of Alaska, eastern Bering Sea, eastern Kamchatka (Fig. 1, Table 1).

Table 1
Location, sample data, and spawning habitat of walleye pollock examined in this study.

Major region and population	Spawning habitat	Date	*n*	N Lat.	W Long.
North America–Gulf of Alaska					
Shelikof Strait	shelf	1 Mar 97	100	57°55′	154°35′
		19 Mar 98	100	57°55′	154°40′
Prince William Sound	fjord	6 Feb 97	100	60°05′	148°20′
		6 Feb 98	100	60°05′	148°19′
Middleton Island	shelf	5 Mar 98	100	59°26′	145°45′
North America–Bering Sea					
Bogoslof Island	basin	1 Mar 97	100	53°15′	169°00′
		7 Mar 98	100	53°08′	169°11′
Unimak Pass	shelf	11 Apr 98	40	54°33′	165°38′
Asia–eastern Kamchatka					
Kronotsky Bay	shelf	1 Feb 99	96	52°00′	161°00′E

In total, nine samples were collected from 1977 through 1999: six from each of the major spawning aggregations shown in Figure 1, and three samples that were interannual replicates from Prince William Sound (PWS), Shelikof Strait (SHEL), and Bogoslof Island (BOG). Other samples included Unimak Pass (UNI) and Middleton Island (MID) in 1998 and Kronotsky Bay (KRON) in 1999. Tissue samples from heart, liver, muscle, and eye were taken from 100 individuals per population with the exception of Unimak Pass (n=40) and Kronotsky Bay (n=96). Only muscle tissue was sampled from Bogoslof Island walleye pollock in 1997. Samples were stored at –80°C until analyzed.

Allozyme analysis

Allozyme alleles were resolved by using horizontal starch-gel electrophoresis and enzyme-specific histochemical staining procedures described by Aebersold et al. (1987). Thirty six loci were screened for polymorphism (Table 2): *sAAT-1**, *sAAT-2**, *mAAT-1**, *ADA-1**, *ADA-2**, *AH-1**, *AH-2**, *ALAT**, *CK-A**, *FH**, *GAPDH-1**, *G3PDH-1**, *G3PDH-2**, *G3PDH-3**, *G3PDH-4**, *GPI-1**, *GPI-2**, *IDHP-1**, *IDHP-2**, *IDHP-3**, *LDH-2**, *LDH-3**, *MDH-A**, *MEP-1**, *MEP-2**, *MPI**, *PEPA**, *PEPB**, *PEPD**, *PEPLT**, *PGDH**, *PGM-1**, *PGM-2**, *SOD-1**, *SOD-2**, and *TPI**. Twenty-four loci were polymorphic in at least one of nine populations, and these loci were used in the tests of spatial and temporal genetic variation (Table 3).

DNA analysis

Total genomic DNA was isolated from 20–30 mg of heart tissue by using a Gentra Systems™ (Minneapolis, MN) Puregene DNA isolation kit. Precipitated DNA was hydrated in 50–100 µL tris-ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and heated at 55°C for approximately 12 h. Approximately 1 µL of hydrated DNA was used from each sample for polymerase chain reaction (PCR) amplification of mtDNA and microsatellite fragments.

mtDNA For an initial screen, six segments of the walleye pollock mitochondrial genome were examined for restriction fragment length polymorphism in a sample of 12 walleye pollock. The mtDNA segments examined were ND5/6 (~2400 base pairs *[*bp*]*; Cronin et al., 1993), cytochrome *b* (~1200 bp and~800 bp; Bickham et al., 1995), cytochrome oxidase I (~700 bp; Powers[1]), D-loop (~1400 bp; Cronin et al., 1993), and 16S (~600 bp; Palumbi et al., 1991). We used the following restriction enzymes: *Alu* I, *Apa* I, *Ase* I, *Ava* I, *Ava* II, *BamH* I, *Bcl* I, *Bgl* I, *Bgl* II, *BstE* II, *BstU* I, *Dpn* II, *EcoR* I, *EcoR* V, *Hae* II, *Hae* III, *Hha* I, *Hinf* I, *Hind* III, *Kpn* I, *Mse* I, *Msp* I, *Nci* I, *Pst* I, *Rsa* I, *Sac* I, *Sac* II, *Sau96* I, *Sca* I, *Stu* I, *Taq* I, *Xba* I, and Xho I.

PCRs were performed in 25–100 µL volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0–3.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.05 units/µL *Taq* polymerase, 0.3–1.2 µM primer, and about 250 ng DNA template. The PCR profile was 92°C (2 min) + 30–40 cycles of (92°C (30 sec) + *X*°C (30 sec) + 72°C (140 sec)) + 72°C (5 min), where the annealing temperature *X* varied among primer pairs. Restriction digests followed manufacturer's specifications (New England Biolabs, Beverly, Maine).

The mtDNA fragment and enzyme combinations ND5/6 (~2400 bp)—*Ase* I, *Ava* I, *Msp* I, *Rsa* I; cytochrome *b* (~1200 bp)—*Alu* I, *Hae* III, *Mse* I; and cytochrome oxidase (~700 bp)—*Alu* I revealed polymorphism and were used to test for spatial and temporal genetic variation. The PCR annealing temperatures for these fragments were the following: (for ND5/6) 50°C; (for cytochrome *b*) 54°C; and (for cytochrome oxidase) 50°C. Restriction fragments

[1] Powers, D. A. 1997. The use of molecular techniques to dissect the genetic architecture of pollock populations. Unpubl. rep. to the National Marine Fisheries Service, 11 p. Hopkins Marine Station, Stanford University, Pacific Grove, CA, 93950.

Table 2

Buffers and tissues used to resolve enzyme-coding loci in walleye pollock. Enzyme nomenclature and Enzyme Commission (EC) number follow IUBNC (1984); locus nomenclature follows Shaklee et al., (1990).

