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Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure

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Abstract

Microsatellites have proved to be useful for the detection of weak population structure in marine fishes and other species characterized by large populations and high gene flow. None the less, uncertainty remains about the net effects of the particular mutational properties of these markers, and the wide range of locus polymorphism they exhibit, on estimates of differentiation. We examined the effect of varying microsatellite polymorphism on the magnitude of observed differentiation in a population survey of walleye pollock, *Theragra chalcogramma*. Genetic differentiation at 14 microsatellite loci among six putative populations from across the North Pacific Ocean and Bering Sea was weak but significant on large geographical scales and conformed to an isolation-by-distance pattern. A negative relationship was found between locus variability and the magnitude of estimated population subdivision. Estimates of F_{ST} declined with locus polymorphism, resulting in diminished power to discriminate among samples, and we attribute this loss to the effects of size homoplasy. This empirical result suggests that mutation rates of some microsatellite loci are sufficiently high to limit resolution of weak genetic structure typical of many marine fishes.

Keywords: genetic differentiation, heterozygosity, isolation by distance, microsatellites, marine fish, mutation

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Introduction

Observed levels of genetic differentiation are often very low among populations of abundant and widespread marine fish species. The apparent genetic homogeneity of many marine species is thought commonly to be due to two factors that minimize accumulation of genetic differences among populations: large effective population sizes that limit genetic drift (DeWoody & Avise 2000) and life history characteristics that favour dispersal (e.g. planktonic eggs and larvae, juvenile and adult vagility) in continuous dynamic oceanic environments (Ward *et al*. 1994; Shaklee & Bentzen 1998; Waples 1998). None the less, considerable evidence has accumulated in recent years to show that abundant marine fishes often exhibit weak but significant population structuring at neutral loci. In particular, nuclear microsatellite loci have proved to be informative for resolving low levels of differentiation in a variety of marine fishes (e.g. Bentzen *et al*. 1996; Lundy *et al*. 1999, 2000; Naciri *et al*. 1999; Ruzzante *et al*. 1999, 2000, 2001; Shaw *et al*. 1999; Nesbø *et al*. 2000; Hutchinson *et al*. 2001; McPherson *et al*. 2001; Wirth & Bernatchez 2001; Withler *et al*. 2001; Knutsen *et al*. 2003).

Although studies such as these suggest a useful role for microsatellites in analyses of population structure of species characterized by large populations and high potential

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for gene flow, questions remain about the interpretation of estimates of differentiation obtained with these markers. For example, high mutation rates may have a significant effect on the magnitude of population differentiation detected at microsatellite loci. High levels of locus polymorphism that characterize microsatellites may lead to increased statistical power (Goudet *et al*. 1996; Hedrick 1999) but may also constrain estimates of differentiation, because F_{ST} cannot exceed the homozygosity of the markers used to estimate it (Hedrick 1999). The practical significance of the homozygosity limit on F_{ST} remains unclear for marine organisms, because most F_{ST} estimates reported for most marine fishes and invertebrates tend to fall well below the theoretical maximum F_{ST} values for these hypervariable markers.

Perhaps a more important negative bias on estimates of differentiation arises from the particular mutational properties of microsatellite loci. Microsatellites generally appear to evolve according to a stepwise mutational model (SMM), where mutations result in the addition or deletion of one to several repeat units (Weber & Wong 1993; Di Rienzo *et al*. 1994). More complex patterns of variation, such as the presence of insertions/deletions in regions flanking the repeat array (Zardoya *et al*. 1996; Grimaldi & Crouau-Roy 1997; Colson & Goldstein 1999), nucleotide substitutions or indels within the arrays (Estoup *et al*. 1995; Garza & Fremier 1996; Angers & Bernatchez 1997; van Oppen *et al*. 2000), mutational bias related to array length (Weetman *et al*. 2002) and allele size constraints (Garza *et al*. 1995) indicate that mutational patterns at microsatellite loci may deviate significantly from a strict SMM, adding unknown levels of bias to both frequency-based (F_{ST}) and size-based (R_{ST}) estimators of population differentiation. Size homoplasy is expected to be an inherent characteristic of microsatellites and this may introduce a downward bias in estimates of population differentiation by making allelic distributions more similar than they would be under an infinite alleles model (Estoup & Cornuet 1999). Uncertainty about the effects of mutational processes on the magnitude of differentiation is compounded by the wide variability in levels of polymorphism among individual microsatellite loci. In a recent simulation study, Estoup *et al*. (2002) concluded that homoplasy is most prevalent when populations are large and mutation rates are high, conditions likely to characterize some microsatellite loci in marine fish populations. The estimated slippage mutation rate of some microsatellite loci increases exponentially with the number of uninterrupted repeats (Brinkmann *et al*. 1998; Lai & Sun 2003). Thus, the highly polymorphic loci often observed in marine fishes (DeWoody & Avise 2000) suggest that their mutation rates may be high. On the other hand, Estoup *et al*. (2002) also found that two other conditions that are likely to apply to microsatellites in marine fishes, increased locus polymorphism and high levels of gene flow, tend to counteract the negative bias of homoplasy on estimates of differentiation. The complexities associated with varying and incompletely understood mutational rates and processes, presumed enormous population sizes and potentially complex patterns of gene flow suggest that the ability of simulations to realistically capture the effects of varying microsatellite locus polymorphism on estimates of differentiation in many marine organisms is limited, and that empirical studies are needed. Here we examine the influence of widely varying levels of microsatellite polymorphism on estimates of differentiation in a marine fish characterized by very large populations and very weak genetic structure, the walleye pollock (*Theragra chalcogramma*).

Walleye pollock are abundant and broadly distributed across the coastal shelves and slopes in the northern Pacific Ocean and Bering Sea and exhibit weak population structuring typical of pelagic marine fishes. Pollock populations are large; for example, estimated numbers of age 1–10 pollock for 2002 in Shelikof Strait (Dorn *et al*. 2002) and eastern Bering Sea (Ianelli *et al*. 2002) are ~1.3 and 40 billion individuals, respectively. Genetic heterogeneity at ocean basin-scales in this species was first recognized in studies of allozyme variation (Iwata 1973, 1975a, 1975b; Grant & Utter 1980) and subsequent studies of mitochondrial DNA (mtDNA) variation have found low or insignificant levels of genetic differentiation within the Bering Sea and between the Bering Sea and the Gulf of Alaska (Mulligan *et al*. 1992; Shields & Gust 1995; Kim *et al*. 2000; Olsen *et al*. 2002). A recent study using several classes of genetic markers (Olsen *et al*. 2002) reported differentiation among Asian, Bering Sea and North American populations and within the Gulf of Alaska using allozyme and mtDNA restriction fragment length polymorphism (RFLP) data, but three moderately polymorphic microsatellite loci (mean = 20 alleles) used in their study did not detect structuring among these samples.

