Genetic differentiation in walleye pollock (*Theragra chalcogramma*) in response to selection at the pantophysin (*Pan*I) locus

Michael F. Canino, Patrick T. O'Reilly, Lorenz Hauser, and Paul Bentzen

Abstract: Samples of walleye pollock (*Theragra chalcogramma*) from the North Pacific Ocean and Bering Sea were screened for variation at the pantophysin (*Pan*I) locus. Global genetic differentiation across samples (F_{ST} = 0.038) was considerably greater than reported in previous population studies using allozymes, mtDNA, or microsatellite loci and significantly greater than F_{ST} distributions of neutral loci simulated over a large range of locus heterozygosity. *Pan*I allele frequencies varied over a broad latitudinal gradient and were correlated with estimated mean surface temperatures, resulting in the greatest levels of genetic divergence between the northern Bering Sea and the southernmost locations in the temperate Pacific Ocean (Puget Sound, Japan). The discordance between estimates of population differentiation estimated from *Pan*I and other neutral marker classes, both in magnitude and in geographic patterns, could arise from temperature-mediated effects of natural selection over broad geographic scales. Our empirical results suggest that loci subject to directional selection may prove to be useful markers for stock identification in weakly structured marine fishes.

Résumé : Nous avons évalué la variation au locus de la pantophysine (*Pan*I) dans des échantillons de goberges de l'Alaska (*Theragra chalcogramma*) du nord du Pacifique et de la mer de Bering. La différentiation génétique globale parmi les échantillons (F_{ST} = 0,038) est considérablement plus élevée que celle qui a été signalée dans les études démographiques antérieures portant sur les allozymes, l'ADN mitochondrial (ADNmt) ou les locus microsatellites et significativement plus grande que les distributions simulées de F_{ST} de locus neutres sur une gamme étendue d'hétérozygotie des locus. Les fréquences de l'allèle *Pan*I varient sur un large gradient de latitudes et sont en corrélation avec les températures moyennes de surface estimées; le niveau le plus élevé de divergence génétique s'observe entre le nord de la mer de Bering et les sites les plus au sud du Pacifique tempéré (Puget Sound, Japon). La discordance entre les estimations de différentiation des populations faites au moyen de *Pan*I et les autres classes de marqueurs neutres, tant au niveau de l'importance que de la répartition géographique, peut être due à des effets de la sélection naturelle sur de grandes échelles géographiques, modulés par la température. Nos résultats empiriques laissent croire que les locus sujets à la sélection directionnelle peuvent être des marqueurs utiles pour l'identification des stocks dans les populations de poissons marins peu structurées.

[Traduit par la Rédaction]

Introduction

Assessments of the degree of demographic connectivity among stock components of marine fishes have become more important as levels of exploitation increase in world fisheries. Detection of genetic differentiation among putative demographic populations provides information on the extent of self-recruitment and is thus useful in managing and conserving fish stocks. The low levels of divergence commonly observed among marine fish or invertebrate populations have often been cited as evidence for the effects of high gene flow on restricting the extent of spatial genetic heterogeneity in species with life history characteristics conducive to dispersal (e.g., planktonic developmental stages, high vagility, migratory behavior) in environments lacking obvious physical barriers (Hauser and Ward 1998). However, a growing number of studies, many using highly polymorphic microsatellite markers, have detected subtle yet significant genetic structuring over varying spatial scales: Atlantic cod (*Gadus morhua*) (Ruzzante et al. 2001; Knutsen et al. 2003; Nielsen

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et al. 2003), veined squid (*Loligo forbesi*) (Shaw et al. 1999*a*), sea bass (*Dicentrarchus labrax*) (Naciri et al. 1999), European hake (*Merluccius merluccius*) (Lundy et al. 1999, 2000), European flounder (*Platichthys flesus*) and starry flounder (*Platichthys stellatus*) (Borsa et al. 1997), Atlantic mackerel (*Scomber scombrus*) (Nesbø et al. 2000), Atlantic herring (*Clupea harengus*) (Shaw et al. 1999*b*; McPherson et al. 2001), European eel (*Anguilla anguilla*) (Wirth and Bernatchez 2001), and Pacific ocean perch (*Sebastes alutus*) (Withler et al. 2001). Although many studies have revealed statistically significant estimates of differentiation, the genetic divergences are often so small that the biological significance of this variation is not always obvious (Waples 1998).

The effects of genetic drift and of gene flow are expected to be largely similar across nuclear genetic markers, although differences in mutation rate, mode of inheritance, and response to selection among loci can result in different patterns of population differentiation (Buonaccorsi et al. 2001). For example, variation at protein-coding loci deviating from neutral expectations can be used to infer the role of selection in creating population differentiation (Baer 1999; Pogson 2001; Dufresne et al. 2002) and can sometimes identify the life history stage when such selection occurs (Lemaire et al. 2000). Geographic patterns of differentiation exhibited by different marker loci may vary with the type and magnitude of selective forces. Some allozyme loci appear to be under balancing selection and may show less differentiation among populations than neutral markers if environments are similar (Pogson et al. 1995). On the other hand, those subjected to directional selection may reveal greater levels of genetic heterogeneity at the same spatial scales (Pogson et al. 2001), potentially reflecting local adaptation over a number of generations among semiisolated groups (Conover 1998).