Enzyme or protein	EC number	Locus	Tissue[1] (buffer[2])
Aspartate aminotransferase	2.6.1.1	*sAAT-1**	L(AC6.5)
		*sAAT-2**	H(AC6.1), M(AC6.1)
		*mAAT-1**	M(AC6.1)
Adenosine deaminase	3.5.4.4	*ADA-1**	L(AC6.5)
		*ADA-2**	L(AC6.5)
Aconitate hydratase	4.2.1.3	*AH-1**	L(AC6.5)
		*AH-2**	H(AC6.5), M(AC6.1, AC6.5), L(AC6.5)
Alanine aminotransferase	2.6.1.2	*ALAT**	M(TBE)
Creatine kinase	2.7.3.2	*CK-A**	M(TBE)
Fumarate hydratase	4.2.1.2	*FH**	M(ACEN7.0)
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	M(AC6.9)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	L(AC6.5)
		*G3PDH-2**	M(AC6.5), L(AC6.5)
		*G3PDH-3**	H(AC6.5), L(AC6.5)
		*G3PDH-4**	L(AC6.5)
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	H(TBCL)
		*GPI-2**	H(TBCL)
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*IDHP-1**	L(ACE7.0), L(TC7.0)
		*IDHP-2**	M(ACEN7.0)
		*IDHP-3**	L(ACE7.0)
L-Lactate dehydrogenase	1.1.1.27	*LDH-2**	H(TBCL), M(TBE)
		*LDH-3**	H(TBCL), M(TBE)
Malate dehydrogenase	1.1.1.37	*MDH-A**	H(AC6.1), M(AC6.1, AC6.9)
Malic enzyme (NADP+)	1.1.1.40	*MEP-1**	M(AC6.5, AC6.9, ACEN7.0)
		*MEP-2**	M(AC6.5, AC6.9, ACEN7.0)
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H(TBCL), M(TBE)
Dipeptidase	3.4.-.-	*PEPA**	H(AC6.5)
Tripeptide aminopeptidase	3.4.-.-	*PEPB**	M(AC6.9)
Proline dipeptidase	3.4.13.9	*PEPD**	M(AC6.1)
Peptidase-LT	3.4.-.-	*PEPLT**	H(AC6.1)
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M(AC6.9, ACEN7.0)
Phosphoglucomutase	5.4.2.2	*PGM-1**	H(TBCL), M(TBE), L(ACE7.0)
		*PGM-2**	M(TBE)
Superoxide dismutase	1.15.1.1	*SOD-1**	M(ACEN7.0, TBE)
		*SOD-2**	H(ACEN7.0)
Triose-phosphate isomerase	5.3.1.1	*TPI**	H(AC6.1), M(AC6.1)

[1] H = heart; E = eye; M = muscle; L = liver.

[2] Buffers: AC, ACN, ACE, ACEN: amine-citric acid buffer (Clayton and Tretiak, 1972) modified with EDTA (E), NAD (N), or both (Aebersold et al., 1987), values indicate pH; TBCL: tris-citric acid gel, pH 8.7 and lithium hydroxide-boric acid electrode buffer, pH 8.0 (Ridgway et al., 1970); TBE: tris-boric acid-EDTA buffer, pH 8.7 (Boyer et al., 1963).

were size fractionated on 3% agarose gels, stained with ethidium bromide, and visualized and photographed under ultraviolet light (312 nm). Fragment sizes as small as 50 bp were estimated by comparison with 100 bp and 250 bp ladders on each gel. A composite haplotype was generated for each individual by recording the presence or absence of restriction sites for all restriction enzymes and mtDNA segments (Lansman et al., 1981). The length of

Table 3

Single locus statistics: number of individuals (n); number of alleles (A); frequency of the most common allele over all populations (P_a); total heterozygosity ($\hat{H}_T$); analogs of Wright's F-statistics F_{IS}, F_{ST}, and F_{IT} ($\hat{f}$, $\hat{\theta}$, $\hat{F}$). Note: The analog of F_{ST} for haplotype data, $\hat{\Phi}_{ST}$, is computed for mtDNA. Values in bold type with an asterisk are statistically significant. The α-level (0.05) was adjusted (α/k) for k tests, where k is the number of loci (allozymes, 24; microsatellites, 3).

Locus	n	A	P_a	$\hat{H}_T$	$\hat{f}$	$\hat{\theta}$	$\hat{F}$
Allozyme							
*sAAT-1**	692	3	0.996	0.008	–0.001	–0.001	–0.003
*sAAT-2**	793	4	0.992	0.016	–0.011	0.006	–0.005
*mAAT-1**	803	2	0.998	0.004	0.001	–0.002	–0.001
*AH-1**	657	4	0.958	0.081	0.060	0.001	0.061
*ALAT**	811	3	0.898	0.188	–0.019	0.002	–0.017
*FH**	814	2	0.999	0.002	0.001	–0.001	–0.001
*G3PDH-2**	811	4	0.994	0.012	–0.002	–0.002	–0.004
*G3PDH-3**	715	4	0.992	0.016	–0.004	–0.002	–0.007
*GPI-1**	815	3	0.992	0.016	–0.008	0.002	–0.006
*GPI-2**	815	4	0.990	0.020	–0.010	0.003	–0.007
*IDHP-1**	714	4	0.976	0.047	–0.018	–0.001	–0.020
*IDHP-2**	814	3	0.996	0.008	–0.002	–0.001	–0.003
*LDH-2**	815	2	0.999	0.002	0.001	–0.001	–0.001
*LDH-3**	814	2	0.999	0.002	0.000	0.000	0.000
*MDH-A**	815	3	0.996	0.008	–0.002	0.000	–0.002
*MEP-1**	815	4	0.994	0.012	–0.004	–0.001	–0.004
*MPI**	810	5	0.788	0.337	0.016	**0.017***	0.032
*PEPB**	802	4	0.962	0.074	–0.001	0.003	0.003
*PEPD**	815	3	0.985	0.030	**0.158***	–0.001	0.157
*PGDH**	814	9	0.544	0.590	0.043	–0.002	0.041
*PGM-1**	815	4	0.993	0.014	–0.004	0.000	–0.004
*SOD-1**	815	2	0.999	0.002	0.000	–0.001	0.000
*SOD-2**	711	3	0.916	0.154	–0.014	**0.088***	0.075
*TPI**	815	3	0.997	0.006	–0.002	0.000	–0.002
Mean	788	4	0.956	0.069	0.024	**0.010***	0.033
SE		1	0.100	0.136	0.014	0.011	0.010
Microsatellite							
Tch10	741	32	0.395	0.811	0.069	0.001	0.070
Tch12	671	12	0.305	0.782	0.035	0.000	0.035
Tch22	637	15	0.367	0.713	0.039	**0.005***	0.043
Mean	683	20	0.356	0.769	**0.048***	**0.002***	0.050
SE		11	0.046	0.050	0.011	0.001	0.011
mtDNA							
ND5/6-CB-CO	684	66	0.371	0.837		**0.022***	