Results from Olsen *et al*. (2002) suggest that high locus polymorphism can lead to lesser rather than greater sensitivity in detecting differentiation, but the interpretation of their results is complicated by the different types of markers involved (allozymes, mtDNA and microsatellites) and associated differences in mutational mechanisms, effective population size and degree of selective neutrality. The current study evaluates the effects of varying locus polymorphism within a single marker class, microsatellites, on estimates of population differentiation in walleye pollock. We examined genetic differentiation among six populations of pollock from across the species range using 14 microsatellite loci developed by O'Reilly *et al*. (2000). Three populations were among those examined by Olsen *et al*. (2002) and included temporal replicates, thus allowing us to evaluate the relative magnitude of spatial and temporal variation, as well as the relative congruence of our results

with those of Olsen *et al*. (2002). The microsatellite loci surveyed here varied widely in numbers of alleles (6–43) and effective heterozygosities (0.68–0.96), permitting evaluation of the influence of locus variability on observed levels of differentiation across levels of polymorphism typical in marine and anadromous fishes (DeWoody & Avise 2000). Because all loci have been subjected to equivalent levels of drift and migration, we might expect more polymorphic loci to resolve weak population structure better, as they sometimes appear to be more sensitive due to higher asymptotic power in exact tests of differentiation (e.g. Estoup *et al*. 1998). However, Ferguson & Danzmann (1998) reported increased probabilities of Type II error (failure to detect significant allele frequency differences) in exact tests of simulated data as the number of alleles increased, and that a locus with two alleles generally outperformed more polymorphic loci across a range of sample sizes (25–100) and varying numbers of alleles at a locus (2–25). They suggested that the tendency of microsatellites to have a greater number of private alleles in populations, and not the total number of alleles, may account for their superior performance over allozymes where empirical comparisons on natural populations have been made. Other simulation results (Kalinowski 2002a) showed that the standard error of the F_{ST} estimator (θ; Weir & Cockerham 1984) decreased with locus polymorphism, suggesting greater precision of highly polymorphic loci in quantifying weak population subdivison. In this study, there was a strong inverse relationship between locus polymorphism and the magnitude of estimates of F_{ST}. Our empirical results indicate that very high mutation rates of some microsatellite loci may limit the power to resolve low levels of genetic structure typical of many large marine populations.

Materials and methods

Sample collection

Walleye pollock samples were obtained from locations across most of the species distribution in the North Pacific Ocean and Bering Sea (Table 1, Fig. 1). Except for age-1 juveniles collected from Puget Sound and a north central Bering Sea sample of adult pollock taken during commercial fishing operations, all samples were obtained from adult spawning aggregates. Prince William Sound, Shelikof Strait and Unimak Pass were sampled in both 1997 and 1998. A second sample was also obtained from Unimak pass in 1997, from a site approximately 200 km east from the first sample.

DNA was extracted by first incubating approximately 50 mg of ethanol-preserved tissue in 1 mL of distilled water for 15–30 min. After aspirating off the water, 150 μL of digestion buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.8% Tween 20, 100 μg/mL proteinase K) was added and samples were incubated at 65 °C for 3–4 h with intermittent vortexing. DNA was purified using either Qiagen DNeasy extraction protocols (Valencia, CA, USA), according to the manufacturer's recommendations, or by protein precipitation methods. In the case of the latter, ammonium acetate was added to a final concentration of 3.0 M, and the sample

Table 1 Sample collection information for walleye pollock

Sample (abbreviation)	Year	N. Latitude	Longitude	Life history stage	Sample size
Funka Bay, Japan (Jpn98)	1998	42.250	142.500 E	Spawning Adults	81
North Central Bering Sea (NCBS97)	1997	61.813	178.500 W	Adults	71
Unimak, Alaska (Uni97A)	1997	54.413	165.728 W	Spawning Adults	99
Unimak, Alaska (Uni97B)	1997	54.450	162.278 W	Spawning Adults	99
Unimak, Alaska (Uni98)	1998	54.331	165.385 W	Spawning Adults	91
Shelikof Strait, Akaska (Shel97)	1997	57.988	154.212 W	Spawning Adults	99
Shelikof Strait, Akaska (Shel98)	1998	57.598	154.233 W	Spawning Adults	97
Prince William Sound, Alaska (PWS97)	1997	60.083	148.317 W	Spawning Adults	98
Prince William Sound, Alaska (PWS98)	1998	60.083	148.333 W	Spawning Adults	99
Puget Sound, Washington (PS98)	1998	48.12	122.770 W	Juveniles	85

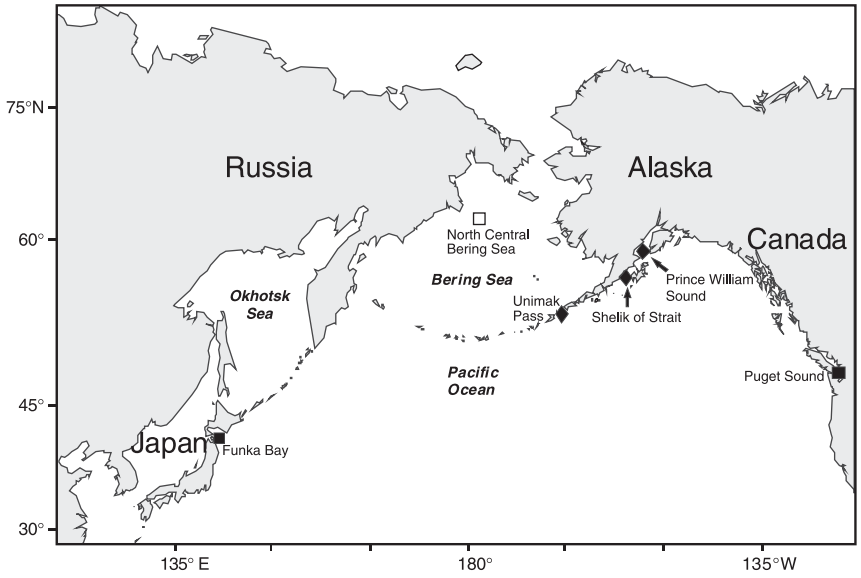

Fig. 1 Sample locations for walleye pollock collected in 1997 (open box), 1998 (solid box) and in both years (solid diamond).

centrifuged at 12 000 *g* for 5 min. The resulting supernatant was removed to a new tube where total DNA was concentrated and the salts removed using standard ethanol precipitation procedures.

Microsatellite analysis

Twelve tetranucleotide and two dinucleotide microsatellite loci were individually amplified using the polymerase chain reaction (PCR), as described in O'Reilly *et al*. (2000). Alleles were size-fractionated using polyacrylamide electrophoresis and visualized using a Molecular Dynamics FluorImager 575 (Amersham Biosciences, Piscataway, NJ), a Hitachi FMBIOII (Hitachi America Ltd, NY, USA) or an ABI 373 (Applied Biosystems, Foster City, CA, USA). Known genotypes and size standards were run on each platform to ensure scoring accuracy and consistency among scoring platforms.

Statistical analyses

Single locus statistics and conformance to Hardy–Weinberg equilibrium. Single-locus estimates of heterozygosity and number of alleles in each sample and over all samples were made using GENETIX version 4.02 (Belkhir 2000) and FSTAT version 2.9.3 (Goudet 2001). The effective number of alleles (n_e) was calculated according to (Kimura & Crow 1964). The analogue of Wright's F_{IS}, $\hat{f}$, was estimated in GENEPOP (version 3.3, Raymond & Rousset 1995). Locus conformance to Hardy–Weinberg equilibrium (HWE) was assessed with exact tests implemented in GENEPOP with specified Markov chain parameters of 5000 dememorization steps followed by 500 batches of 2000 iterations per batch. Significance levels for multiple comparisons of loci across samples were adjusted using a sequential Bonferroni correction (Rice 1989).

In cases where observed genotype frequencies deviated significantly from HWE expectations, the program MICROCHECKER (Shipley 2003) was used to infer the most probable cause of the HWE departures. Null alleles (Pemberton *et al*. 1995) were inferred if an excess of homozygotes occurred over most allele size classes, and upper allele dropout (Wattier *et al*. 1998) was indicated if the deviations were more restricted to the smallest allele size classes. Homozygous excess due to PCR 'stutter' was inferred if there were deficiencies of individuals heterozygous for alleles differing by one repeat unit. Allele and genotype frequencies not conforming to HWE were adjusted according to Brookfield (1996) to contrast with uncorrected data in analyses of population differentiation.