Comparisons between neutral markers and those influenced by selection may provide one of the most powerful approaches for detecting limits to gene flow in marine species that often have very large census population sizes and exhibit low levels of neutral genetic heterogeneity (Ward et al. 1994). One of the most promising nonneutral markers analyzed in marine fishes to date is the pantophysin gene (*Pan*I). Pantophysin is an integral membrane-trafficking protein involved in cellular microvesicle transport (Windoffer et al. 1999; Brooks et al. 2000), although its exact function and the basis for selection at the locus are poorly understood. Analyses of DNA sequence data from the *Pan*I locus in walleye pollock (*Theragra chalcogramma*) (Canino and Bentzen 2004) and related gadid fishes (Pogson 2001; Pogson and Mesa 2004) show strong evidence for the effects of positive (diversifying) selection at this locus, resulting in trans-species polymorphisms that have persisted over long evolutionary periods. Population studies of *Pan*I variation in Atlantic cod have shown striking levels of differentiation (Fevolden and Pogson 1997; Jónsdóttir et al. 2001; Karlsson and Mork 2003) that do not conform to neutral expectations and appear to be largely driven by selection.

In this study, we examine results from a survey of *Pan*I variation over a broad geographic range for evidence of selection in contributing to genetic population structure in walleye pollock. This gadid species is broadly distributed across the arcto-boreal Pacific Ocean, with the largest concentrations on continental shelves and slopes, ranging from central California to the Sea of Japan (Allen and Smith 1988). Walleye pollock has a mean generation time of 3.5 years, exhibits high fecundity, and spawns at predictable locations during the late winter and early spring (reviewed in Bailey et al. 1999). Eggs and larvae are planktonic for up to several months, and juveniles and adults are pelagic or semidemersal. Owing in part to its importance in commercial fisheries, it has been a focal species for genetic stock identification studies. Some previous studies of allozyme and mtDNA variation revealed low levels of population structure across broad spatial scales in the North Pacific (Grant and Utter 1980; Mulligan et al. 1992; Olsen et al. 2002), while others (e.g., Shields and Gust 1995; Kim et al. 2000) detected no significant heterogeneity among putative populations. More recent studies examining allozyme and mtDNA variation (Olsen et al. 2002) or microsatellites (O'Reilly et al. 2004) have provided stronger evidence of population structuring at ocean basin scales, and weak levels of neutral divergence seen at these markers may be interpreted as reflecting high gene flow, as pollock has a high potential for dispersal. Alternatively, gene flow may have been low, but genetic drift in these extremely large populations has been insufficient for significant levels of differentiation to accumulate since separation, probably after the last ice age.

Here, we contrast patterns of genetic structure in walleye pollock inferred from *Pan*I variation with those reported for microsatellites (O'Reilly et al. 2004) over small to broad distances. The two marker types showed some concordance over geographic scales where structuring was evident; however, variation at the *Pan*I locus also revealed a pattern of selective differentiation not previously observed using microsatellites, thus potentially showing adaptive variation in walleye pollock that may be relevant for fisheries conservation and management strategies.

Methods

DNA amplification and single-nucleotide polymorphism (SNP) analyses

Fin clips samples of walleye pollock from six locations examined for microsatellite variation (O'Reilly et al. 2004), plus an additional sample from Kronotsky Bay, Russia, analyzed with microsatellites, allozymes, and mtDNA markers by Olsen et al. (2002), were screened for *Pan*I variation (Fig. 1). Genomic DNA was purified from preserved tissues following lysis in proteinase K (10 mg·mL^{-1} at 60 °C for 1–2 h) using either Qiagen DNeasy isolation protocols (Valencia, California) or protein precipitation methods (Sambrook and Russell 2001). In the latter protocol, ammonium acetate was added to a final concentration of 3.0 mol·L^{-1} and the sample centrifuged for 5 min at 12 000*g*. DNA was precipitated in ethanol and resuspended in 50–100 μL of low-TE buffer (10 mmol Tris·L^{-1}, 0.1 mmol ethylenediamenetetraacetic acid·L^{-1}, pH 8.0).

*Pan*I variation was screened with an SNP protocol developed to detect alleles characterized by amino acid replacement mutations occurring in adjacent codon positions within the second intravesicular domain (IV2) of the *Pan*I locus (Table 1). These codons were previously identified as sites

Fig. 1. Sample locations for walleye pollock (*Theragra chalcogramma*).