the recognition sequence (bp) and the number of recognition sites identified by each enzyme were the following: for ND5/6—*Ase* I (6, 5), *Ava* I (6, 4), *Msp* I (4, 4), *Rsa* I (4, 5); for cytochrome *b*—*Alu* I (4, 2), *Hae* III (4, 2), *Mse* I (4, 7); and for cytochrome oxidase—*Alu* I (4, 4). A total of 150 nucleotides were surveyed for polymorphism or approximately 3.5% of the combined 4300-bp region.

Microsatellites Thirteen microsatellite loci were screened for polymorphism, amplification quality, and null alleles in walleye pollock by using primer pairs derived from Atlantic cod, *Gadus morhua*, (*Gmo1*, *Gmo2*, *Gmo9*, *Gmo10*, *Gmo123*, *Gmo132*, and *Gmo145*; Brooker et al., 1994) and walleye pollock (*Tch5*, *Tch10*, *Tch11*, *Tch12*, *Tch18*, and *Tch22*; O'Reilly et al., 2000). PCRs were performed in 10 µL volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.05 units/µL Taq polymerase, 0.3–1.2 µM primer, and about 250 ng DNA template. The following PCR profile was used: 92°C (2 min) + 30 cycles of (92°C (30 sec) + X°C (30 sec) + 72°C (140 sec)) + 72°C (5 min) where the annealing temperature X varied among microsatellites.

Microsatellites were size fractionated by using an Applied Biosystems Inc. (ABI, Foster City, CA) 377-96 automated DNA sequencer operated in GeneScan™ mode (ABI, 1996a). Data were analyzed by using the internal

lane sizing standard and local Southern sizing algorithm in the GeneScan 672 software vers. 1.1 (ABI, 1996a). Alleles for each locus were scored and data were tabulated for importing into statistical software with Genotyper software, vers. 2.0 (ABI, 1996b).

Of the thirteen loci screened, *Tch10*, *Tch12*, and *Tch22* were used to test for spatial and temporal genetic variation. The PCR annealing temperatures for these loci were the following: (for *Tch10*) 54°C; (for *Tch12*) 47°C; and (for *Tch22*) 52°C. The loci *Gmo10*, *Gmo123*, *Gmo145*, *Tch5*, and *Tch18* did not amplify consistently by the methods above, and *Gmo1*, *Gmo2*, *Gmo9*, *Gmo132*, and *Tch11* appeared to possess null alleles as revealed by significant heterozygote deficits (data not shown).

Statistical analyses

Estimates of allele and haplotype frequency were calculated for each locus and population sample. Heterozygosity estimates ($\hat{H}$) were calculated by using Equation 8.4 of Nei (1987) and haplotype diversity estimates ($\hat{h}$) were computed by using the program ARLEQUIN vers. 1.1 (Schneider et al., 2000). A permutation test of the statistic $\hat{f}$ (Weir and Cockerham, 1984) was used to assess conformity to Hardy-Weinberg equilibrium (HWE) for each locus and over all loci for each population with the computer program FSTAT vers. 2.8 (Goudet 2000). The data set was permuted 1000 times (alleles were permuted among individuals, within population samples), and the threshold for statistical significance (α=0.05) was corrected for simultaneous tests by using the sequential Bonferroni method (Rice, 1989).

Spatial and temporal genetic variation were quantified by estimating analogs of Wright's F_{ST} for each marker class: θ (allozymes and microsatellites, Weir and Cockerham, 1984), R_{ST} (microsatellites, Slatkin, 1995), Φ_{ST} (mtDNA, Excoffier et al., 1992). Estimates of θ, R_{ST}, and Φ_{ST} ($\hat{\theta}$, $\hat{R}_{ST}$, and $\hat{\Phi}_{ST}$) were computed for all nine population samples, for all contemporaneous population samples (1997, 1998), and for the temporal replicates. The haplotype frequency estimates were used to compute $\hat{\Phi}_{ST}$. A hierarchical approach was used to determine if more detailed assessment of spatial variation was necessary. That is, only those statistics ($\hat{\theta}$, R_{ST}, $\hat{\Phi}_{ST}$) that were significant over all samples from a given year were then calculated for populations pooled by region, and for populations within regions. Statistics significant at the regional level were calculated for each population pair. This hierarchical approach was applied over all loci for allozymes and microsatellites as well as for individual loci because in some instances statistically significant spatial variation was evident for a single locus but not for the marker class. The computer programs FSTAT, version 2.8 (Goudet, 2000), RST Calc, version 2.2 (Goodman, 1997), and ARLEQUIN, version 1.1 (Schneider et al., 2000) were used to compute $\hat{\theta}$, $\hat{R}_{ST}$, and $\hat{\Phi}_{ST}$.