Estimates of population structuring. Exact tests of allele (genic) and genotype (genotypic) distributions between pairs of populations were conducted using GENEPOP. Tests of the joint null hypothesis of no heterogeneity in allele or genotype distributions between sample pairs at any locus were made using Fisher's combined method of summing *P*-values for exact tests and comparing the quantity $\Sigma - 2 \ln$ to a χ^2 distribution (28 d.f.) to assess statistical significance. Estimates of F_{ST}, $\hat{\theta}$, following Weir & Cockerham (1984), and the R_{ST} analogue statistic, ρ_{ST} (Rousset 1996), were calculated between sample pairs using FSTAT and significance of those estimates was assessed using 2000 data permutations. Similarly, log-likelihood *G*-tests permuting genotypes between sample pairs were made using 2000 data randomizations in FSTAT. In all cases of multiple

simultaneous tests for genetic heterogeneity or structuring, significance levels were adjusted with sequential Bonferroni correction.

Temporal and regional comparisons of genetic heterogeneity between sampling years and among regions were conducted using analyses of molecular variance (AMOVA) implemented in ARLEQUIN version 2.000 (Schneider *et al*. 2000). Genetic isolation by distance (Slatkin 1993), testing for independence between F_{ST} estimates and geographical distances, was evaluated using a Mantel test available in GENEPOP. Regression matrices of $F_{ST}/1 - F_{ST}$ values vs. the log of distance between sample pairs, the latter estimated as the shortest route along continental margins at depths less than 200 m, were permuted 2000 times and significance of the relationship was assessed by the Spearman rank correlation coefficient.

Microsatellite polymorphism and fixation indices. Because sample sizes ranged considerably among loci, the relationships of locus polymorphism and F_{IS} with F_{ST} were examined by incorporating allelic richness, an estimate of the number of alleles that is independent of sample size, with single locus $\hat{f}$ and $\hat{\theta}$ values in a general linear model (GLM) using SYSTAT version 10 (SPSS Inc., Chicago, IL, USA). Estimates of allelic richness were made using FSTAT. The number of alleles per locus for each sample was standardized to the smallest number of single-locus genotypes by sampling 53 pairs of alleles 1000 times, following El Mousadik & Petit (1996).

Results

Genetic variation and Hardy–Weinberg equilibrium

The 14 microsatellites varied widely in number of alleles (6–43, mean = 20.9) and expected heterozygosities (0.68–0.96, mean = 0.85) across populations (Table 2). The number of individuals genotyped at each locus ranged from 53 to 99 (median 84.8) per sample. This was due, in part, to variation in the number of individuals collected from the different locations surveyed (Table 1) but may also be related to tissue degradation prior to preservation in some samples (e.g. NCBS97) that prevented successful PCR amplification of both alleles in all individuals. Significant departures from HWE were observed in 54 of 140 single locus exact tests and all deviations except one were towards heterozygote deficiencies (Table 2). Loci *Tch6*, *Tch11*, *Tch14* and *Tch19* showed significant heterozygote deficits in every sample, while *Tch8* and *Tch15* exhibited deficits in four or five samples. Loci *Tch5*, *Tch10*, *Tch12*, *Tch13*, *Tch18*, *Tch20* and *Tch22* were in agreement with HWE expectations in eight or more of the 10 samples. Analyses of genotype and allele frequencies confirmed excess homozygosity at one or more alleles in loci characterized by significant positive $\hat{f}$ values. Analyses of homozygote size classes with MICRO-CHECKER did not indicate the effects of upper allele dropout that might result from degraded DNA template or stuttering during PCR amplification as causes for the observed heterozygote deficiencies. These results indicate that true null alleles (i.e. alleles that failed to amplify because of base substitutions or deletions in PCR priming sites flanking microsatellite arrays) were the primary cause of departures from HWE. An alternative explanation, population admixtures within samples (i.e. a Wahlund effect) seems unlikely because all but two samples were collected from spawning aggregations and the observed levels of spatial heterogeneity would appear to be too small to account for the large F_{IS} values.

Genetic differentiation among populations

The joint null hypothesis of no heterogeneity between sample pairs for any locus was rejected in 42 and 39 of 45 possible tests of genic differentiation using Fisher's combined method on uncorrected data, and data corrected for null alleles, respectively. Similarly, data corrected for null alleles resulted in fewer significant pairwise tests of genotypic variation (17 of 45) compared with tests using uncorrected data (39 of 45). Exact tests of genic (Table 3) and genotypic (Table 4) variation between sample pairs showed that significant heterogeneity was detected most often between the Jpn98 sample and other locations. Differentiation was also found in pairwise comparisons between the north central Bering Sea and locations in the Gulf of Alaska, between Shelikof Strait and Unimak, and between Unimak and Puget Sound, although these relationships were not consistent across sampling years where temporal collections had been made.

Multilocus estimates of F_{ST} ($\hat{\theta}$) and R_{ST} ($\hat{\rho}_{ST}$) for population pairs were low but indicated significant population structuring over broad spatial scales (Table 5). Correcting the data for null alleles decreased the number of significant pairwise estimates but concordant patterns of differentiation were found with both estimators between the Jpn98 sample and other locations (excluding the central Bering Sea). Pairwise values of $\hat{\rho}_{ST}$ were significantly higher than $\hat{\theta}$ (Wilcoxon paired-sample test, $P < 0.001$) but did not result in a greater number of significant comparisons among sample pairs (Table 5).

Temporal samples were grouped across years for Shelikof Strait (Shel97 and Shel98), Unimak Pass (Uni97A, Uni97B and Uni98) and Prince William Sound (PWS97 and PWS98) to compare interannual heterogeneity within and among sample locations. There was no evidence of significant regional heterogeneity among these proximate groups of samples but a small and significant temporal component within sampling locations was detected by AMOVA (Table 6), a result concordant with pairwise tests of

Table 2 Summary statistics for 14 microsatellite loci in 10 walleye pollock samples. Sample abbreviations are as in Table 1; other abbreviations are as follows: number of individuals (N), number of alleles observed per locus (N_A), number of alleles per locus standardized to the smallest sample size, $N = 53$ individuals (Ns); allelic range in bp (R), expected (H_E) and observed (H_O) heterozygosities, analogue of Wright's F_{IS} ($\hat{f}$), effective number of alleles (n_e), and single-locus estimates of F_{ST} ($\hat{\theta}$) over all samples. Italic $\hat{f}$ values indicate significant genotypic deviation from expected Hardy–Weinberg proportions ($P < 0.05$) after sequential Bonferroni correction for multiple ($k = 10$) tests