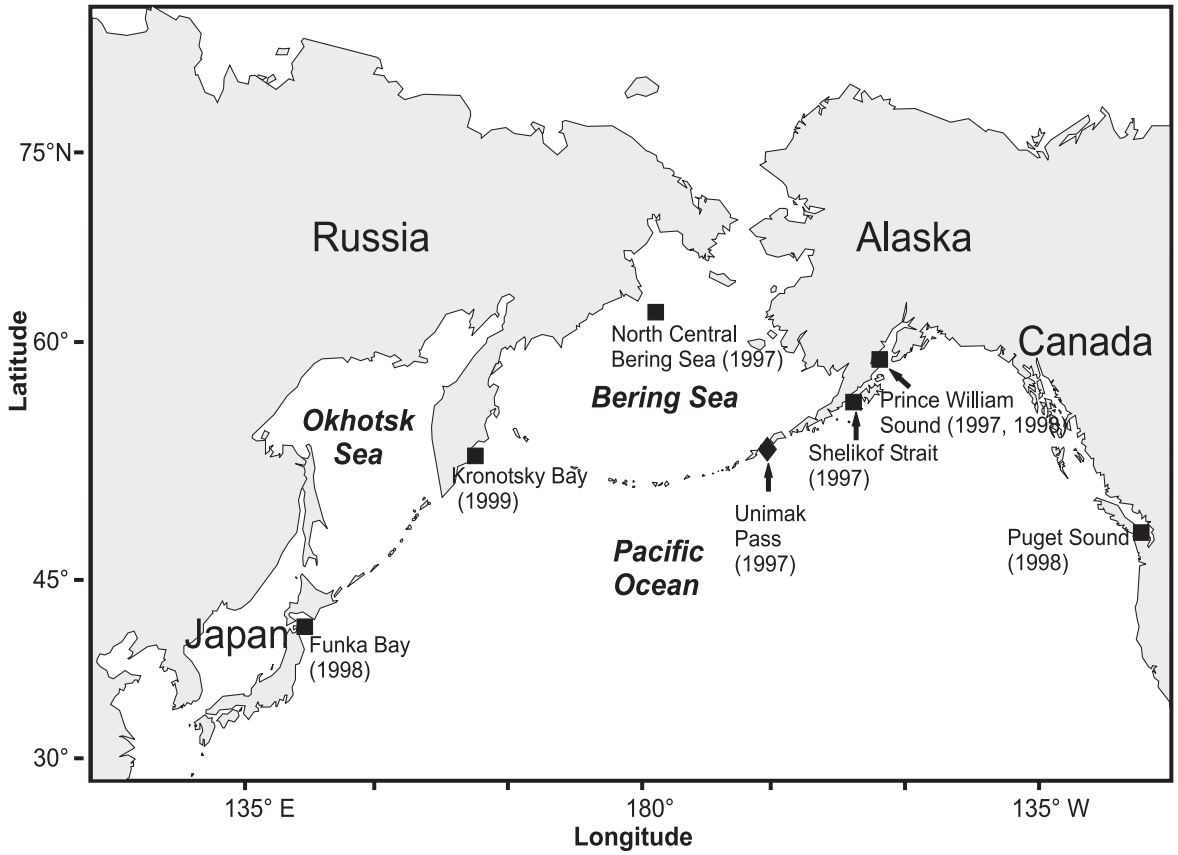


Table 1. Adjacent codons and amino acid residues (in parentheses) segregating in the second intravesicular (IV2) domain of the *Pan*I locus in walleye pollock (*Theragra chalcogramma*).

	Codon position	
Allele	158	159
1	A**G**T (S)	G**G**G (G)
2	A**G**T (S)	G**T**G (V)
3	A**T**T (I)	G**T**G (V)
4	A**A**T (N)	C**T**G (L)
5	A**G**T (S)	A**T**G (M)

Note: Bolded nucleotides were screened with an SNP protocol.

with high potential for positive selection using maximum likelihood analyses (Canino and Bentzen 2004). First, a 985 base pair (bp) region of the *Pan*I locus was amplified via the polymerase chain reaction (PCR) in 10-μL volumes containing 10 mmol Tris–HCl·L^{-1} (pH 8.3), 20 mmol KCl·L^{-1}, 1.5 mmol $MgCl_2$·L^{-1}, 0.2 mmol each dNTP·L^{-1}, approximately 100 ng template DNA, 0.8 U Gene Choice *Taq* DNA polymerase (PGC Scientifics Corp., Frederick, Maryland), and 0.5 μmol oligonucleotide primers·L^{-1} (F: 5′-TCTACAAATGCGTGAAAGTGG – 3′; R: 5′-CCAGACGCTACAGGGATCAT-3′). The PCR thermal cycling profile consisted of a denaturation step of 94 °C for 2 min, followed by 35 cycles of 94 °C (30 s), 57 °C (30 s), and 72 °C (60 s) and a final extension at 72 °C for 5 min.

Next, PCR amplicons were treated with ExoSAP-IT (USB Corp., Cleveland, Ohio) to degrade unincorporated primers and dNTPs. Two single nucleotide polymorphism primers (SNP1: 5′-GACGGAAATTGCACGGCGGGCA-3′; SNP2: 5′-ACGGAAATTGCACGGCGGGCADTG-3′), where D is a wobble position for A, T, or C, were designed to anneal to the 5′ flanking sequences immediately next to two variable second-position nucleotide sites in adjacent codons (Table 1). Primers were subsequently end-labeled via PCR with a single fluorescent dideoxynucleotide complementary to the variable nucleotide positions using the MegaBACE SNuPe genotyping kit (Amersham Pharmacia Biotech, Inc., Piscataway, New Jersey) according to the manufacturer's protocols and then detected with a MegaBACE 1000 automated sequencer (Amersham Pharmacia Biotech, Inc.). *Pan*I genotypes were scored manually from electropherogram output and verified by comparison with 117 known individual genotypes from a geographic survey of *Pan*I DNA sequences (Canino and Bentzen 2004). Two alleles (2 and 5) that occurred at low frequencies (1–2%) in that study had identical nucleotides at second-position sites (Table 1) and thus could not be resolved using the SNP protocol described above. These alleles were collectively assigned as allele 2 in subsequent analyses.

Statistical analyses

Observed and expected heterozygosities were calculated using Genetix version 4.02 (Belkhir 2000) and genotype frequency conformance to Hardy–Weinberg equilibrium and

genotypic linkage equilibrium was examined using exact tests implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). Tests were conducted with specified Markov chain parameters of 5000 dememorization steps followed by 500 batches of 2000 iterations per batch. Significance levels for multiple tests were adjusted to an $\alpha = 0.05$ level using sequential Bonferroni correction (Rice 1989). Exact tests for differences in genic and genotypic frequencies among samples were conducted using GENEPOP. Estimates of F_{ST}, $\hat{\theta}$, between sample pairs, following Weir and Cockerham (1984), were calculated using FSTAT version 2.9.3 (Goudet 2002) and the significance of those estimates was determined with *G*-based likelihood tests in FSTAT using 2000 permutations of the data.