The statistical significance of spatial and temporal variation in genetic diversity of walleye pollock was estimated by using F_{ST} estimator tests and allelic (haplotypic) goodness-of-fit tests (Goudet et al., 1996). First, the statistical significance of estimates for each F_{ST} analog ($\hat{\theta}$, $\hat{R}_{ST}$, and $\hat{\Phi}_{ST}$) was determined by using a permutation test option in the respective computer programs. In each case the data set was permuted 1000 times (alleles or haplotypes among population samples). Second, allele and haplotype frequency homogeneity was tested for the same population comparisons by using a *G*-test (allozymes and microsatellites) and probability test (mtDNA). The threshold for statistical significance (α=0.05) for multiple comparisons was determined by using the sequential Bonferroni method (Rice, 1989).

Results

Single-locus statistics and a comparison of marker classes

The degree of polymorphism indicated by the number of alleles per locus, allele frequency, and total heterozygosity ($\hat{H}_T$) varied across loci and marker classes and was generally lower for allozymes than for microsatellites (Table 3). The number of alleles per allozyme locus ranged from two to nine (mean=four) and the frequency of the common allele in the pooled population sample was greater than 0.950 for all but four loci (*ALAT**, *MPI**, *PGDH**, and *SOD-2**, Table 3). In contrast, the number of alleles per locus was higher for microsatellites (range=12–32, mean=20), and the maximum allele frequency did not exceed 0.400 (Table 3). Relatively high polymorphism was also detected from restriction digests of ND5/6, cytochrome *b*, and cytochrome oxidase I regions of mtDNA (66 composite haplotypes, maximum haplotype frequency 0.371, Table 3). Values of mean $\hat{H}_T$ were an order of magnitude greater for microsatellites (0.769) than for allozymes (0.069); however, some overlap in range was evident because of the large variation in $\hat{H}_T$ among loci (Table 3). The estimate of haplotype diversity ($\hat{h}$=0.837) was slightly greater than the $\hat{H}_T$ for microsatellites (Table 3).

Values for $\hat{\theta}$ and $\hat{\Phi}_{ST}$ varied among loci and failed to expose a single marker class, regardless of overall variability, as most informative for detecting population structure in walleye pollock (Table 3). Permutation tests revealed two allozyme loci (*MPI**, *SOD-2**) and one microsatellite (*Tch22*) with $\hat{\theta}$ values significantly greater than zero over all population samples (Table 3, Fig. 2). The $\hat{\theta}$ of 0.088, for *SOD-2** was exceptionally high. The $\hat{\Phi}_{ST}$ for mtDNA was within the range of $\hat{\theta}$ for allozymes and microsatellites and was significantly greater than zero (P<0.001).

Intrapopulation genetic variation and Hardy-Weinberg equilibrium

Estimators of intrapopulation diversity (alleles per locus, $\hat{H}_E$ and $\hat{h}$) differed among marker types within populations but generally showed little variation between populations (Table 4). As expected, the values for the diversity estimators were usually lowest in the population with the smallest sample sizes (e.g. UNI, n=40). This was most notable in haplotype number (range 12–27) because most

