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[Article]

Temporal and Spatial Genetic Structure among Some Pacific Herring Populations in Puget Sound and the Southern Strait of Georgia

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Abstract.—We investigated temporal and spatial genetic variation in Pacific herring *Clupea pallasii* collections from six sites in Puget Sound (PS) and the southern Strait of Georgia (SOG), using 12 microsatellite loci. Loci were highly variable with up to 70 alleles per locus (mean = 30.67 alleles), and observed heterozygosity was high (mean = 0.823). Analysis of molecular variance (AMOVA) indicated significant structure, with over twice as much variance among sites as among collection years, although among-site variance was mainly due to Cherry Point and Squaxin Pass collections. In an AMOVA excluding Cherry Point and Squaxin Pass, only temporal variance was significant. With the exception of the Cherry Point and Squaxin Pass collections, pairwise genotypic and F_{ST} tests show some differences among collection years within a site and some genetic overlap among most PS and SOG collections. The Cherry Point and Squaxin Pass collections had no differences in genotypic distributions among collection years, and in cluster analyses the Cherry Point and Squaxin Pass collections each formed groups separate from other PS and SOG collections. Cherry Point herring have a later spawning time than other PS and SOG herring, and Squaxin Pass is physically isolated in southern Puget Sound. We hypothesize that spawn timing differences and spatial isolation generated genetic structure among some Pacific herring in PS and SOG. We suspect that, as in the case of Atlantic herring *C. harengus*, population genetic structure in Pacific herring in PS and southern SOG is a combination of a larval retention model and a metapopulation model. Because Cherry Point and Squaxin Pass herring are genetically and behaviorally differentiated from other PS and SOG herring populations, this unique variation should be preserved through careful management.

Identifying populations in marine fish species is an important challenge to biologists. In ecologically important species that are commercially exploited, understanding genetic structure provides a foundation for effective conservation and management of genetic diversity. However, genetic differentiation is often low in marine species capable of long-distance dispersal because migration and gene flow among populations is often high (Shaklee and Bentzen 1998; Waples 1998). Yet, isolation by distance or geographic features may prohibit free mixing among some populations, promoting differentiation through genetic drift and accumulated mutations. Highly polymorphic markers with high mutation rates, such as microsatellite DNA, have proven useful for identifying genetically divergent populations (Waples 1998; Hedrick 1999). However, in marine species such as herring, with population sizes numbering in the millions, genetic drift may be slow to nonexistent and, although mutation is important for introducing new alleles, mutational constraints on microsatellite DNA evolution cause size homoplasy (Nauta and Wiessing 1996), thus decreasing measured genetic divergence (Estoup et al. 2002). Further, genetic structure in marine species utilizing coastal areas for part of their life cycle, such as spawning herring, has been episodically perturbed during glacial epochs, when glaciers at high latitudes and lowered sea-level worldwide altered coastal habitat. Despite factors countering population genetic divergence in marine species, differentiation has been documented in several marine species (Shaklee and Bentzen 1998). Concerns have arisen recently about identifying population genetic structure in Pacific herring *Clupea pallasii*, because some herring populations in the Pacific Northwest, specifically Cherry Point herring, have declined to the point that they are being considered for listing under the Endangered Species Act (NOAA 2004).

Pacific herring are small marine fish with a

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broad distribution around the Pacific Rim, from California to Japan. Herring are critical components in oceanic and nearshore food chains: their large schools compose up to 70% of the prey base for other marine fish, birds, and mammals (Hart 1973). Starting near the end of January to as late as June in northern areas, herring aggregate in schools of millions of fish near inshore spawning habitat in preparation to spawn a few weeks later (Hay 1985). Males broadcast milt while females attach adhesive eggs to eelgrass, algae, and solid substrate in sheltered lower intertidal and upper subtidal shoreline areas (Haegele and Schweigert 1985). Spawning sites and associated spawning aggregations have patchy distributions, some spawning areas being separated by unsuitable habitat or by habitat consistently ignored by herring. Eggs hatch within a few weeks and after spending 2 to 3 months drifting in the water column, the pelagic larvae metamorphose into juveniles (Hart 1973). Movements between the juvenile and spawning adult stages are not well known. After metamorphosis, juveniles form schools and generally move inshore for the first summer, after which some move offshore or to estuaries (Hay 1985). Major populations appear to migrate to offshore feeding areas, whereas minor, local populations appear to remain in inland waters (Stout et al. 2001). Tagging studies suggest that some Puget Sound (PS) herring move to feeding grounds off the west coast of Vancouver Island (Stout et al. 2001). Herring in PS mature sexually at 2 to 4 years and return annually to their natal spawning area for up to 10 years (Bargmann 1998). According to tagging studies in British Columbia (BC), herring return to spawning sites with levels of fidelity varying from 64% to 96% (Ware et al. 2000; Hay et al. 2001, Ware and Schweigert 2001, 2002). Fidelity (and straying) appears to be influenced by the biomass of the spawning stock—the larger the stock, the higher the exportation of individuals (Ware and Schweigert 2001). The genetic structure of herring in BC reflects this straying rate in a high connectivity among BC herring spawning sites (Beacham et al. 2002). In many comparisons, temporal variation exceeded spatial variation (Beacham et al. 2002; McPherson et al. 2004), supporting a metapopulation structure (McQuinn 1997). Genetic differentiation has been detected on a large geographic scale in Alaskan herring, using allozymes (Grant and Utter 1984), microsatellite DNA (O'Connell et al. 1998b), and mitochondrial DNA (Bentzen et al. 1998). Atlantic herring *C. harengus* have also been differentiated over large scales (Shaw et al. 1999; Hauser et al. 2001; McPherson et al. 2001). Pacific and Atlantic herring with different spawn timings or spatial isolation have been genetically differentiated over small geographic scales (O'Connell et al. 1998b; Beacham et al. 2002; McPherson et al. 2004).