We evaluated evidence for the effects of natural selection using a simulation approach developed by Beaumont and Nichols (1996) that they applied to results from a study of *Pan*I variation in Atlantic cod (Pogson et al. 1995). Simulations were conducted using FDist2 and associated programs (Beaumont 2000). A mean weighted F_{ST} for pollock (0.004), calculated from 14 microsatellite loci (O'Reilly et al. 2004), was used to generate distributions of F_{ST} conditional on locus heterozygosity over a range of 0 to 1 in simulations of 50 000 neutral loci. Simulation parameters were for 50 demes and seven populations assuming a stepwise mutational model. Next, a mean F_{ST} from 24 allozyme loci reported in a previous study of pollock (Olsen et al. 2002) was used for the simulations assuming an infinite alleles mutation model. The F_{ST} reported for *SOD2** (0.088) was an exceptionally high estimate relative to the 23 other loci in their study. This locus had previously shown elevated levels of divergence in other studies of pollock (Iwata 1975; Seeb et al. 2002), suggesting that it may potentially be influenced by selection. The putative neutrality of the *SOD2** locus was therefore first evaluated against F_{ST} distributions simulated from the mean of the 23 other loci ($F_{ST} = 0.0007$). Quantiles (5%) for the distributions simulated using microsatellite and allozyme mean F_{ST} estimates were constructed as confidence limits for testing whether genetic divergence at the *Pan*I locus differed significantly from simulated data.

Tests for isolation by distance among pollock samples were conducted using the ISOLDE subprogram in GENEPOP. Linear distances among sampling locations were estimated by following a 200-m contour along continental margins and the significance of the correlation between linearized F_{ST} values ($F_{ST}/1 - F_{ST}$), and the logarithm of geographic distance was assessed using Mantel tests with 3000 permutations of F_{ST} and distance matrices. Mantel tests were also used to test the association of genetic divergence with differences in annual Reynolds (1988) sea surface temperatures estimated for sampling locations in this study during the period 1982–1997 (Comprehensive Ocean–Atmosphere Data Set; Woodruff et al. 1993). To examine the potentially confounded effects of temperature and distance on estimates of genetic differentiation, we conducted partial Mantel tests using the zt program version 1.0 (Bonnet and Van de Peer 2002). The tests computed Spearman rank correlations between matrices of linearized F_{ST} values with those from one independent variable, either log geographic distance or differences in annual mean sea surface temperature, while controlling for effects of the other independent variable. Significance of the tests was assessed using 100 000 data randomizations.

Results

Locus statistics for *Pan*I in the eight samples are provided in Table 2. Fisher's exact tests indicated significant deviation from expected Hardy–Weinberg equilibrium genotypic distributions ($P = 0.043$) across all samples. These deviations consistently involved excesses of two genotypes (Fig. 2): homozygotes for the most common allele (genotype 1/1) and heterozygotes for alleles 3 and 4 (genotype 4/3). Tests across individual samples revealed significant heterozygote excesses only in the JPN98 sample ($P = 0.024$), but this result was not significant after adjustment for multiple tests.

Genetic differentiation at the *Pan*I locus was highly significant over all samples combined (Fisher's exact test, log-likelihood *G* test, both $P < 0.001$) and for a number of pairwise comparisons involving the north-central Bering Sea sample (NCBS97) and the two southernmost samples in Puget Sound and Japan (Table 3). Significant genic divergence was also found within the Bering Sea and between NCBS97 and samples collected in the Gulf of Alaska. Temporal heterogeneity was not observed in *Pan*I genic or genotypic distributions in Prince William Sound samples (PWS97 and PWS98), which were subsequently pooled for additional analyses. Pairwise estimates of F_{ST} were significant in seven of 21 comparisons, with three comparisons involving samples from Puget Sound and Japan (Table 4).

Simulations using the FDist2 program indicated that variation at both the allozyme locus *SOD2** and the *Pan*I locus deviated significantly from neutral expectations. The *SOD2** F_{ST} (0.088) was determined to be a significant outlier ($P < 0.01$) and was excluded in further simulations. The F_{ST} estimate for *Pan*I over all samples ($F_{ST} = 0.038$) exceeded the 95% confidence intervals for distributions simulated using either the mean from 14 microsatellite loci ($F_{ST} = 0.004$; O'Reilly et al. 2004) or the mean from 23 allozyme loci, excluding *SOD2** ($F_{ST} = 0.0007$; Olsen et al. 2002) (Fig. 3).

*Pan*I allele frequencies appeared to follow a latitudinal gradient, resulting in a 30% decrease in the frequency of the most common allele towards the southernmost ends of the sample distribution (Fig. 4). Allelic distributions strongly indicated a possible relationship with water temperature as well as geographic distance. Partial Mantel tests showed a significant association of *Pan*I variation with differences in temperature when controlling for the effect of geographic distance ($r = 0.746$, $P = 0.002$) but not for distance while controlling for temperature ($r = -0.238$, $P = 0.116$). Pairwise *Pan*I F_{ST} values were highly correlated with differences in annual sea surface temperatures (Fig. 5). Likewise, frequencies of the three common *Pan*I alleles were significantly correlated ($P < 0.030$ in each comparison) with mean annual sea surface temperatures (Fig. 6) but not with geographic distances, with Spearman correlation coefficients ranging from 0.47 to 0.78. In contrast, a partial Mantel test of multilocus F_{ST} estimates derived from 14 microsatellite loci (O'Reilly et al. 2004) showed no significant correlation with temperature difference while controlling for distance but did show a significant association with distance when controlling for temperature difference ($r = 0.636$, $P = 0.003$). How-

Table 2. Sample sizes (n), allelic frequencies, expected (H_E) and observed (H_O) heterozygosities, and F_{IS} of alleles at the *Pan*I locus.