		Locus (repeat type)														
Location		*Tch3* (di)	*Tch5* (tetra)	*Tch6* (tetra)	*Tch8* (tetra)	*Tch10* (tetra)	*Tch11* (tetra)	*Tch12* (tetra)	*Tch13* (di)	*Tch14* (tetra)	*Tch15* (di)	*Tch18* (di)	*Tch19* (tetra)	*Tch20* (tetra)	*Tch22* (tetra)	Mean all loci
Jpn98	N	67	81	76	70	80	80	80	76	79	80	80	78	71	80	77
	N_A	19	24	43	31	20	19	10	8	26	12	16	19	39	9	21.1
	N_S	18.14	20.95	30.19	29.67	16.93	17.71	7.78	6.04	21.92	11.13	14.54	18.25	28.25	8.17	17.83
	R	226–290	212–296	97–349	128–296	127–239	125–213	118–158	76–106	100–248	77–103	71–107	70–146	116–336	76–112	
	H_E	0.83	0.93	0.96	0.95	0.79	0.92	0.83	0.68	0.94	0.68	0.86	0.93	0.96	0.72	0.86
	H_O	0.72	0.90	0.83	0.93	0.70	0.76	0.79	0.75	0.65	0.54	0.81	0.71	0.99	0.66	0.77
	$\hat{f}$	0.14	0.03	*0.14*	0.03	0.12	*0.17*	0.05	*–0.11*	*0.31*	0.21	0.06	*0.24*	*–0.02*	0.08	
	n_e	6	14.5	25.8	21.5	4.8	12	5.8	3.1	16.4	3.2	7.1	14.3	27.2	3.6	11.8
NCBS97	N	53	60	54	65	63	64	64	70	61	59	60	63	57	68	61.5
	N_A	21	25	32	35	15	18	8	7	20	13	13	18	32	7	18.9
	N_S	19.25	19.79	31.39	31.16	19.30	20.38	7.66	5.86	21.66	12.96	15.97	18.59	34.30	5.93	18.87
	R	230–282	176–300	117–341	104–292	143–227	125–193	118–146	76–88	108–224	77–109	67–101	82–143	124–348	80–112	
	H_E	0.78	0.94	0.95	0.96	0.78	0.91	0.78	0.65	0.94	0.66	0.77	0.93	0.96	0.69	0.84
	H_O	0.76	0.88	0.74	0.91	0.68	0.69	0.73	0.77	0.62	0.54	0.67	0.60	0.98	0.57	0.73
	$\hat{f}$	0.03	0.06	*0.22*	0.05	0.12	*0.24*	0.06	–0.19	*0.34*	*0.18*	0.14	*0.35*	–0.02	0.17	
	n_e	4.5	15.3	20.5	22.2	4.5	10.6	4.6	2.9	15.8	2.9	4.4	14.3	24.6	3.2	10.7
Uni97A	N	60	85	56	56	98	96	99	99	89	99	99	99	60	99	85.3
	N_A	19	22	31	30	21	20	8	7	24	14	17	20	29	10	19.4
	N_S	17.39	22.85	36.70	37.77	18.32	16.02	8.42	6.48	23.51	9.54	14.21	18.51	28.85	7.36	18.99
	R	230–282	188–280	117–313	136–304	139–243	121–197	122–162	74–86	124–236	77–111	67–109	74–158	116–316	80–152	
	H_E	0.77	0.94	0.94	0.96	0.79	0.92	0.78	0.68	0.94	0.62	0.78	0.93	0.96	0.69	0.84
	H_O	0.65	0.87	0.70	0.89	0.71	0.76	0.78	0.75	0.73	0.45	0.70	0.70	0.95	0.62	0.73
	$\hat{f}$	0.16	0.08	*0.26*	*0.07*	0.10	*0.17*	0.00	–0.10	*0.22*	*0.26*	0.11	*0.25*	0.01	0.11	
	n_e	4.4	17.2	17.1	23.8	4.8	12.4	4.6	3.1	16.1	2.6	4.5	14.6	24.4	3.3	10.9
Uni97B	N	58	86	54	79	91	91	89	91	83	91	91	89	55	89	81.2
	N_A	18	25	37	42	23	18	9	7	27	11	16	20	29	9	20.8
	N_S	21.00	24.02	31.83	32.28	14.16	17.10	7.83	6.70	19.67	12.58	12.62	17.48	31.35	6.55	18.23
	R	232–284	184–292	97–353	128–352	143–251	129–209	122–154	76–90	116–240	77–101	71–109	78–154	144–296	76–108	
	H_E	0.82	0.94	0.96	0.97	0.82	0.90	0.75	0.68	0.93	0.66	0.77	0.93	0.96	0.68	0.84
	H_O	0.78	0.92	0.76	0.89	0.85	0.68	0.74	0.79	0.71	0.53	0.78	0.80	0.91	0.67	0.77
	$\hat{f}$	0.06	0.02	*0.21*	*0.09*	–0.03	*0.24*	0.01	–0.17	*0.24*	0.20	–0.02	*0.15*	0.06	0.01	
	n_e	5.6	5.5	26.6	32.4	5.5	9.6	4	3.1	14.9	2.9	4.3	15.2	28.4	3.1	12.2

Table 2. *Continued*

Location		Locus (repeat type)														
		Tch3 (di)	*Tch5* (tetra)	*Tch6* (tetra)	*Tch8* (tetra)	*Tch10* (tetra)	*Tch11* (tetra)	*Tch12* (tetra)	*Tch13* (di)	*Tch14* (tetra)	*Tch15* (di)	*Tch18* (di)	*Tch19* (tetra)	*Tch20* (tetra)	*Tch22* (tetra)	Mean all loci
Uni98	N	90	78	96	74	88	93	95	96	98	98	85	94	97	85	86.2
	N_A	20	24	38	37	25	18	8	7	24	15	14	24	33	8	21.1
	N_S	18.25	20.54	32.79	34.65	17.67	18.42	9.27	6.28	22.33	11.57	12.55	17.95	32.02	8.56	18.77
	R	226–288	200–304	117–341	132–352	139–307	121–193	122–150	76–90	96–240	75–111	77–105	78–170	120–320	80–112	
	H_E	0.83	0.94	0.95	0.96	0.75	0.92	0.76	0.69	0.93	0.63	0.79	0.94	0.96	0.70	0.84
	H_O	0.62	0.99	0.74	0.91	0.73	0.77	0.78	0.69	0.78	0.40	0.75	0.69	0.89	0.58	0.74
	$\hat{f}$	*0.25*	–0.05	*0.22*	0.06	0.03	*0.22*	–0.03	0.00	*0.17*	0.02	0.04	*0.26*	0.07	0.18	
	n_e	5.7	16.4	20.2	27.9	4	12.2	4.1	3.2	14.3	2.7	4.7	16.2	23.5	3.4	11.3
Shel97	N	93	73	97	77	83	90	79	57	92	89	88	76	94	94	84.4
	N_A	22	21	35	34	23	23	8	6	25	15	19	20	42	7	21.4
	N_S	17.56	21.51	37.68	28.54	16.99	17.87	9.32	6.76	23.22	11.02	14.52	18.11	35.06	7.88	19.00
	R	230–286	188–280	117–313	124–296	127–247	121–217	122–150	76–86	96–248	77–139	75–139	70–158	104–328	80–108	
	H_E	0.86	0.93	0.96	0.96	0.83	0.92	0.78	0.69	0.94	0.75	0.85	0.92	0.97	0.64	0.86
	H_O	0.83	0.93	0.81	0.83	0.70	0.76	0.77	0.68	0.75	0.51	0.74	0.68	0.96	0.52	0.75
	$\hat{f}$	0.03	0.00	*0.15*	*0.14*	*0.16*	*0.18*	0.02	0.01	*0.20*	*0.33*	0.13	*0.26*	0.03	0.18	
	n_e	6.9	15.2	24.8	25.3	6	12.9	4.6	3.2	16.1	4	6.8	12	28.6	2.8	12.1
Shel98	N	89	81	87	92	84	93	94	91	91	91	94	86	83	97	89.5
	N_A	23	23	43	34	22	21	9	6	30	14	15	20	40	6	21.8
	N_S	20.60	20.67	35.36	29.69	17.81	17.92	8.50	7.72	21.79	9.99	15.62	18.75	34.48	5.66	18.90
	R	226–292	192–288	117–345	140–352	147–303	109–229	122–154	76–86	92–280	75–103	77–107	74–150	116–324	80–104	
	H_E	0.83	0.94	0.97	0.96	0.75	0.92	0.78	0.70	0.94	0.78	0.80	0.93	0.97	0.68	0.85
	H_O	0.79	0.94	0.79	0.88	0.71	0.79	0.71	0.67	0.76	0.68	0.73	0.77	0.96	0.68	0.78
	$\hat{f}$	0.06	0.01	*0.18*	0.08	0.04	*0.15*	0.09	0.04	*0.19*	0.12	0.08	*0.18*	0.00	–0.01	
	n_e	6	17.9	29	25.4	3.9	12.9	4.6	3.3	16.4	4.4	4.9	14.2	29.8	3.1	12.6
PWS97	N	87	96	75	91	78	97	94	85	93	80	81	86	86	91	87.1
	N_A	22	22	38	41	20	20	10	7	24	12	14	19	37	10	21
	N_S	19.29	21.45	37.22	30.39	18.67	19.25	8.72	5.94	25.48	12.47	13.22	18.93	35.08	5.51	19.40
	R	216–288	196–284	73–313	120–320	143–231	117–197	122–158	76–88	116–236	77–101	75–107	82–174	124–300	80–116	
	H_E	0.82	0.94	0.96	0.95	0.82	0.91	0.77	0.72	0.95	0.74	0.80	0.93	0.96	0.72	0.86
	H_O	0.75	0.95	0.76	0.91	0.76	0.71	0.76	0.77	0.82	0.55	0.73	0.71	0.95	0.68	0.77
	$\hat{f}$	0.09	–0.01	*0.21*	*0.05*	0.08	*0.16*	0.02	–0.07	*0.14*	*0.25*	0.09	*0.24*	0.01	0.05	
	n_e	5.6	16.8	28	21.9	5.5	11.2	4.3	3.5	18.1	3.8	5.1	13.7	27.2	3.5	12