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Herring genetic structure within PS and the southern region of the Strait of Georgia (SOG) has not been examined closely. Eighteen herring populations have been identified in PS and in the portion of the SOG within Washington State (WA; Bargmann 1998). Populations were identified by spawning location, prespawning behavior, age structure, and spawn timing. Except for Cherry Point herring, which spawn in April and May, most WA herring spawn in February and March (Bargman 1998). Although tagging data suggest that herring mingle and move throughout PS (Markiewicz et al. 2001), differences in spawn timing and spatial isolation could act as reproductive barriers that promote genetic differentiation in some populations. In this study, we use microsatellite DNA to examine temporal and regional population structure in herring over a small spatial scale in PS and SOG. We focus in particular on Cherry Point herring, which have a late spawning time, and Squaxin Pass herring, which spawn in an isolated region in south PS. We document a degree of genetic differentiation that is remarkable, given the large population sizes, hypothesized stray rates, and the recent postglacial founding of populations (12,000 years before present; Stout et al. 2001). We hypothesize that temporal and spatial isolation have served as reproductive barriers, creating genetic structure within Pacific herring in PS and SOG.

Methods

Samples.—Midwater rope trawls were employed to collect samples of sexually mature herring from prespawning holding areas near spawning grounds at three sites in PS (Fidalgo Bay, Port Gamble, and Squaxin Pass; Table 1; Figure 1), three sites in southern SOG (Northumberland Passage, BC; Semiahmoo Bay; and Cherry Point; Table 1, Figure 1), and from San Francisco Bay in California (Table 1). Cherry Point was sampled in 1999, 2000, 2002, and 2003. Port Gamble, Squaxin Pass, and Semiahmoo Bay were sampled in 1999 and 2002; other sites were sampled a single year (Table 1). Because all samples were sexually mature, spawning was imminent but exact spawning dates were unknown. DNA was extracted with Chelex (Small et al. 1998).

TABLE 1.—Summary information for Puget Sound and southern Strait of Georgia herring collections, including the sample size per collection (N) and observed and expected heterozygosity (H_{obs} and H_{exp}) over all loci. Hardy–Weinberg equilibrium (HWE) P-values indicate whether collections deviated from Hardy–Weinberg expectations with an excess of homozygotes; values significant before correction are underlined and those significant after correction are in bold italics. Allelic richness is the number of alleles per collection corrected for sample size.

Location	Abbreviation	Date	N	H_{obs}	H_{exp}	HWE P	Allelic richness
Northumberland, British Columbia	99North	25 Feb 1999	94	0.8204	0.8325	0.0276	12.018
Cherry Point, Washington	99ChP	28 Apr 1999	95	0.8211	0.8325	0.0456	12.165
	00ChP	02 May 2000	94	0.8054	0.8266	0.3102	12.012
	02ChP	30 Apr 2002	92	0.8150	0.8329	0.1385	12.556
	03ChP	29 Apr 2003	96	0.8212	0.8252	0.4493	11.618
Semiahmoo Bay, Washington	99Semi	17 Feb 1999	96	0.8204	0.8320	0.2911	12.077
	02Semi	20 Feb 2002	96	0.8189	0.8328	0.3963	12.113
Fidalgo Bay, Washington	99Fidal	18 Feb 1999	91	0.8202	0.8326	0.0300	11.728
Port Gamble, Washington	99PtG	09 Feb 1999	96	0.8190	0.8313	***0.0013***	12.222
	02PtG	05 Feb 2002	62	0.8230	0.8279	0.8004	10.604
Squaxin Pass, Washington	99SqP	01 Jan 1999	96	0.8188	0.8301	0.0727	12.485
	02SqP	16 Jan 2002	96	0.8193	0.8303	0.0339	12.000
San Francisco Bay, California	98SFBay	[a]	61	0.8199	0.8301	0.1673	11.729
Total			1,165				11.948

[a] Not available.