	Sample location							
	Puget Sound, Washington	Shelikof Strait, Alaska	Prince William Sound, Alaska	Prince William Sound, Alaska	Unimak Pass, Alaska	North Central Bering Sea	Kronotsky Bay, Russia	Funka Bay, Japan
Year	1998	1997	1997	1998	1997	1997	1999	1998
Abbreviation	PS98	SHEL97	PWS97	PWS98	UNI97	NCBS97	KRON99	JPN98
n	54	75	73	76	89	82	87	76
Allele frequencies								
1	0.602	0.867	0.740	0.763	0.770	0.909	0.764	0.599
2	0.028	0.000	0.000	0.000	0.000	0.006	0.011	0.026
3	0.194	0.087	0.164	0.138	0.180	0.061	0.155	0.204
4	0.176	0.047	0.096	0.099	0.051	0.024	0.069	0.171
H_E	0.574	0.241	0.420	0.391	0.375	0.171	0.389	0.574
H_O	0.407	0.173	0.342	0.355	0.360	0.146	0.345	0.605
F_{IS}	0.292*	0.282	0.185	0.093	0.041	0.146	0.114	–0.055

Note: Deviation from Hardy–Weinberg equilibrium expectations: *, $P < 0.05$.

ever, tests of single-locus F_{ST} values revealed that three microsatellite loci (*TCH3*, $P = 0.039$; *TCH8*, $P = 0.010$; TCH12, $P = 0.003$) positively correlated and two (*TCH14*, $P = 0.008$; *TCH19*, $P = 0.025$) negatively correlated with water temperature difference when the effect of distance was controlled.

Discussion

The influence of selection in contributing to genetic differentiation in marine fishes is poorly understood and rarely investigated (Conover 1998). Spatial variation at protein-coding loci results from the dynamic interaction of gene flow, drift, and selection differentials occurring at varying spatial scales. Although many marine species have large populations and potentially high dispersal capabilities, high gene flow per se does not preclude the potential for localized adaptation (Conover 1998). There is increasing evidence for locus-specific selection in marine species (e.g., Powers and Schulte 1998; Lemaire et al. 2000; Sotka et al. 2004) that is particularly compelling when relatively strong geographical patterns of allozyme variation are discordant with weak structure inferred from neutral loci (e.g., Lemaire et al. 2000; Dufresne et al. 2002).

In recent years, a number of studies have suggested that population structuring observed at the *Pan*I locus in gadid fishes, and in particular the Atlantic cod, is largely the result of natural selection. Two lines of evidence support this conclusion. First, analyses of pantophysin DNA sequences in cod (Pogson 2001), walleye pollock (Canino and Bentzen 2004), and related gadids (Pogson and Mesa 2004) strongly infer the effects of positive (diversifying) Darwinian selection in the evolution of this locus. Second, potential selection effects inferred from nonsynonyous substitution patterns at the nucleotide level are corroborated by population genetic studies showing relatively high levels of geographic heterogeneity at the *Pan*I locus that are discordant with results derived from other putative neutral markers in Atlantic cod (e.g., Fevolden and Pogson 1997; Pogson et al. 2001; but see Lage et al. 2004) and in walleye pollock (Grant and Utter 1980; Olsen et al. 2002; O'Reilly et al. 2004). Departures of *Pan*I genotype frequencies from Hardy–Weinberg equilibrium in pollock towards an excess of heterozygotes, similar to that found in cod (Jónsdóttir et al. 1999, 2001; Beacham et al. 2002), indicates that selection dynamics may favor heterozygotes. Other studies have shown complex cohort and gender-specific effects (Karlsson and Mork 2003) and a clear distinction between Arctic and more southern coastal cod populations based on geographic *Pan*I variation (Fevolden and Pogson 1997; Pogson 2001; Pogson and Fevolden 2003).

The broad, weak cline of *Pan*I allele frequencies observed in walleye pollock is consistent with a lack of strong thermal or salinity barriers between the Bering Sea and North Pacific Ocean and suggests that weak, spatially variable selection occurs over broad distances. Similarities in *Pan*I allelic frequencies at comparable latitudes could be attributed to convergent directional selection, which may explain the observed homogeneity of *Pan*I distributions across both large (e.g., Japan and Puget Sound) and small geographic distances with similar temperature regimes. Thus, historical

Fig. 2. Expected genotypes (solid bars) and observed genotypes (open bars) at the *Pan*I locus in walleye pollock (*Theragra chalcogramma*). Sample abbreviations are as in Table 2. Genotypes are composed from alleles defined in Table 1.