Table 2. *Continued*

Location		Locus (repeat type)														
		Tch3 (di)	*Tch5* (tetra)	*Tch6* (tetra)	*Tch8* (tetra)	*Tch10* (tetra)	*Tch11* (tetra)	*Tch12* (tetra)	*Tch13* (di)	*Tch14* (tetra)	*Tch15* (di)	*Tch18* (di)	*Tch19* (tetra)	*Tch20* (tetra)	*Tch22* (tetra)	Mean all loci
PWS98	N	70	84	93	90	89	88	89	88	88	88	90	86	91	89	87.4
	N_A	21	25	42	37	27	21	10	9	30	13	14	21	40	8	22.7
	N_S	18.05	22.85	37.59	31.45	22.91	18.43	8.50	7.85	25.88	11.83	12.90	19.32	34.48	7.16	19.94
	R	226–284	188–280	113–337	120–296	139–303	125–209	118–154	70–90	116–264	75–125	77–125	70–170	116–352	80–112	
	H_E	0.78	0.93	0.96	0.96	0.83	0.92	0.79	0.67	0.94	0.72	0.82	0.93	0.96	0.75	0.85
	H_O	0.70	0.92	0.63	0.90	0.76	0.72	0.78	0.77	0.68	0.51	0.79	0.77	0.91	0.72	0.75
	$\hat{f}$	0.11	0.02	*0.34*	0.06	0.09	*0.22*	0.01	–0.15	*0.28*	*0.29*	0.03	*0.18*	0.05	0.05	
	n_e	4.6	14.8	24.8	25.3	6	11.7	4.7	3	17.7	3.5	5.4	14.2	25.9	4	11.8
PS98	N	67	79	68	68	81	75	83	77	77	79	80	79	60	80	75.2
	N_A	22	22	38	32	20	19	9	9	24	11	18	20	36	6	20.4
	N_S	17.50	22.62	31.53	33.48	20.72	15.68	7.90	6.46	21.61	12.42	12.31	21.13	28.17	6.87	18.46
	R	230–286	192–288	117–321	132–312	143–227	129–205	122–154	74–94	124–264	81–101	77–115	82–170	112–308	84–112	
	H_E	0.83	0.93	0.97	0.95	0.80	0.92	0.80	0.62	0.93	0.72	0.84	0.92	0.96	0.72	0.85
	H_O	0.66	0.90	0.75	0.93	0.78	0.73	0.80	0.66	0.68	0.61	0.75	0.61	1.00	0.68	0.75
	$\hat{f}$	*0.21*	0.04	*0.23*	0.03	0.03	*0.20*	0.00	–0.07	*0.27*	0.15	0.10	*0.34*	–0.04	0.06	
	n_e	5.7	14.6	29.5	21.1	5.1	12.5	4.9	2.6	13.4	3.5	6	12.6	25.6	3.5	11.5
Mean all pops	N	73.4	80.3	75.6	76.2	83.5	80.3	86.6	83	85.1	79.4	84.8	83.6	75.4	87.2	81.5
	N_A	20.5	23.3	37.7	35.3	21.5	17.9	8.9	7.3	25.4	13	15.6	20.1	35.7	8	20.9
	N_S	19.21	22.27	35.68	33.10	18.99	18.17	8.51	6.64	23.71	11.94	14.00	19.12	32.94	6.88	19.37
	R	216–292	176–304	73–349	104–352	127–307	109–229	118–162	70–106	92–280	75–139	67–139	70–174	104–352	76–152	
	H_E	0.82	0.94	0.96	0.96	0.80	0.92	0.79	0.68	0.94	0.71	0.81	0.93	0.96	0.71	0.85
	H_O	0.73	0.92	0.75	0.90	0.74	0.74	0.76	0.73	0.72	0.56	0.75	0.71	0.94	0.63	0.75
	n_e	5.5	14.8	24.6	24.7	5	10.6	4.6	2.9	14.4	3.4	5.3	14.1	26.5	3.4	11.6
	$\hat{\theta}$	–0.000	0.002	0.002	0.002	0.002	0.002	0.006	0.006	0.002	0.009	0.006	0.003	0.000	0.011	0.004

Table 3 Probability values for Fisher's combined test of genic (allelic) differentiation at 14 microsatellite loci using data that were uncorrected (above diagonal) and corrected (below diagonal) for the presence of null alleles. Bold *P* values are significant after sequential Bonferroni correction for 45 multiple tests. Sample abbreviations are as in Table 1

	Jpn98	NCBS97	Uni97A	Uni97B	Uni98	Shel97	Shel98	PWS97	PWS98	PS98
Jpn98	—	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
NCBS97	**<0.001**	—	**0.023**	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
Uni97A	**<0.001**	0.122	—	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
Uni97B	**<0.001**	0.011	**0.007**	—	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Uni98	**<0.001**	**<0.001**	**0.001**	**<0.001**	—	**<0.001**	0.042	**0.002**	**<0.001**	**<0.001**
Shel97	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	—	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Shel98	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.061	**<0.001**	—	0.274	**<0.001**	**<0.001**
PWS97	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**	**<0.001**	0.345	—	**0.002**	**<0.001**
PWS98	**<0.001**	**0.005**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.007**	—	**0.001**
PS98	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.022	—

Table 4 Probability values for Fisher's combined test of genotypic differentiation at 14 microsatellite loci using data that were uncorrected (above diagonal) and corrected (below diagonal) for the presence of null alleles. Bold *P* values are significant after sequential Bonferroni correction for 45 multiple tests. Sample abbreviations are as in Table 1

	Jpn98	NCBS97	Uni97A	Uni97B	Uni98	Shel97	Shel98	PWS97	PWS98	PS98
Jpn98	—	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
NCBS97	0.002	—	0.345	0.067	0.007	**0.001**	**<0.001**	**<0.001**	0.019	0.031
Uni97A	**<0.001**	0.675	—	0.050	0.014	**<0.001**	**0.001**	0.004	0.008	**0.003**
Uni97B	**<0.001**	0.127	0.146	—	**0.001**	**<0.001**	**<0.001**	**0.001**	**0.003**	**<0.001**
Uni98	0.006	0.011	0.050	0.006	—	**<0.001**	0.230	0.057	**0.003**	**<0.001**
Shel97	**<0.001**	0.018	0.002	**<0.001**	**<0.001**	—	**<0.001**	**<0.001**	**0.002**	**0.002**
Shel98	**<0.001**	**<0.001**	0.003	**<0.001**	0.263	0.007	—	0.501	**0.001**	**0.001**
PWS97	**<0.001**	**0.001**	0.029	0.012	0.089	0.015	0.750	—	0.015	**0.001**
PWS98	0.009	0.083	0.053	0.021	0.009	0.256	0.047	0.060	—	0.118
PS98	**<0.001**	0.211	0.045	**0.001**	0.035	0.127	0.007	0.010	0.562	—