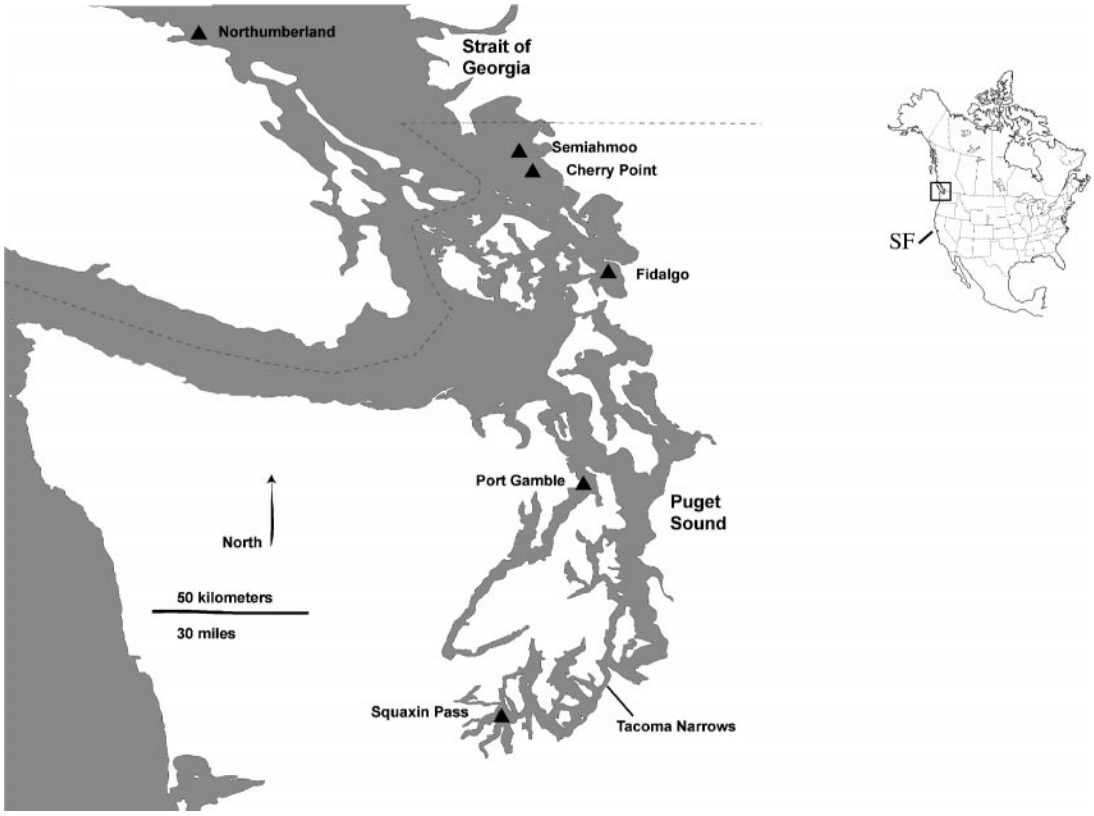

FIGURE 1.—Map of collection sites for Pacific herring in Puget Sound and the Strait of Georgia. San Francisco Bay (SF) is indicated on the map of North America.

TABLE 2.—Multiplexes, loci, and cycling parameters, including annealing temperature (temp. [°C]), number of PCR cycles, dye type, concentration of $MgCl_2$, number of alleles in this study, size range (base pairs), observed heterozygosity (H_{obs}), repeat unit size (base pairs), and *P*-value for deviation from Hardy–Weinberg equilibrium (HWE). Values out of equilibrium are underlined and values out of equilibrium after Bonferroni corrections are in bold italics. Publications with primer sequences are referenced under ''Source''.