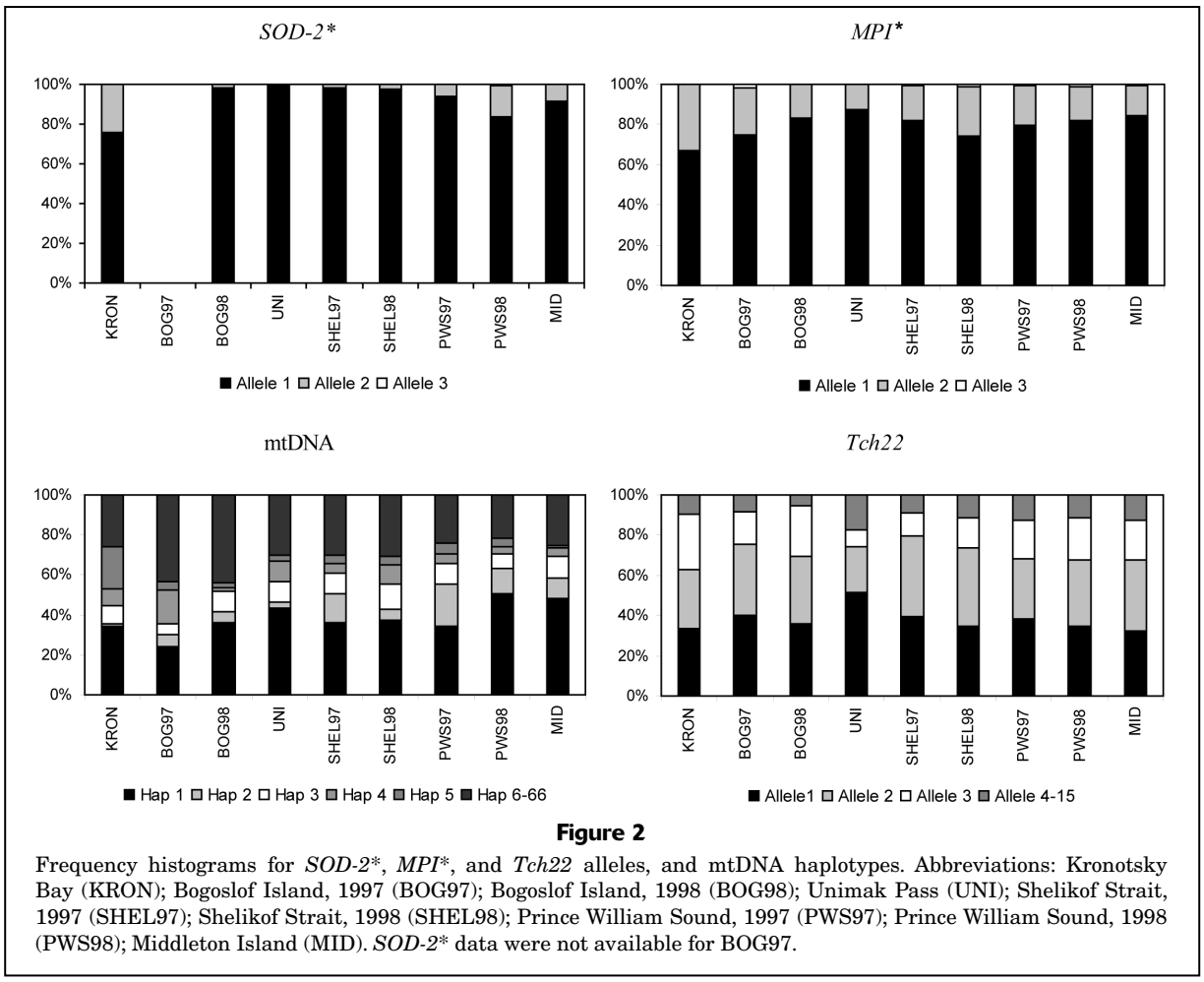

Figure 2

Frequency histograms for *SOD-2**, *MPI**, and *Tch22* alleles, and mtDNA haplotypes. Abbreviations: Kronotsky Bay (KRON); Bogoslof Island, 1997 (BOG97); Bogoslof Island, 1998 (BOG98); Unimak Pass (UNI); Shelikof Strait, 1997 (SHEL97); Shelikof Strait, 1998 (SHEL98); Prince William Sound, 1997 (PWS97); Prince William Sound, 1998 (PWS98); Middleton Island (MID). *SOD-2** data were not available for BOG97.

Table 4

Population statistics: mean number of individuals (n); mean number of alleles (A); expected heterozygosity ($\hat{H}_E$); analog of Wright's F_{IS} ($\hat{f}$); haplotype diversity ($\hat{h}$). Values in bold type with an asterisk are statistically significant. The α-level (0.05) was adjusted (α/k) for k tests, where k is the number of populations (9). SHEL = Shelikof Strait: PWS = Prince William Sound; MID = Middleton Island; BOG = Bogoslof Island; UNI = Unimak Pass; and KRON = Kronotsky Bay.

		Allozyme				Microsatellite				mtDNA		
Population	Year	n	A	$\hat{H}_E$	$\hat{f}$	n	A	$\hat{H}_E$	$\hat{f}$	n	A	$\hat{h}$
North America–Gulf of Alaska												
SHEL	1997	79.7	2.0	0.061	–0.002	80.0	12.0	0.762	0.037	99	27	0.832
	1998	99.7	2.1	0.064	0.023	84.3	12.3	0.774	0.021	72	22	0.828
PWS	1997	99.4	2.2	0.065	0.012	75.0	14.7	0.757	0.002	99	20	0.820
	1998	99.8	2.2	0.069	0.020	95.3	14.0	0.778	0.066	65	16	0.718
MID	1998	99.6	2.3	0.064	0.033	91.7	13.7	0.735	0.018	75	19	0.743
North America–Bering Sea												
BOG	1997	99.5	2.3	0.079	0.015	44.3	11.0	0.777	0.100	99	25	0.885
	1998	99.1	2.2	0.068	0.073	91.7	13.3	0.773	**0.094***	75	21	0.838
UNI	1998	39.8	1.5	0.062	0.007	40.0	10.3	0.746	**0.159***	30	12	0.786
Asia–eastern Kamchatka												
KRON	1999	92.0	2.0	0.080	–0.011	81.7	14.0	0.766	0.008	70	19	0.822
Mean		89.8	2.1	0.068	0.020	76.0	12.8	0.763	0.048	76.0	20.1	0.808

of the rare haplotypes were found in large samples. For example, 52 of 66 composite haplotypes had frequencies of 1% or less and only three of these haplotypes were present in the UNI sample, but 8, 14, and 15 rare haplotypes were present in the three largest samples (PWS 1997, BOG 1997, SHEL 1997; Table 4).

Population genotypic frequencies generally did not deviate from Hardy-Weinberg expectations. Permutation tests for the statistic $\hat{f}$, conducted independently for allozymes and microsatellites, were concordant for all but two populations (BOG 1998; UNI 1998). The observed value of $\hat{f}$ for microsatellites in these two populations was significantly greater than zero (P<0.005) when α=0.05 was adjusted for nine simultaneous tests (Table 4).

Spatial structure in genetic variation

1997 Gulf of Alaska and Bering Sea Population structure among the three North America samples was evident from mtDNA (overall $\hat{\Phi}_{ST}$=0.017, P=0.002; Table 5). The hierarchical analysis indicated that the two Gulf of Alaska populations were genetically different from the Bering Sea population ($\hat{\Phi}_{ST}$=0.028, P<0.001) but were not distinguishable from each other (Table 5). These results were confirmed by goodness-of-fit tests of haplotype homogeneity. Spatial genetic structure among the 1997 samples was not evident from values of $\hat{\theta}$ over all allozymes and microsatellites (Table 5).

1998 Gulf of Alaska and Bering Sea, and 1999 eastern Kamchatka Indices of population structure for allozymes ($\hat{\theta}$=0.017, P<0.001) and mtDNA ($\hat{\Phi}_{ST}$=0.021, P<0.001) were similar over all population samples and larger than for microsatellites ($\hat{\theta}$=0.002, P>0.050; $\hat{R}_{ST}$=0.001, P>0.050; Table 5). Because the values of $\hat{\theta}$ and $\hat{R}_{ST}$ over all microsatellites and pop-ulations were not significant, analyses of population structure were conducted with only the allozyme and mtDNA data. Significant genetic differences were revealed between pooled samples from North America and Asia with allozymes ($\hat{\theta}$=0.030, P<0.001) and mtDNA ($\hat{\Phi}_{ST}$=0.035, P<0.001; Table 5), between samples from the Gulf of Alaska and Bering Sea with allozymes ($\hat{\theta}$=0.005, P=0.006), and within the Gulf of Alaska with allozymes ($\hat{\theta}$=0.009, P<0.001) and mtDNA ($\hat{\Phi}_{ST}$=0.018, P=0.009).