Table 5 Multilocus estimates of F_{ST}, $\hat{\theta}$, (above diagonal) and R_{ST}, $\hat{\rho}_{ST}$, (below diagonal) between sample pairs from 14 microsatellite loci. Values in bold or denoted by asterisks are significantly greater than zero for data that were uncorrected or corrected for the presence of null alleles, respectively, after sequential Bonferroni adjustment for 45 simultaneous tests. Sample abbreviations are as in Table 1

	Jpn98	NCBS97	Uni97A	Uni97B	Uni98	Shel97	Shel98	PWS97	PWS98	PS98
Jpn98	—	0.006	**0.007***	**0.007***	0.006*	**0.008***	**0.005***	**0.005**	**0.004***	**0.005***
NCBS97	0.011	—	0.000	0.002	0.003	0.006	**0.006***	0.004	0.003	0.004
Uni97A	**0.011**	−0.001	—	0.001	0.003	0.005	0.003	0.004	0.002	0.005
Uni97B	**0.020***	0.020	0.020	—	0.003	0.004	**0.003**	0.002	0.002	**0.004**
Uni98	**0.010**	0.005	0.007	0.031	—	0.005	0.000	0.002	0.003	**0.005***
Shel97	**0.015***	0.001	0.004	0.022	0.006	—	0.003	0.002	0.003	0.004
Shel98	**0.025***	**0.028***	0.003	**0.028***	0.006	0.001	—	0.000	−0.001	0.004
PWS97	**0.014***	0.005	0.004	0.013	0.016	0.002	0.009	—	−0.001	0.003
PWS98	**0.011**	−0.001	−0.003	0.031*	−0.001	0.005	0.003	0.010	—	0.002
PS98	**0.014***	−0.003	−0.002	**0.029***	0.004	0.001	0.002	0.006	−0.003	—

genic differentiation between years for Shel and PWS samples (Table 3) but not with results from genotypic tests. Mantel regression tests showed a significant ($P = 0.003$) pattern of isolation by distance across the entire geographical range (Fig. 2). Test results remained statistically significant when excluding either of the two most distant samples (Jpn98 or PS98) but not when excluding both ($P = 0.10$).

Table 6 Analysis of molecular variance (AMOVA) results from pooled samples collected in 1997 and 1998 at three locations (Shelikof Strait, Unimak Pass and Prince William Sound)

Source of variation	Sum of squares	% variation
Among locations	19.589	0.09
Between years within locations	34.868	0.55**
Within samples	3541.299	99.36**
Total	3595.756	

**$P < 0.001$.

Table 7 Results from generalized linear model (GLM) regression analysis comparing the effects of allelic richness and F_{IS} upon estimates of F_{ST}

Effect	Coefficient	SE	*t*	*P*
Constant	0.005	0.001	4.865	<0.001
Allelic richness	−0.000	0.000	−2.558	0.012
F_{IS}	−0.005	0.008	−0.604	0.547
Allelic richness × F_{IS}	0.000	<0.001	0.249	0.800

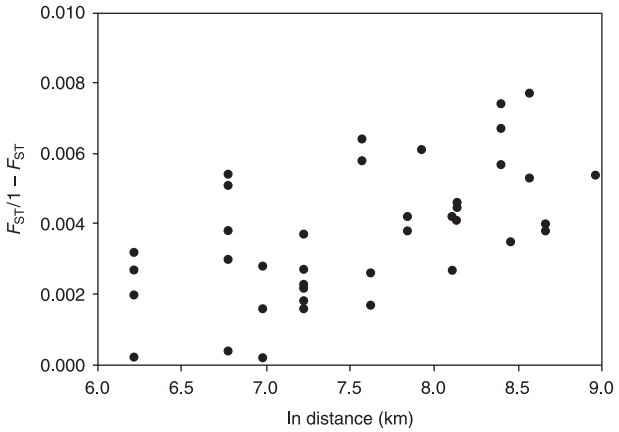

Fig. 2 Genetic isolation by distance in walleye pollock inferred from multilocus estimates of F_{ST} vs. geographical distance.

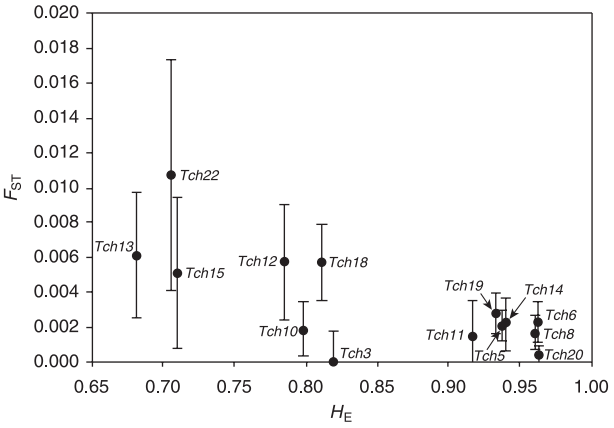

Fig. 4 Single-locus estimates of F_{ST} vs. expected heterozygosity for 14 microsatellite loci in 10 samples.

Relationship between locus polymorphism and detection of differences among populations

Estimates of population differentiation ($\hat{\theta}$) were inversely correlated with microsatellite polymorphism. GLM results showed that allelic richness, but not F_{IS}, had a significant effect in diminishing single-locus estimates of F_{ST} among all populations (Fig. 3, Table 7). Estimates of F_{ST} also declined with increasing expected heterozygosity ($P = 0.003$, Fig. 4). The observed range of $\hat{\theta}$ is well below the theoretical maximum values that θ could attain, suggesting that other factors (e.g. nonequilibrium migration-drift conditions, mutational homoplasy), and not constraints related to locus homozygosity, are the principal causes for low estimates of population structuring. The precision of $\hat{\theta}$ values appeared to improve with locus polymorphism. Excluding one anomalous value of 41.0, coefficients of variation for $\hat{\theta}$ declined significantly from approximately 2.4–0.3 as a function of allelic richness (ANOVA $F = 14.20$, $P = 0.003$). The number of significant pairwise log-likelihood *G*-tests of population differences tended to decline with increasing locus polymorphism (Fig. 5), although the correlation was not statistically significant. Similarly, the numbers of significant $\hat{\theta}$ and $\hat{\rho}_{ST}$ test results between sample pairs also tended to decrease with increasing microsatellite polymorphism, but again the correlations were not statistically significant (data not shown).

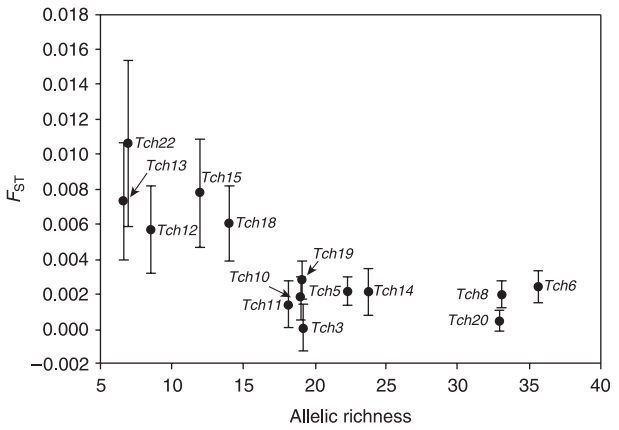

Fig. 3 Single-locus estimates of F_{ST} (+ 95% CI) vs. allelic richness for 14 microsatellite loci in 10 samples.