Multiplex	Temp.	Cycles	Dye	$MgCl_2$ (μM)	Locus	No. of alleles	Size range	H_{obs}	Repeat	HWE *P*-value	Source
Cpa-A	52	34	Hex	0.3	*Cpa-6*	22	158–258	0.724	4	0.9353	Miller et al. (2001)
			6fam	0.04	*Cpa-103*	23	175–263	0.86	4	0.0452	Olsen et al. (2002)
			Ned	0.2	*Cpa-27*	19	97–209	0.876	4	0.0567	Miller et al. (2001)
Cpa-B	52	36	6fam	0.07	*Cpa-107*	30	108–168	0.872	2	0.5993	Miller et al. (2001)
			Hex	0.07	*Cha-113*	26	77–133	0.875	2	0.667	O'Connell et al. (1998a)
			Ned	0.045	*Cpa-107a*	27	204–308	0.82	4	0.428	Olsen et al. (2002)
Cpa-C	52	32	6fam	0.4	*Cpa-8*	34	87–259	0.852	4	***0***	Miller et al. (2001)
			Ned	0.07	*Cpa-134*	46	116–222	0.718	2	0.0036	Miller et al. (2001)
Cpa-D	58	34	6fam	0.2	*Cpa-106*	21	136–232	0.597	4	0.1511	Olsen et al. (2002)
			Ned	0.25	*Cpa-114*	26	192–292	0.896	4	0.5591	Olsen et al. (2002)
Cpa-E	58	31	Hex	0.1	*Cpa-104*	70	182–490	0.858	4	***0***	Olsen et al. (2002)
			Ned	0.1	*Cpa-111*	23	228–316	0.929	4	0.2364	Olsen et al. (2002)

Microsatellite survey.—Genotypes for 1,165 herring from PS, SOG, and San Francisco Bay (Table 1; Figure 1) were assessed at 12 microsatellite DNA loci (Table 2). Laboratory conditions and primer sequences for loci are outlined in Table 2. Polymerase chain reactions (PCRs) were conducted on a MJResearch PTC-200 thermocycler in 10-μL volumes with 1 μL of template at final concentrations of 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.05 units of *Taq* polymerase, and 1× Promega PCR buffer. Samples were run on ABI 377 and 3730 automated sequencers. We standardized allele mobilities on the two platforms by running a subsample on both platforms. Alleles were binned by using GENEMAPPER software (Applied Biosystems).

Variation within samples.—Collections were tested for deviations from Hardy–Weinberg equilibrium (HWE) at each locus and over all loci by using GENEPOP 3.3 (Raymond and Rousset 1995) with 1,000 dememorizations, 100 batches, and 2,000 iterations. F_{IS} values (relationship of alleles within individuals, an indicator of nonrandom mating) were calculated for each locus within each population by using FSTAT2.9.3 (Goudet 2001). MICRO-CHECKER (Van Oosterhout et al. 2004) was employed to test loci for null alleles, large-allele drop out, and scoring errors. Genotypic disequilibrium was tested by using GENEPOP 3.3 with 2,000 dememorizations, 400 batches, and 4,000 iterations, to assess whether loci were inherited independently and to see if collections included more than one spawning group. Allelic richness was estimated by using rarefaction implemented in HP-Rare (Kalinowski 2005a). Observed and expected heterozygosity for each collection was calculated by using Microsatellite Analyzer (Dieringer and Schlötterer 2003). Results in all analyses were adjusted for multiple simultaneous tests to an overall alpha value of 0.05 by using Bonferroni corrections (Rice 1989).

Variation among samples.—Genetic structure was examined with several tests. FSTAT2.9.3 (Goudet 2001) was used to calculate θ (θ, an estimate of F_{ST}, from Weir and Cockerham 1984, hereafter referred to as F_{ST}), and the 95% confidence interval surrounding the value. The San Francisco Bay collection was removed before calculating F_{ST} so that only structure within PS and SOG was estimated. Partitioning of molecular variance within PS and SOG was examined with a locus-by-locus analysis of molecular variance (AMOVA; Excoffier et al. 1992) and using ARLEQUIN 2.001 (Schneider et al. 2000) with 10,000 randomizations. The PS and SOG collections from 1999 and 2002 (Cherry Point, Semiahmoo Bay, Port Gamble, and Squaxin Pass) were grouped by location to examine how much molecular variance was partitioned among locations and how much variance was partitioned among collection years within locations. Because earlier tests had indicated that Cherry Point and Squaxin Pass collections were significantly different from other collections, we conducted the AMOVA with only Semiahmoo and Port Gamble collections to look at temporal and spatial differences in the absence of Cherry Point and Squaxin Pass collections. Because O'Reilly et al. (2004) showed a negative correlation between F_{ST} values and locus polymorphism in marine fish, we tested locus F_{ST} values for dependence on the number of alleles per

TABLE 3.—Pairwise tests of genotypic differentiation among Pacific herring collections from Puget Sound and the Strait of Georgia over all loci (below diagonal) and number of significant pairwise genotypic tests between collections (above diagonal). The upper portion of the matrix shows the number of loci with significantly different genotypic distributions before Bonferroni correction and after correction (in parentheses). *P*-values calculated over all loci are below the diagonal, and significant values are in bold italics. The entry <0.0001 in the lower portion of the matrix indicates an undefined but significant *P*-value. The chi square value for tests over all loci was infinity, and the *P*-value was highly significant. Abbreviations are given in Table 1.