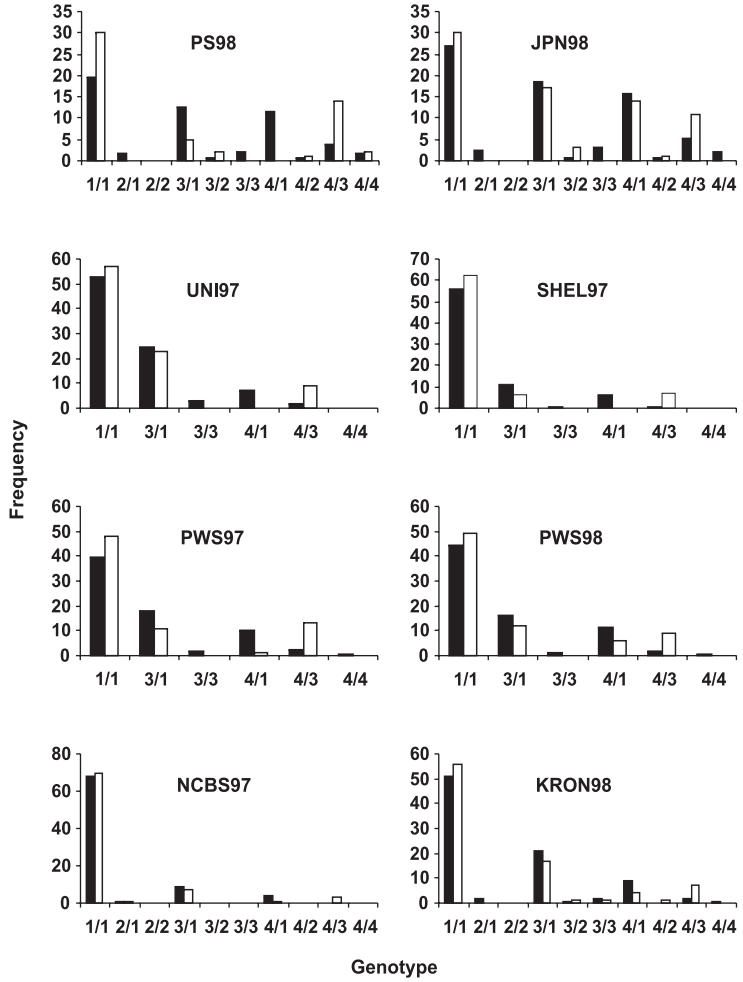


Table 3. Probability (*P*) values for exact tests of genic (above diagonal) and genotypic (below diagonal) differentiation between sample pairs at the pantophysin (*Pan*I) locus.

	PS98	SHEL97	PWS	UNI97	NCBS97	KRON99	JPN98
PS98	—	**<0.001**	**0.002**	**<0.001**	**<0.001**	0.010	0.998
SHEL97	**<0.001**	—	0.017	0.041	0.411	0.079	**<0.001**
PWS	0.005	0.024	—	0.158	**<0.001**	0.248	**0.001**
UNI97	**0.001**	0.053	0.135	—	**0.001**	0492	**<0.001**
NCBS97	**<0.001**	0.368	**<0.001**	**0.002**	—	**0.003**	**<0.001**
KRON99	0.025	0.069	0.185	0.380	0.007	—	**0.004**
JPN98	0.994	**<0.001**	**0.001**	**<0.001**	**<0.001**	0.007	—

Note: Sample abbreviations are as in Table 2. Bolded values are significant ($\alpha < 0.05$) after sequential Bonferroni correction for 21 multiple tests.

or contemporary temperature-mediated selection pressures on the *Pan*I locus may account for the observed geographic distributions of pantophysin alleles in the two best studied gadid fishes, Atlantic cod and walleye pollock. While the agent of selection at the locus is unknown, the role of temperature in contributing to patterns of variation could be further investigated by conducting crosses and rearing offspring with different *Pan*I genotypes in "common garden" environments and monitoring growth and survival at various stages of development under the range of temperatures observed in this study. The role of other segregating genes can be minimized by designing crosses so that comparisons can be made between full sibs that exhibit different genotypes at the locus under study (Lohm et al. 2002).

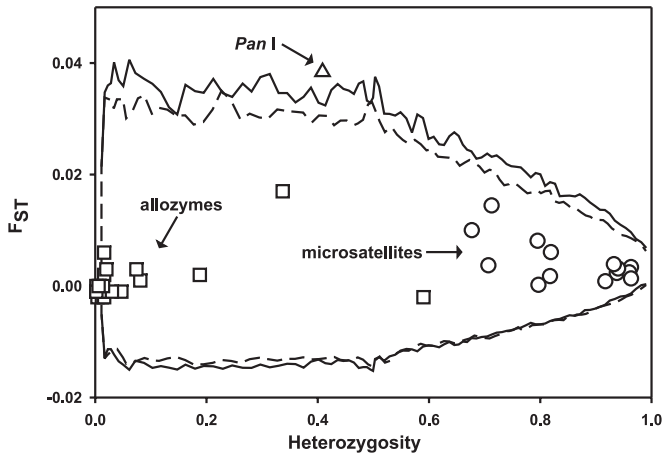
The range of pairwise F_{ST} estimates for the *Pan*I locus (0.000–0.163) was greater than for multilocus estimates derived from microsatellites (0.001–0.008) in comparisons between most of the same samples (O'Reilly et al. 2004). Both the magnitude and the number of significant pairwise F_{ST} estimates from *Pan*I variation were greatest in north–south comparisons involving the two most southern samples (Japan and Puget Sound) with other locations, particularly with

Table 4. Pairwise estimates of F_{ST} ($\hat{\theta}$) from the *Pan*I locus (below diagonal) and from 14 microsatellite loci (above diagonal; data from O'Reilly et al. 2004) for walleye pollock (*Theragra chalcogramma*).

	PS98	SHEL97	PWS	UNI97	NCBS97	KRON99	JPN98
PS98	—	0.004	0.004	**0.004**	0.004	na	**0.005**
SHEL97	**0.107**	—	0.003	0.005	0.006	na	**0.008**
PWS	0.027	0.022	—	0.002	0.004	na	**0.005**
UNI97	**0.04**	0.021	–0.001	—	0.000	na	**0.007**
NCBS97	**0.163**	–0.001	**0.051**	0.052	—	na	0.006
KRON99	0.033	0.017	–0.004	–0.005	0.047	—	na
JPN98	–0.009	**0.105**	0.029	**0.040**	**0.156**	0.035	—

Note: The PWS sample consisted of pooled individuals from 1997 and 1998. Sample abbreviations are as in Table 2. Bolded comparisons are significant ($P < 0.05$) after sequential Bonferroni corrections for multiple tests. na, not available.