Discussion

This study yielded results on two distinct topics. First, it provided a description of the pattern and degree of population structuring across the entire range of walleye

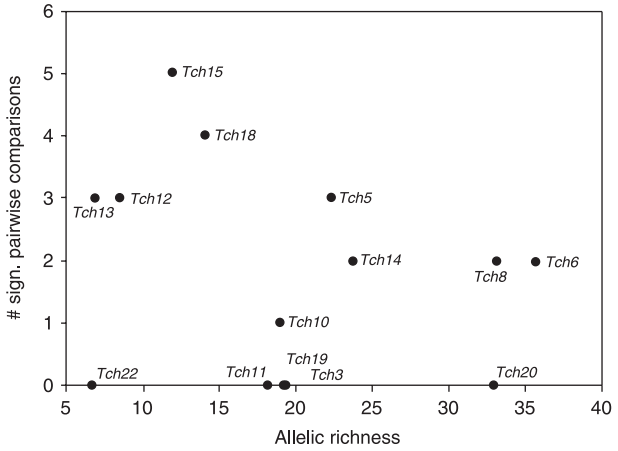

Fig. 5 Number of significant log-likelihood *G*-tests as a function of allelic richness in pairwise comparisons between samples.

pollock based on a substantial panel of microsatellite markers. Second, it provided evidence that estimates of population differentiation and the probability of detecting genetic differences among populations both diminish when allelic richness and locus heterozygosity are high. We consider these major results in turn below.

Walleye pollock population structure

We observed weak, but significant genetic structuring among pollock populations. Population structure followed an isolation-by-distance pattern, but even on transoceanic spatial scales, pairwise estimates of F_{ST} were less than 0.008 (Fig. 2). These levels of differentiation are similar in magnitude to those reported for other marine fish species with potentially high gene flow (e.g. Elliot & Ward 1992; Gold *et al*. 1994; Bentzen *et al*. 1996; Borsa *et al*. 1997; Ruzzante *et al*. 1999, 2000, 2001; Shaw *et al*. 1999; Lundy *et al*. 2000; Nesbø *et al*. 2000; De Innocentiis *et al*. 2001; McPherson *et al*. 2001; Wirth & Bernatchez 2001; Withler *et al*. 2001; Knutsen *et al*. 2003). Our results corroborate previous findings of significant differentiation between populations in the Western Pacific Ocean and those in the Bering Sea and Gulf of Alaska using allozyme and mtDNA data (Iwata 1975a, b; Grant & Utter 1980; Shields & Gust 1995; Olsen *et al*. 2002). Some evidence for population structuring at closer geographical scales, as reported for allozymes (Grant & Utter 1980) and allozymes combined with mtDNA (Olsen *et al*. 2002), is suggested by the microsatellite data (e.g. between Unimak and Shelikof in exact genotypic tests), although the data are not consistent across sampling years and were not a significant source of variation in the AMOVA.

Departures from HWE expectations in half the loci used in this study appear to be due to the presence of null alleles, resulting possibly from high levels of sequence heterogeneity in regions flanking microsatellite arrays. Findings of increased numbers of alleles observed in marine fishes relative to freshwater fishes have been attributed to increased effective populations sizes in the former, and reduced loss of variation due to drift (Dewoody & Avise 2000). This same mechanism may account for increased sequence heterogeneity in populations of pollock surveyed here and suggest that null alleles may be common in large marine populations. We emphasize that data should be examined carefully and, when possible, corrected for the presence of null alleles prior to estimating weak levels of divergence expected among samples, as several pairwise tests between samples were no longer significant after correction. The variable numbers of individuals successfully genotyped in this study (53–99) may also have contributed to some loss of power in discriminating among samples, although that effect appears to be minimal. For example, the Jpn98 sample averaged 77 genotyped individuals across all loci, the third lowest mean in the study, but yielded the highest number of significant pairwise exact tests of differentiation or estimates of population structuring. Ruzzante (1998) showed that samples of 50 or greater are sufficient to produce relatively precise estimates of F_{ST} with highly variable microsatellites and simulation results (Kalinowski 2002b) indicate that the precision of estimates of θ for large populations (N = 5000) is not affected adversely by sample sizes in the range of 60–100 individuals. Hence, the variation in numbers of individuals genotyped per locus is unlikely to have biased our results, although the potential effects of sample size could be greater in marine species with higher levels of locus polymorphism.

We did not find significant heterogeneity among samples collected within the Gulf of Alaska or between these and samples from the Unimak area. Similarly, Olsen *et al*. (2002) also did not detect genetic structure within or between these two regions using three microsatellite loci (*Tch10*, *Tch12* and *Tch22*) that were also used in this study. However, they did report significant differentiation between pollock from the Unimak area in the Bering Sea and two locations (Shelikof Strait and Prince William Sound) in the Gulf of Alaska based upon allozyme data. They also found genetic heterogeneity within the Gulf of Alaska (between Shelikof Strait and Prince William Sound) with both allozyme and mtDNA. Similar discordance between allozyme and microsatellite-based estimates of F_{ST} observed in other marine fishes has been attributed, at least in part, to the effects of selection acting on allozyme loci (Lemaire *et al*. 2000; De Innocentiis *et al*. 2001). The reasons for somewhat discordant estimates of genetic structure in walleye pollock among different marker classes are unclear. The most informative allozyme locus in their study, *SOD-2**, may be influenced by selection (Olsen *et al*. 2002) but this would not explain significant differentiation among Gulf of

Alaska populations found at the *MPI** allozyme locus and from mtDNA RFLP data.

The temporal heterogeneity between years in Unimak, Shelikof Strait, and Prince William Sound samples detected using AMOVA was very weak but significant (Table 7). Comparisons between sampling years using Fisher's exact test showed that only a single locus in this study produced a significant test statistic supporting temporal differentiation, and the informative locus was different at all three locations. Olsen *et al*. (2002) also reported weak temporal heterogeneity in allozyme (but not mtDNA) variation in the same Prince William Sound samples we analysed but the evidence from both studies was equivocal. Temporal variation has been detected in the related European hake, *Merluccius merluccius* (Lundy *et al*. 2000) and may arise from life history characteristics common to many marine invertebrates and fishes. Pollock spawn at predictable times but the extent of natal or spawning site fidelity is unknown (Bailey *et al*. 1999) and movement of adults or juveniles could contribute to temporal instability. Gene flow may also occur through planktonic drift of eggs and larvae, potentially resulting in source and sink populations mediated by prevailing current patterns (Bailey *et al*. 1997, 1999). Finally, pollock are highly fecund and spawn in dynamic and patchy oceanic environments where recruitment may be strongly affected by variable reproductive success among a limited number of spawners (Hedgecock 1994). We could not evaluate the importance of variation in reproductive success in contributing to the results because the fish analysed in this study were not aged, but our observations of temporal heterogeneity within spawning sites are consistent with recent reports of very low ratios (10^{-3}–10^{-5}) of genetic effective size to census size (Hauser *et al*. 2002; Turner *et al*. 2002) and the highly variable recruitment dynamics of pollock may contribute to significant temporal fluctuations in gene frequencies.