Location	99North	99ChPt	00ChPt	02ChPt	03ChPt	99Semi
99North		4 (2)	4 (3)	4 (2)	3 (2)	2 (0)
99ChPt	***<0.0001***		0 (0)	0 (0)	1 (0)	3 (0)
00ChPt	***<0.0001***	0.7454		0 (0)	0 (0)	5 (1)
02ChPt	***<0.0001***	0.5681	0.3147		0 (0)	7 (1)
03ChPt	***<0.0001***	0.3786	0.5825	0.5113		7 (1)
99Semi	0.0671	0.0004	***<0.0001***	***<0.0001***	***<0.0001***	
02Semi	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***0.0002***	***<0.0001***
99Fidal	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
99PtG	0.1000	***<0.0001***	***<0.0001***	***0.0001***	***<0.0001***	0.2073
02PtG	0.0884	0.0010	***<0.0001***	***0.0005***	***<0.0001***	0.1337
99SqP	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
02SqP	***<0.0001***	***<0.0001***	***<0.0001***	***0.0001***	***<0.0001***	***<0.0001***
98SFBay	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***

locus in a regression analysis using FSTAT2.9.3 (Goudet 2001).

Relationships among collections were explored with pairwise tests. Collections were tested for differences in genotypic distributions at each locus and over all loci by using genotypic randomization tests implemented in GENEPOP 3.3 with 1,000 dememorizations, 200 batches, and 2,000 iterations. To explore the magnitude of differentiation, we tested collections in pairwise tests for deviations from expected heterozygosity, using pairwise F_{ST} statistics in FSTAT2.9.3 (Goudet 2001), with 78,000 permutations.

Genetic distances (Cavalli-Sforza and Edwards [1967] chord distance; CSE) among collections were calculated by using PHYLIP3 (Felsenstein 1993). To examine distances in a nonhierarchical analysis, we plotted distances among collections in a multidimensional scaling analysis, using NTSYS-pc (Rohlf 1993). CSE distances were also employed in a consensus neighbor-joining tree by using PHYLIP3.5c. To estimate the reliability of branching in the neighbor-joining tree, we bootstrapped the data 10,000 times and generated a consensus tree.

Results

Genetic Variation within Collections and HWE

Loci varied widely in the number of alleles (19–70, mean = 30.1; Table 2) and observed heterozygosities (0.6–0.93) across collections (Table 2). Deviations from HWE for homozygote excess were detected in 15 out of 156 tests of loci within collections (data not shown), but none of these deviations remained significant after Bonferroni corrections. MICRO-CHECKER suggested a possible null allele at *Cpa-8*, and because *Cpa-8* was out of HWE in most collections before corrections and out of HWE over all collections (Table 2), we removed this locus from the rest of the analyses to satisfy assumptions (e.g., HWE) of other tests. When calculated over all loci (except *Cpa-8*), four collections deviated from HWE with homozygote excesses, but only the deviation in 1999 Port Gamble remained significant after corrections (Table 1). When calculated over all loci, only 1999 Port Gamble had significant F_{IS} value after corrections (Table 1). In 1999 Port Gamble, *Cpa-134* and *Cpa-27* were out of HWE before corrections, which probably explains the HW disequilibrium in 1999 Port Gamble over all loci. In linkage disequilibrium tests within collections, 86/858 tests were significant before corrections, and 8 tests were significant after corrections (adjusted $P = 0.0000583$ [0.05/858]), but none of the same locus pairs was linked in different collections. Three of the eight significant tests were in the Fidalgo Bay collection and two were in the San Francisco Bay collection, suggesting a possible mixture in these collections, such as sampling multiple spawning waves (McPherson et al. 2003). The 2000 Cherry Point, 1999 Semiahmoo, and 1999 Northumberland samples each had one significant test. Because different loci were linked in different populations, loci appeared to be inherited independently.

Genetic Variation among Collections

The overall F_{ST} value from FSTAT (0.003, excluding San Francisco Bay collection) was signif-

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TABLE 3.—Extended.