Values for $\hat{\theta}$ were considerably larger for *SOD-2** than for the two other informative markers *MPI**, and mtDNA (Table 6, Fig. 2). Estimates of F_{ST} from mtDNA and *MPI** for each of the regional comparisons were similar but the most informative microsatellite locus, *Tch22*, did not vary significantly among regions.

Based on permutation test results from the hierarchical analyses, estimates of genetic differentiation were computed for all population pairs by using both allozyme and mtDNA data, except for Gulf of Alaska versus Bering Sea populations (allozyme data only). Values of $\hat{\theta}$ (allozymes) and $\hat{\Phi}_{ST}$ for most pairwise comparisons from North America and Asia were relatively large and highly significant (Table 5). In contrast, values of $\hat{\theta}$ (allozymes) for most population pairs from the Gulf of Alaska and Bering Sea were relatively small, and only the UNI × SHEL and UNI × PWS pairs were significant ($\hat{\theta}$=0.021, P<0.003; $\hat{\theta}$=0.017, P<0.006). Pairwise comparisons within the Gulf of Alaska were significant for SHEL × PWS (allozymes, $\hat{\theta}$=0.017, P<0.002) and for SHEL × MID (mtDNA, $\hat{\Phi}_{ST}$=0.025, P<0.007; Table 5).

In goodness-of-fit tests, allele and haplotype frequency homogeneity were concordant with the statistically significant values of $\hat{\theta}$ (allozymes) and $\hat{\Phi}_{ST}$ for all populations and pooled samples within and between regions (Table 5). However, results from the two types of tests conflicted for some population pairs. For example, G-tests of allozyme data revealed significant genetic variation for two population pairs (BOG × PWS; BOG × MID; Table 5) for which $\hat{\theta}$ was not significant. In contrast, $\hat{\theta}$ was significant for two population pairs, KRON × PWS and UNI × SHEL, which were not genetically different based on G-test results. One population pair, PWS × MID, was genetically different in the probability test of mtDNA data, but $\hat{\Phi}_{ST}$ for this pair was not significant (Table 5).

Temporal change in genetic variation: Bogoslof Island, Shelikof Strait, and Prince William Sound

Significant temporal change in mtDNA variation was detected in one population, BOG, by using the probability test of haplotype homogeneity (P<0.001) and $\hat{\Phi}_{ST}$ (P=0.003, Table 5). The $\hat{\Phi}_{ST}$ was within the range of values described for spatial variation and indicated a significant interannual change in haplotype frequency. Interestingly, this interannual shift in genetic variation was not evident from allozymes and microsatellites. However, the single most-informative locus *SOD-2** was not analyzed in the BOG 1997 sample because heart tissue was not available. Also, variation at the locus *MPI**, although not statistically significant, suggested that the BOG replicates were genetically different (θ=0.011, P=0.066; Table 6). A similar indication of temporal genetic variation was detected in the PWS population for all allozymes ($\hat{\theta}$=0.005, P=0.046), but $\hat{\theta}$ was not significant when α was adjusted for three simultaneous tests (initial α=0.016, Table 5). In this case *SOD-2** was resolved and exhibited significant genetic variation ($\hat{\theta}$=0.047, P=0.004; Table 6). The mtDNA data, although not significant, also provided an indication of temporal variation in the PWS population ($\hat{\Phi}_{ST}$=0.012, P=0.055; Table 6).

Discussion

Comparison of marker classes

Our empirical results support two tentative conclusions regarding the usefulness of these marker classes for detecting population structure in walleye pollock. First, high overall heterozygosity such as that observed in some microsatellites may not be a reliable indicator of power for detecting population differences in walleye pollock, regardless of spatial scale. This conclusion contrasts with some simulation studies that have shown a positive relationship between locus polymorphism and the power to

Table 5

Tests for spatial and interannual genetic variation in walleye pollock. Abbreviations are as follows: number of populations (N); Gulf of Alaska (GOA); Bering Sea (BS); North America (NA); Bogoslof Island (BOG); Shelikof Strait (SHEL); Prince William Sound (PWS); Kronotsky Bay (KRON); Unimak Pass (UNI); Middleton Island (MID). Values in bold type with an asterisk are statistically significant. The α-level (0.05) for pairwise comparisons was adjusted (α/k) for k tests, where k is the number of population pairs per grouping strategy. Allo = allozyme; msat = microsatellite. Pr - P = the P-value for the probability test.

		F_{ST} analog tests				Goodness-of-fit tests		
						G-test P		Pr - P
Grouping strategy	N	$\hat{\theta}$ allo	$\hat{\theta}$ msat	$\hat{R}_{ST}$ msat	$\hat{\Phi}_{ST}$ mtDNA	allo	msat	mtDNA
1997 populations	3	0.000	0.000	–0.005	**0.017***	0.711	0.585	**<0.001**
Between GOA and BS	3				**0.028***			**<0.001**
BOG × SHEL					**0.024***			**<0.001**
BOG × PWS					**0.029***			**<0.001**
Within GOA	2							
SHEL × PWS					–0.004			0.876
1998 populations, Asia	6	**0.017***	0.002	0.001	**0.021***	**<0.001**	0.106	**<0.001**
Between NA and Asia	6	**0.030***			**0.035***	**<0.001**		**<0.001**
KRON × BOG		**0.039***			**0.035***	**<0.001**		**<0.001**
KRON × UNI		**0.057***			0.024	**<0.001**		0.028
KRON × SHEL		**0.028***			**0.026***	**<0.001**		**<0.001**
KRON × PWS		**0.014***			**0.051***	0.016		**<0.001**
KRON × MID		**0.029***			**0.048***	**<0.001**		**<0.001**
Between GOA and BS	5	**0.005***			0.004	**<0.001**		0.070
BOG × SHEL		0.004				0.216		
BOG × PWS		0.010				**<0.001**		
BOG × MID		0.001				**0.007**		
UNI × SHEL		**0.021***				0.017		
UNI × PWS		**0.017***				**<0.001**		
UNI × MID		0.011				0.011		
Within BS	2							
BOG × UNI		0.000			–0.008	0.454		0.754
Within GOA	3	**0.009***			**0.018***	**0.040**		**<0.001**
SHEL × PWS		**0.017***			0.007	**0.006**		0.504
SHEL × MID		0.006			**0.025***	0.109		**<0.001**
PWS × MID		0.002			0.019	0.722		**<0.001**
Interannual (1997–98)								
Bogoslof Island	2	0.001	–0.002	–0.007	**0.023***	0.162	0.479	**<0.001**
Prince William Sound	2	0.005	0.002	0.003	0.012	0.212	0.363	0.641
Shelikof Strait	2	0.001	0.001	0.000	–0.003	0.618	0.030	0.804