Fig. 3. Estimates of F_{ST} from 23 allozyme loci (Olsen et al. 2002) (squares), 14 microsatellite loci (from O'Reilly et al. 2004) (circles), and the *Pan*I locus (triangle) versus locus heterozygosity for walleye pollock (*Theragra chalcogramma*). Confidence limits (95%) were estimated from 50 000 simulations of neutral loci with an expected mean F_{ST} equal to the mean from the 23 allozyme loci (broken line) and the 14 microsatellite loci (solid line).



the central Bering Sea. In contrast, microsatellite-based pairwise estimates of F_{ST} followed a weak isolation-by-distance pattern (O'Reilly et al. 2004) with no significant differentiation between southernmost samples and the central Bering Sea. The discordant results derived from these different genetic markers support our conclusion that temperature-mediated selection may have influenced the geographic pattern of *Pan*I variation. Results from partial Mantel tests indicated that the geographic patterns observed at the *Pan*I locus are associated with temperature differentials between sample locations and that levels of divergence estimated from microsatellites appear to be largely dependent on distance. However, significant correlations (both positive and negative) of F_{ST} values with temperature observed for five microsatellite loci suggest that the covariant effects of temperature and distance were not completely controlled by the partial Mantel test analyses or that the extremely low F_{ST} estimates produced spurious results.

Simulation results indicated that the global F_{ST} estimate from *Pan*I was significantly greater than expected for presumptively neutral loci with both lesser (allozyme) and greater locus heterozygosities (microsatellites). In addition, the F_{ST} estimated from *SOD2** significantly exceeded neutral expectations, suggesting that the *SOD2** locus may also be influenced by selection. Simulations using the weighted mean F_{ST} from 14 microsatellites ($F_{ST} = 0.004$) to generate neutral F_{ST} distributions showed the *Pan*I F_{ST} to be a significant outlier, but this result potentially reflects some mutational bias in estimating this parameter using microsatellites. O'Reilly et al. (2004) concluded that size homoplasy, caused by high mutation rates in this marker class, probably introduced a significant downward bias in F_{ST} estimates among these samples, a result supported by several recent studies reporting empirical evidence for this effect when contrasting patterns of microsatellite and allozyme variation (De Innocentiis et al. 2001; Freville et al. 2001; Olsen et al. 2004). The differences between *Pan*I and microsatellite estimates could thus be due, at least in part, to higher mutation rates of microsatellites and argue for a cautious interpretation of empirical or simulation results derived from markers with different mutational properties.

Many pelagic finfish species, including walleye pollock, are characterized by high larval mortality, presenting at least in theory the potential for the development of "nursery stocks" (Smith et al. 1990), where different selection pressures at different locations of a panmictic population could produce the impression of several isolated subpopulations (Ward and Grewe 1994). For example, environmental conditions specific to different nursery (Smith et al. 1990) or retention areas (Iles and Sinclair 1982) may result in unique selection pressures, which in turn could bring about differences in allele frequencies at one or more loci influencing survival. Analysis of these (or closely linked) loci might then provide discordant results with panmixia inferred from neutral markers. Screening *Pan*I and microsatellite variation in larval pollock cohorts over smaller geographic distances (i.e., at the "nursery" scale for known spawning aggregates) would be required to address this question. Overall, the weak cline in *Pan*I allele frequencies and the simulation results suggest that selection coefficients at the *Pan*I locus may be fairly low and, if so, the dangers of falsely identifying subpopulations (type 1 error) would be greatly reduced.

Even small selection pressures, however, may be of considerable importance for the interpretation of genetic data in a historical context. Most sites in the present study are thought to have been colonized following Pleistocene glaciations 15 000 – 18 000 years before present when ice covered the outer edges of the continental shelf in the Gulf of Alaska (Mann and Peteet 1994) and sea levels were ap-

Fig. 4. Frequencies of *Pan*I alleles in walleye pollock (*Theragra chalcogramma*). Samples taken in Prince William Sound in 1997 and 1998 are indicated by year.