Location	02Semi	99Fidal	99PtG	02PtG	99SqP	02SqP	98SFBay
99North	3 (2)	3 (1)	1 (0)	2 (0)	4 (1)	2 (1)	5 (3)
99ChPt	3 (1)	4 (1)	3 (0)	3 (0)	2 (1)	5 (3)	6 (4)
00ChPt	5 (1)	3 (1)	3 (2)	3 (1)	4 (1)	4 (1)	5 (2)
02ChPt	3 (1)	2 (1)	3 (2)	3 (1)	4 (1)	3 (1)	6 (4)
03ChPt	2 (0)	4 (1)	5 (1)	3 (1)	4 (2)	2 (1)	6 (3)
99Semi	3 (1)	4 (1)	1 (0)	1 (0)	4 (2)	5 (2)	8 (3)
02Semi		3 (0)	2 (1)	3 (1)	5 (1)	3 (1)	6 (2)
99Fidal	0.0066		4 (1)	3 (1)	5 (1)	5 (0)	7 (3)
99PtG	***<0.0001***	***<0.0001***		3 (0)	2 (1)	2 (1)	4 (2)
02PtG	***<0.0001***	***<0.0001***	0.0280		3 (2)	4 (1)	4 (3)
99SqP	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***		0 (0)	7 (2)
02SqP	***<0.0001***	***0.0001***	***<0.0001***	***<0.0001***	0.2321		5 (2)
98SFBay	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	

icantly different from zero. The 95% confidence interval spanned 0.001–0.006 when bootstapped over all loci, indicating low but significant genetic structure among PS and SOG herring collections.

Most pairwise tests indicated significant differences in genotype distributions among collections from different sites and overlap between collections from different years at the same sites: Cherry Point, Squaxin Pass, Port Gamble (Table 3, lower matrix). After Bonferroni correction, there were no significant pairwise within-locus comparisons between Cherry Point samples in 66 tests and at least one significant test in pairwise comparisons with other PS and SOG collections (Table 3, upper matrix). In simultaneous tests of no heterogeneity between sample pairs over all loci (Table 3, lower matrix), the null hypothesis was rejected in 66 (uncorrected) and 63 (corrected) of 78 possible tests. Significant overlap in genotypic distributions (summed over all loci) was found among at least one year-class from all PS and SOG collections except Semiahmoo Bay (Table 3, lower matrix).

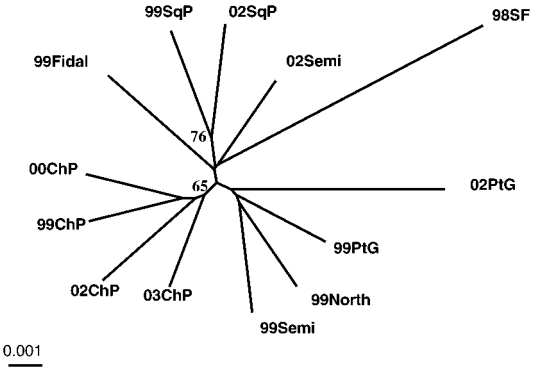

FIGURE 2.—Consensus neighbor-joining tree of Cavalli-Sforza and Edwards's genetic chord distances (1967) among Pacific herring collections from Puget Sound and the southern Strait of Georgia. Numbers at the nodes (only values >60% are shown) indicate the percentage of 10,000 trees in which collections beyond the nodes grouped together. Abbreviations are given in Table 1.

Pairwise, multilocus estimates of F_{ST} indicated population structuring over large and small geographic scales (Table 4). All comparisons between San Francisco Bay and the PS and SOG collections were significant, as were several comparisons between PS and SOG collections (Table 4). Results were generally concordant with pairwise tests of genotypic heterogeneity: Cherry Point and Squaxin Pass collections were indistinguishable from other collection years from their own site and different from other PS and SOG collections. In comparisons of other collections with Cherry Point, 31/32 and 19/32 tests were significant before and after corrections, respectively (versus 31/32 tests significant after corrections in genotypic tests). In comparisons of other collections with Squaxin Pass, 22/22 and 17/22 tests were significant before and after corrections, respectively (versus 22/22 tests significant after corrections in genotypic tests). The 2002 Port Gamble collection, the smallest sample, was the least distinguished from other collections: Only comparisons with Squaxin Pass and San Francisco Bay were significant after corrections (Table 4). Northumberland, Fidalgo Bay,

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TABLE 4.—The lower portion of the matrix contains pairwise F_{ST} values for Puget Sound and Strait of Georgia herring collections. Values significant before Bonferroni corrections are underlined and values significant after corrections are indicated with asterisks ($P < 0.05$*; $P < 0.001$**). The upper portion of the matrix contains pairwise Cavalli-Sforza and Edwards distances (1967). Abbreviations are given Table 1.