resolve population differentiation (e.g. Rousset and Raymond, 1995; Goudet et al., 1996). Nevertheless, empirical support for this relationship is mixed (e.g. Pogson et al., 1995; Estoup et al., 1998; Scribner et al., 1998). We believe our results suggest that mutation, which varies among these marker classes, has relatively little bearing on detecting genetic variation among walleye pollock populations. Instead, spatial differentiation may be limited because populations are large (i.e. genetic drift is weak) and gene flow among populations is high. The fact that only one private allele was found at the most variable microsatellite locus (Middleton Island, locus *Tch10,* allele 147) is an argument for high gene flow. Further, the index of population structure $\hat{R}_{ST}$ (Slatkin, 1995) developed to reflect the high mutation rate and mutation process of microsatellites is no larger than $\hat{\theta}$. The similarity of these values suggests that gene flow is relatively high and that mutation has relatively little influence on population differentiation (Slatkin, 1995).

Our second conclusion is that balancing selection may influence some but certainly not all allozymes in walleye pollock, as posited for marine species exhibiting low variation in allozyme allele frequencies (Pogson et al., 1995). Our conclusion is supported by two observations. First, microsatellites and mtDNA provide no greater resolution of overall population structure than allozymes (Tables 5

Table 6

F_{ST} estimates for *SOD-2**, *MPI**, and mtDNA. Abbreviations are as follows: number of populations (*N*); Gulf of Alaska (GOA); Bering Sea (BS); North America (NA). Values in bold type with an asterisk are statistically significant.

		F_{ST} analog tests		
Grouping strategy	*N*	$\hat{\theta}$ *SOD-2**	$\hat{\theta}$ *MPI**	$\hat{\Phi}_{ST}$ mtDNA
1997 populations	3	N/A	0.001	**0.017***
1998 populations, Asia	6	**0.095***	**0.028***	**0.021***
Between NA and Asia	6	**0.155***	**0.059***	**0.035***
Between GOA and BS	5	**0.043***	0.002	0.004
Within BS	2	0.001	0.000	0.000
Within GOA	3	**0.051***	**0.013***	**0.018***
Interannual (1997–98)				
Bogoslof Island	2	N/A	0.011	**0.023***
Prince William Sound	2	**0.047***	0.000	0.012
Shelikof Strait	2	0.000	0.011	–0.003

and 6). Second, two allozyme loci (*SOD-2**; *MPI**) provided the strongest evidence of regional genetic variation among the 1998 samples (Table 6). In fact, the locus *SOD-2** had exceptionally high values of $\hat{\theta}$ for most regional comparisons in contrast to the other nuclear loci and mtDNA. Diversifying selection may be acting on this locus (e.g. Hudson et al., 1997); however, the general concordance between *SOD-2**, *MPI**, and mtDNA data suggests that the values of $\hat{\theta}$ most likely reflect the influence of genetic drift (Table 6, Fig. 2).

Spatial structure in genetic variation

North America to Asia The signature of a major east–west division between Asian and North American walleye pollock was evident in our allozyme and mtDNA data but not in our microsatellite data. The results of our F_{ST} analog tests and goodness-of-fit tests confirm the findings of earlier studies (e.g. Iwata, 1975a, 1975b; Grant and Utter, 1980; Shields and Gust, 1995, Bailey et al., 1999) and add some new insight regarding genetic heterogeneity in walleye pollock from different geographic regions. For example, we confirm the significant *SOD-2** variation observed in the past but we also reveal that a second allozyme locus, *MPI**, exhibits substantial spatial variation at this geographic scale (Table 6, Fig. 2). Nevertheless, only these two loci and the mtDNA reveal genetic variation between these two regional groups although some of the other 25 nuclear loci screened were equally or more polymorphic (e.g. *ALAT**, *PGDH**, *Tch10*, *Tch11*, and *Tch22*). These results suggest that, despite broad spatial separation, Asian and North American walleye pollock populations are remarkably genetically similar. This outcome supports the notion that the effective population size in walleye pollock is large, the rate of genetic drift is very low, and migration despite physical distance is a strong homogenizing force.

North America–Bering Sea and Gulf of Alaska The allozyme and mtDNA provided discordant results with respect to inter- and intraregional population structure within North American walleye pollock. The most noteworthy instance was the contradictory evidence of genetic variation between populations from the Bering Sea and the Gulf of Alaska. That is, significant genetic differentiation between populations from these two regions was evident from both the allozymes and mtDNA, but in different years (Table 5). We believe that the intraannual differences between marker classes is an artifact due primarily to the lack of *SOD-2** data for the Bogoslof Island sample in 1997. The values of $\hat{\theta}$ and $\hat{\Phi}_{ST}$ were similar in magnitude for the 1998 Bering Sea and Gulf of Alaska comparison, but only $\hat{\theta}$ was statistically significant (Table 5). This result appears to be due to the influence of *SOD-2** for which $\hat{\theta}$ was 0.043 and highly significant (P<0.002, Table 6). The value of $\hat{\theta}$ over all allozymes for the 1997 Bering Sea and Gulf of Alaska comparison might have been similar to or larger than the $\hat{\Phi}_{ST}$ for mtDNA if the *SOD-2** data had been available for the 1997 Bogoslof Island sample.