The weak isolation-by-distance pattern exhibited by walleye pollock is consistent with a stepping-stone mode of gene flow occurring along continental margins and contemporary levels of genetic structuring appear to have arisen in recent evolutionary time. Most of the sampling areas were thought to have been colonized following Pleistocene glaciation 15 000–18 000 years BP, when ice cover reached the outer edges of the continental shelf in the Gulf of Alaska (Mann & Peteet 1994) and sea levels were reduced by 85–100 m in current spawning areas in the Bering Sea and northern Japan (CLIMAP 1976). Census estimates of populations in this study (excluding Prince William Sound and Puget Sound) range from hundreds of millions to billions of individuals (e.g. $\sim 1.7 \times 10^9$ in the eastern Bering Sea from 1979 to 2001, Ianelli *et al*. 2002). The Puget Sound population probably arrived *c*. 6000–7000 years ago based upon scale deposition patterns in sediments from nearby Saanich Inlet (Tunnicliff *et al*. 2001) and, until recently, exceeded seven million individuals (Gustafson *et al*. 2000). Given these large census estimates, the amount of time since colonization may have been insufficient for a significant approach towards migration-drift equilibrium to occur and may explain, in part, the low magnitude of genetic structure revealed by microsatellites. Nonequilibrium conditions are also indicated from an analysis of several previous mtDNA studies (W. S. Grant, personal communication) that shows a star phylogeny characteristic of young populations that had experienced a bottleneck followed by more recent expansion (Ingman *et al*. 2000). In large populations of recent origin, detectable levels of differentiation at neutral loci may be realized only at the largest geographical scales.

The effect of locus polymorphism on the detection of differentiation

We found a significant negative correlation between estimates of F_{ST} and two measures of locus polymorphism, allelic richness and expected heterozygosity (Figs 3 and 4). The inverse relationship between F_{ST} and locus polymorphism occurred even though the largest single-locus estimates of F_{ST}, obtained with the least polymorphic loci, were only ~ 1/3 of the maximum F_{ST} (0.034) that could theoretically be reached by the most polymorphic loci used in this study for a set of six populations. This result, and the fact that the numbers of significant test results in pairwise tests of population differences also tended to decrease with allelic richness (Fig. 5) suggests that it was the particular mutational properties of the microsatellites, and not merely the effect of locus polymorphism *per se*, that led to reduced detection of genetic differences among populations.

Our results thus provide empirical evidence that the detection of low levels of neutral genetic divergence in large populations may be limited by the mutational characteristics of highly polymorphic microsatellites. This observation is qualitatively similar to those reported in recent studies, where less polymorphic allozymes (Lemaire *et al*. 2000; De Innocentiis *et al*. 2001; Freville *et al*. 2001; Olsen *et al*. 2004) and moderately polymorphic microsatellites (mean sample heterozygosity, H_S, = 0.33–0.57; Olsen *et al*. 2004) provided substantially greater estimates of F_{ST} than highly polymorphic microsatellites.

The inverse relationship between locus polymorphism and F_{ST} appears to be a consequence of size homoplasy resulting from the stepwise mutational mode of microsatellites. If there are constraints on allelic size variation (Garza *et al*. 1995) an increase in mutation rate, μ, is expected to bring an increase in the number of homoplasious alleles and to reduce estimates of genetic differentiation between individuals from different populations more strongly than differences between individuals in the same population, thus making different populations appear

more similar (Hellberg *et al*. 2002). Simulation studies (Estoup *et al*. 2002; Kalinowski 2002b) have shown that very high mutation rates (e.g. > 10^{-3}) characteristic of many microsatellite loci are likely to produce a significant downward bias in estimates of F_{ST}. However, high mutation rates can also result in greater locus polymorphism thereby offsetting, to some degree, the negative effects of homoplasy in detecting shallow genetic divergence (Estoup *et al*. 2002). Our results appeared to show the combined effects of increased amounts of homoplasy and polymorphism on estimates of differentiation with increasing μ. Coefficients of variation for estimates of θ decreased with allelic richness, an empirical result consistent with simulations using increasing numbers of independent alleles (Kalinowski 2002a). Thus, the precision of the estimates appeared to increase due to greater polymorphism while homoplasy may be responsible for the decline in their magnitude.

The effects of homoplasy on reducing estimates of population differentiation are most pronounced when effective population sizes are large, mutation rates are high and there are strong constraints on allelic ranges (Estoup *et al*. 2002). The first two conditions are likely to be common in large marine fish populations for loci exhibiting high heterozygosities. The potential effect of constraining allele sizes is less clear. In this study, two loci had 40 or more alleles. Our data suggest that even the highest levels of polymorphism reported for marine fishes (up to ~60 alleles) may constitute sufficient allelic constraint when presumed effective population sizes are large, μ is likely to be high, and population structuring is weak. Such cases may warrant increased sample sizes and the use of microsatellites with moderate levels of polymorphism if size homoplasy is suspected.

The complex processes generating homoplasious and nonhomoplasious variation in microsatellites may deviate substantially from the mutational models assumed when using conventional indices of population subdivision. Theoretical (Rousset 1996) and simulation (Estoup *et al*. 2002; Kalinowski 2002b) studies indicate that the choice of mutational model has less effect upon estimates of differentiation than the mutation rate, although these effects may become more problematic in weakly structured populations. Slatkin (1995) introduced the F_{ST} analogue statistic, R_{ST}, to account for the underlying stepwise mutational model (SMM) of evolution in microsatellites. Under this model, the expected value of R_{ST} is independent of the mutation rate and always greater than the expected value of F_{ST}. Pairwise $\hat{\rho}_{ST}$ estimates in this study averaged 52% higher than $\hat{\theta}$ (Table 5), suggesting that some fraction of the genetic 'signal' lost through homoplasy for the frequency-based estimator $\hat{\theta}$ had been captured by $\hat{\rho}_{ST}$ when comparing allelic size distributions. The greater overall magnitude of $\hat{\rho}_{ST}$ estimates compared to $\hat{\theta}$ did not result in a greater number of significant comparisons between sample pairs, perhaps because of the high variance associated with estimating the latter parameter (Slatkin 1995; Balloux *et al*. 2000) and the low overall level of genetic differentiation among the samples.

Discrepancies between $\hat{\rho}_{ST}$ and $\hat{\theta}$ values also suggest that mutation has had a more significant effect than migration in determining population structure over long distances. Balloux & Goudet's (2002) simulation study using a finite island model of gene flow showed that the expected values of these two estimators became more similar as migration rates increased. The isolation-by-distance pattern in pollock appears to indicate that gene flow occurs most probably in a stepping-stone pattern between adjacent population components, and thus the relative strength of migration will vary in response to distance. Levels of population subdivision estimated by $\hat{\rho}_{ST}$ and $\hat{\theta}$ are generally similar and not significant at small to moderate geographical distances (Table 5) where the migration rate (m) is likely to be greater relative to μ. The significant pairwise $\hat{\rho}_{ST}$ values found between the Jpn98 sample and the most distant locations in the eastern Bering Sea and northeastern Pacific are uniformly higher than corresponding estimates of θ, suggesting the relatively greater influence of μ on the degree of population differentiation at large geographical scales.

In summary, these empirical results corroborate most theoretical and simulation studies that indicate reduced likelihood of highly polymorphic microsatellites, relative to less variable loci, to detect population differences in weakly structured marine species. Furthermore, we suggest that this difference is due to homoplasy, and not locus polymorphism *per se*. The high levels of polymorphism shown by microsatellite loci make them a promising marker class for detecting low levels of population structuring in marine fishes yet also imply limitations on their resolving power and warrant caution in their use to infer other population parameters such as migration. Single-locus comparisons across a range of locus heterozygosities using additional marker classes (e.g. Freville *et al*. 2001), binning of alleles to reduce allelic variation (e.g. Buonaccorsi *et al*. 2001; Olsen *et al*. 2004) and the use of alternative estimators of F_{ST} should be used to assess the degree of mutation bias introduced by microsatellites.

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