Location	99North	99ChP	00ChP	02ChP	03ChP	99Semi
99North		0.0079	0.008	0.0083	0.0078	0.0063
99ChP	0.0047**		0.0058	0.0068	0.0062	0.0078
00ChP	0.0037**	−0.0005		0.0071	0.0065	0.0082
02ChP	0.0045	−0.0009	−0.0001		0.007	0.009
03ChP	0.0045*	0.0008	−0.0008	0.0011		0.0079
99Semi	0.0006	0.0017	0.0038	0.0037	0.0038	
02Semi	0.0042**	0.0046**	0.0036*	0.0019	0.0031	0.0029
99Fid	0.0062*	0.0066*	0.006*	0.006*	0.0051	0.0056
99PtG	0.0003	0.0022**	0.0044**	0.0024*	0.0044**	−0.0002
02PtG	−0.0005	0.0056	0.0036	0.0054	0.0037	0.0012
99SqP	0.0045**	0.003	0.0043**	0.0045*	0.0029*	0.0035**
02SqP	0.006**	0.0044**	0.0034**	0.0022	0.002*	0.0055**
98SF	0.0163**	0.0161**	0.0151**	0.0131**	0.0126**	0.016**

Semiahmoo Bay, and 1999 Port Gamble displayed more genetic overlap in pairwise F_{ST} tests than in pairwise genotypic tests (Tables 3 and 4).

The AMOVA analysis also indicated low but significant genetic structure within PS and SOG collections (Table 5). The variance among sites was an order of magnitude greater than variance among collection years within sites (in Table 5), supporting a hypothesis that some herring populations in PS and southern SOG are partitioned into breeding groups according to spawning site. However, without Cherry Point and Squaxin Pass in the AMOVA, temporal variance exceeded spatial variance (Table 5), suggesting high genetic connectivity among other PS and southern SOG populations. When locus F_{ST} values from the AMOVA analysis (Table 4) were regressed on the number of alleles at the locus (Table 2), locus variability appeared to be uncorrelated with population subdivision within this relatively small geographic region ($r^2 = 2.72$).

The multidimensional scaling plot (not shown) and consensus neighbor-joining dendrogram (Figure 2) of CSE distances (Table 4, upper matrix) indicated groups based on location. In the multidimensional scaling plot, distances involving San Francisco Bay were so large that the other collections were compressed together; subsequently, genetic distances were plotted without San Francisco Bay (not shown). Although genetically indistinct, 2002 Port Gamble separated from PS collections along the first and second axes, suggesting that the small sample size poorly characterized the population. Cherry Point collections formed a group separated from PS and SOG collections and in the dendrogram (Figure 2) formed a branch with 65% bootstrap support (69% without San Francisco Bay in analysis). Squaxin Pass collections grouped in the far corner of the first and second axes separate from other PS collections; in the dendrogram (Figure 2), Squaxin Pass collections formed a branch with 76% bootstrap support (93% without San Francisco Bay in analysis). These groupings indicate that temporal and spatial isolation generated genetic divergence. Collections from Semiahmoo Bay, Port Gamble, and Northumberland in 1999 grouped together near the center and formed an unsupported branch in the dendrogram, suggesting genetic similarity within collection year for collections with similar spawning times.

Discussion

Our survey has demonstrated temporally stable genetic divergence among some Pacific herring populations over a small spatial scale in PS and the SOG. This divergence is remarkable, given that mutational constraints in microsatellite DNA should increase homoplasy (Nauta and Wiessing 1996; Balloux and Lugon-Moulin 2002; Estoup et al. 2002); large population sizes should minimize genetic drift; and high stray rates (Hay et al. 2001; Ware and Schweigert 2001) should counteract genetic differences accumulating among spawner groups and maintained by natal homing. We hypothesize that temporal and spatial isolation promoted reproductive isolation in the Cherry Point and Squaxin Pass spawner groups, fostering genetic divergence. We also hypothesize that straying promoted genetic cohesion among PS and SOG groups with similar spawn timing. Similar to Atlantic herring (McPherson et al. 2004), population genetic structure in Pacific herring in PS and south-

TABLE 4.—Extended.

Location	02Semi	99Fid	99PtG	02PtG	99SqP	02SqP	98SF
99North	0.008	0.0081	0.0063	0.0098	0.0083	0.0089	0.0135
99ChP	0.0075	0.0087	0.008	0.011	0.0075	0.0092	0.0145
00ChP	0.0076	0.0084	0.0084	0.0104	0.009	0.0083	0.0142
02ChP	0.008	0.0094	0.0082	0.0116	0.0092	0.0085	0.0134
03ChP	0.0069	0.0085	0.0074	0.0098	0.0086	0.0083	0.0124
99Semi	0.0077	0.0082	0.0065	0.0097	0.0088	0.0096	0.0146
02Semi		0.0074	0.0071	0.0106	0.0076	0.0073	0.0119
99Fid	0.0016		0.0088	0.0109	0.0086	0.0085	0.0133
99PtG	0.0021	0.0062**		0.0098	0.008	0.0084	0.0126
02PtG	0.0039	0.0052	0.0019		0.012	0.0115	0.0166
99SqP	0.0042	0.0079	0.0028*	0.0067**		0.0068	0.0136
02SqP	0.0024	0.0063*	0.0043**	0.0078**	0.0003		0.0128
98SF	0.0101**	0.013**	0.0142**	0.0207**	0.0147**	0.0124**	

ern SOG is, we suspect, a combination of a larval retention model (Smedbol and Stephenson 2001) and a metapopulation model (McQuinn 1997).