The 1998 samples provided the first indication of statistically significant genetic variation among populations in the Gulf of Alaska with both allozymes and mtDNA. Populations from Prince William Sound and Middleton Island were genetically distinguishable from the Shelikof Strait population, but they were not distinguishable from each other. These results are important for two reasons. First, this is the first indication of limited gene flow between a fjord spawning population (Prince William Sound) in the Gulf of Alaska and the numerically dominant Shelikof Strait population which spawns over the ocean shelf. Obvious factors that may limit gene flow between these two populations are the differences in spawning habitat preference and the counterclockwise flow of the Gulf of Alaska gyre which prevents larval drift from Shelikof Strait to the Prince William Sound (Bailey et al., 1999).

Second, the Prince William Sound and Middleton Island populations may warrant consideration as a distinct stock, which should be managed independently of the Shelikof Strait population.

The significant genetic differences between population pairs in the Gulf of Alaska are alternately supported by allozymes and mtDNA. That is, the Shelikof Strait and Prince William Sound populations differ significantly according to the allozyme data but not the mtDNA data, and the Shelikof Strait and Middleton Island populations differ significantly according to the mtDNA data but not the allozyme data (Table 5). We believe that the significant variation observed for each population pair is, in fact, real but is not supported by both marker types because these populations differ at only three loci (*SOD-2**, *MPI**, mtDNA). Thus, it is reasonable to expect differences between the populations at one locus (in this case, the marker class) and not the others based solely on the random effects of genetic drift.

Temporal change in genetic variation: Bogoslof Island, Prince William Sound

In addition to evidence of spatial structure within the Gulf of Alaska, we found evidence of interannual variation in genetic diversity among replicate samples from Bogoslof Island (mtDNA) and Prince William Sound (allozyme *SOD-2**). In each case the statistically significant variation was evident at only one marker, although in each case a second marker showed noticeable, but not statistically significant, variation between years (e.g. Bogoslof Island, *MPI**—$\hat{\theta}$=0.011, P=0.066; Prince William Sound, mtDNA—$\hat{\Phi}_{ST}$=0.012, P=0.055). We believe there are four likely sources for this apparent temporal shift.

First, walleye pollock may experience high variability in reproductive success among spawning adults as has been shown, for example, in oysters (Hedgecock, 1994). Walleye pollock have many life history traits such as semiplanktonic larvae, high fecundity, and external fertilization in a variable environment (Hedgecock, 1994), that may result in highly variable reproductive success and may contribute to temporal instability, particularly in small census populations such as that in Prince William Sound.

Second, walleye pollock may have varying degrees and types of philopatry. For example, juvenile walleye pollock may recruit to any one of a number of spawning aggregations and then exhibit repeat spawning behavior as an adult (adult philopatry). Adult philopatry has been used to explain the temporal instability observed in Atlantic herring (*Clupea harengus*, [McQuinn, 1997]) and could explain the apparent heterozygote deficit at microsatellites in the Bogoslof Island 1998 and Unimak Pass 1998. Alternatively, the degree of philopatry in walleye pollock may be extremely low despite the spatial and temporal predictability of spawning aggregations.

Third, a source-sink type metapopulation relationship may exist between some walleye pollock populations (e.g., Shelikof Strait and Prince William Sound; Unimak Pass and Bogoslof Island). Bailey et al., (1999) suggested that a metapopulation theory may explain existing data on population structure and dynamics of walleye pollock. The temporal genetic variation we observed in Prince William Sound and Bogoslof Island is consistent with this theory. Other circumstantial evidence supporting a source-sink relationship among some walleye pollock populations includes the discovery of walleye pollock in Prince William Sound (sink) concurrent with the increase in biomass of walleye pollock in the neighboring Shelikof Strait (source).

Finally, our supposed temporal replicates may actually represent samples from different spawning aggregates that spawn in the same location but at different times of the year. This finding suggests that fine-scale, intra-annual, genetic variation exists among walleye Pollock that spawn in the same area but perhaps days or weeks apart.

Summary

Our results show that the genetic variation, as measured by F_{ST}, among spatially distinct spawning aggregations of walleye pollock is generally low and is similar to the spatial genetic variation observed in other gadids such as Atlantic cod and European hake, *Merluccius merluccius* (e.g. Ruzzante et al., 1998; Lundy et al., 2000). In contrast, evidence of temporal genetic variation in gadid fishes is equivocal. Although some populations of European hake exhibit interannual variation in gene frequencies (Lundy et al., 2000), Ruzzante et al. (1997) found no evidence of temporal instability in Atlantic cod. For walleye pollock more detailed studies are needed to determine the extent to which all or some of the factors described above explain the temporal variability observed within some spawning aggregations. A priority of future genetic studies of walleye pollock should be to examine both fine-scale temporal variation (days and weeks within a year) and the genetic relationship among age cohorts from spatially distinct spawning aggregations. Because these studies will require extensive sampling, we recommend detailed examination of one or two spawning aggregations, such as those at Prince William Sound and Shelikof Strait. To further evaluate the metapopulation theory, candidate samples should include potential source populations (e.g. Shelikof Strait and Unimak Pass) and sink populations (e.g. Bogoslof Island and Prince William Sound). Future studies should also include a statistical examination of single locus $\hat{\theta}$ values to assess if *SOD-2** exceeds expectation and is perhaps influenced by a locus-specific force such as diversifying selection (e.g. Beaumont and Nichols, 1996).

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