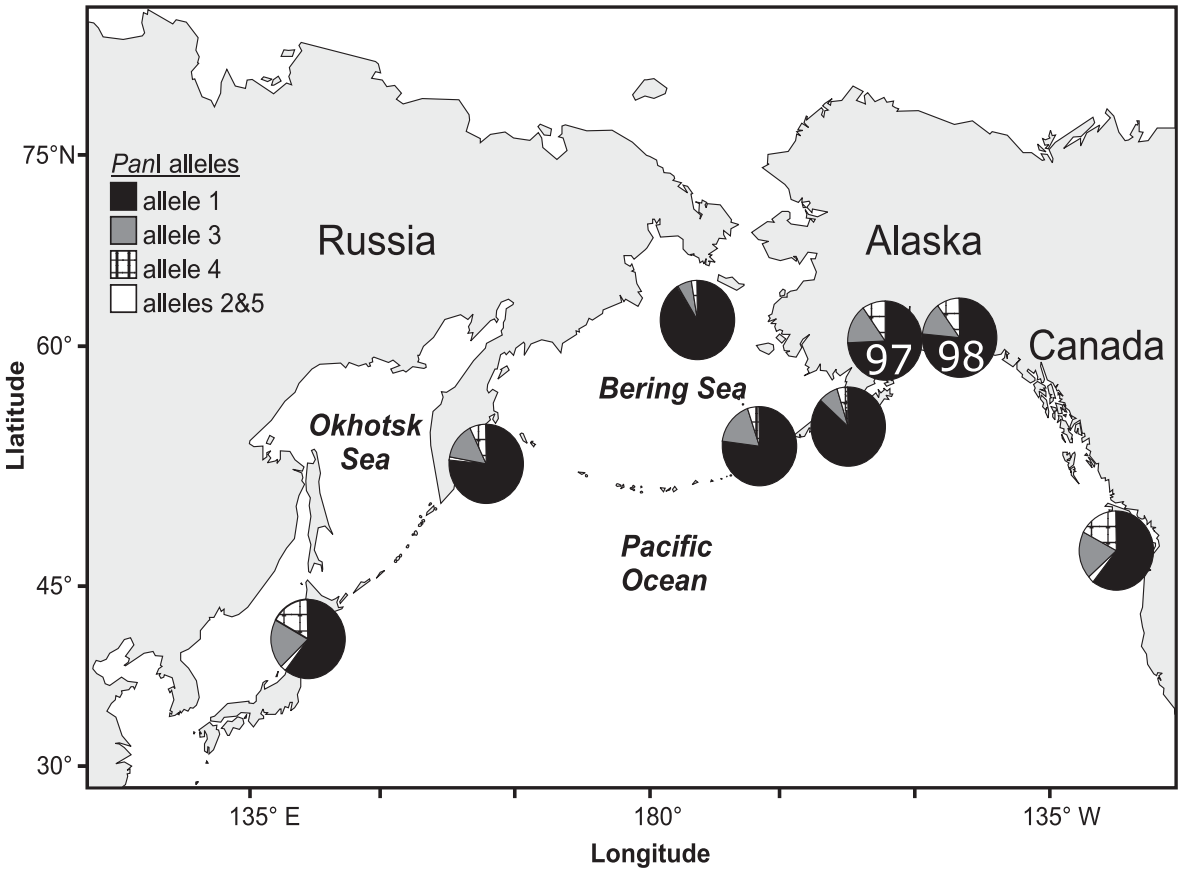


Fig. 5. Pairwise estimates of F_{ST} from 14 microsatellite loci (from O'Reilly et al. 2004) (circles) and the *Pan*I locus (squares) in walleye pollock (*Theragra chalcogramma*) versus difference in estimated annual mean surface temperature between samples.

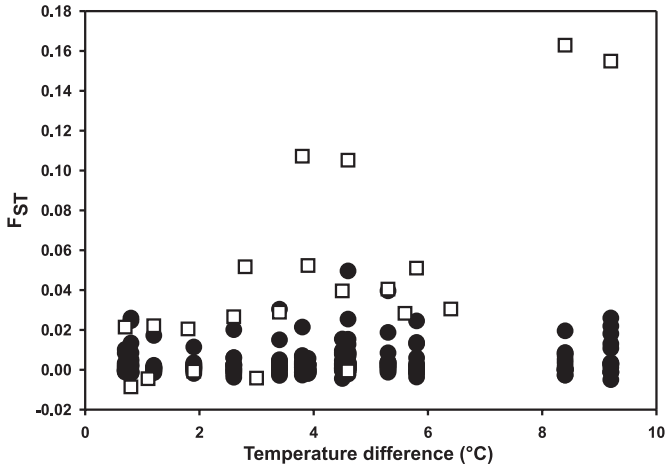
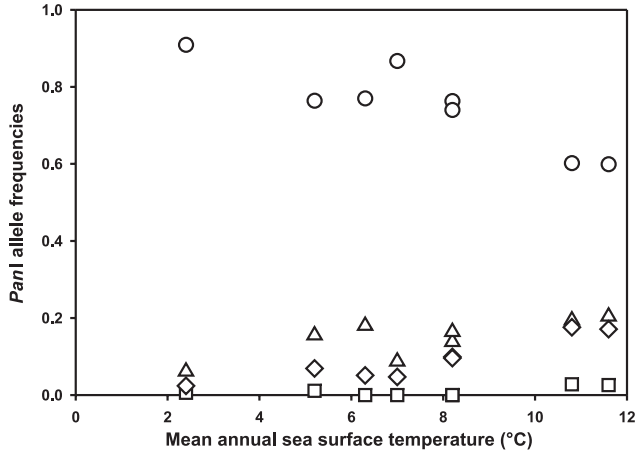


Fig. 6. Frequency occurrence of *Pan*I alleles in walleye pollock (*Theragra chalcogramma*) versus estimated annual mean surface temperature. Circles, allele 1; squares, alleles 2 and 5; triangles, allele 3; diamonds, allele 4.



proximately 85–100 m lower in current spawning areas in the North Pacific Ocean and Bering Sea (CLIMAP 1976). Pollock appears to have colonized Puget Sound approximately 6000–7000 years ago (Tunnicliffe et al. 2001). Populations have therefore separated very recently, and low levels of differentiation at neutral loci may be more a reflection of this recent separation than of high contemporary gene flow, as inferred for Atlantic cod (Pogson et al. 2001). As the rate of approach to migration–drift equilibrium, where maximum F_{ST} values are realized, is inversely proportional to the effective population size for neutral loci (Crow and Aoki 1984), the problem presented by recent divergence is exacerbated in marine species with very large population sizes. This is typified in walleye pollock, where

estimated numbers for year classes 1–10 in Shelikof Strait, Gulf of Alaska (Dorn et al. 2002), and the eastern Bering Sea (Ianelli et al. 2002) are approximately 1.3 and 40 billion individuals, respectively. For loci under selection, allele frequencies may approach equilibrium levels much more quickly than neutral markers, even when selection is weak (e.g., = 1%). This level of selection is significant over evolutionary time scales but is effectively neutral over scales of interest to humans and does not seriously bias their specific practical application as markers (Ferguson 1994), thus making them potentially more useful in detecting structure in young, large populations.

The broad cline of *Pan*I allele frequencies observed in walleye pollock challenges the assumption that gene flow is always the predominant force in limiting genetic structure in marine species and suggests the existence of self-recruiting populations at moderate geographic scales. More discrete sampling should be conducted to determine whether the patterns of spatial and temporal variation at the *Pan*I locus reflect some degree of localized adaptation, historical processes, or some combination of both. Regardless, our empirical results suggest that estimating genetic differentiation using markers under weak selection, such as *Pan*I, in conjunction with more conservative neutral markers (e.g., microsatellites) may provide a useful approach for resolving stock structure in managed marine fish populations that are often young (in an evolutionary sense), abundant, and highly dispersive. In this context, a search for additional genetic markers experiencing directional selection in marine fishes may prove to be valuable.

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