Comparison with Pacific Herring Structure on the Canadian Coast

The Pacific coast of BC is highly dissected by numerous inlets, bays, and fjords. About 5,000 km of the coast is utilized (Hay et al. 2001) by at least 75 herring spawner groups (Beacham et al. 2002). Homing to natal sites and straying was documented through tagging studies (Hay et al. 2001; Ware and Schweigert 2001; Hay and McKinnell 2002). Along the BC coast, most herring population structure appeared to follow a metapopulation model, with episodic straying (Ware and Schweigert 2001, 2002), which caused high genetic connectivity among spawning groups, often separated by hundreds of kilometers (Beacham et al. 2002). Straying depended on aggregation size, which in turn depended on productivity and climatic conditions: Straying increased with aggregation size, which increased under warmer oceanic conditions (Ware and Schweigert 2001). If spawn timing is under genetic control, straying would most effectively prevent genetic divergence among popula-

TABLE 5.—Analysis of molecular variance (AMOVA) averaged over 11 loci from collections from 1999 and 2002 and locus-by-locus AMOVA results for analysis with four sites.

Comparison	df	Source of variation	Variance	% Variation	Fixation index[a]	*P*-value
By site	1	Among sites	−0.0041	−2.560	−0.0009	<0.853
Semiahmoo and Port Gamble only	2	Among years within sites	0.0120	0.810	0.0026	<0.009
	696	Within collections	4.5990	101.76		
Semiahmoo, Port Gamble, Cherry Point, and Squaxin Pass	3	Among sites	0.0118	0.256	0.0026	<0.001
	4	Among years within sites	0.0050	0.122	0.0011	<0.002
	1,450	Within collections	4.5880	99.622		
Per locus AMOVA						
	1,984	*Cpa-6*	0.35500	98.606	0.0139	0.000
	2,028	*Cpa-103*	0.43756	100.044	−0.0004	0.658
	2,044	*Cpa-27*	0.44832	98.685	0.0132	0.000
	1,986	*Cpa-107*	0.45439	99.974	0.0003	0.456
	2,082	*Cha-113*	0.29697	99.694	0.0031	0.017
	1,990	*Cpa-107a*	0.44797	99.904	0.0009	0.136
	2,032	*Cpa-134*	0.46383	99.677	0.0032	0.000
	2,010	*Cpa-106*	0.44585	99.893	0.0011	0.158
	2,016	*Cpa-114*	0.35617	99.852	0.0015	0.089
	1,934	*Cpa-104*	0.41446	99.701	0.0030	0.010
	1,928	*Cpa-111*	0.45654	99.990	0.0001	0.639

[a] F_{CT} for comparisons among sites, F_{SC} for comparisons among years within sites, and F_{ST} for individual loci.

tions with the same spawn timing. If spawn timing is learned (McQuinn 1997), but herring remain associated with their natal group (Hay and McKinnell, 2002), spawning aggregates could diverge genetically. Most herring along the Canadian coast spawn in winter, with peak spawning in March. However, Beacham et al. (2002) found a few genetically divergent herring populations with earlier or later spawn times or that spawned in geographically isolated sites. In particular, the Cherry Point collection in their study, with peak spawning in May, was the most genetically divergent of the SOG collections.

Both PS and SOG are convoluted inland waterways created as glaciers retreated at the end of the Pleistocene. Except for Cherry Point herring, all spawning groups utilize protected inlets and bays. Cherry Point is exposed and subject to high winter storm activity at the times when other protected locations in PS and SOG are used for spawning. With the cessation of winter storms in April, Cherry Point becomes available and utilized for spawning. Coincident with decreased wave action, spring thaw in the coastal mountains creates runoff in the Fraser River that stimulates a copepod bloom adjacent to Cherry Point, providing a rich food supply for larval herring (Marshall 2002). That is, environmental factors may select for later spawn timing in Cherry Point herring and the copepod bloom may foster larval retention near their natal site (Smedbol and Stephenson 2001). The winter spawn timing of Squaxin Pass herring overlaps with other PS herring spawn timings, but Squaxin Pass is the most physically isolated of the PS populations in the study (Figure 1). In addition to threading their way down to the southern reaches of PS, Squaxin Pass herring must negotiate a glacial sill at the Tacoma Narrows approximately 30 km northeast of the spawning area (Figure 1). Herring from north of the Tacoma Narrows may be unmotivated to explore into the region if aggregation sizes are small (Ware and Schweigert 2001) and if spawning habitat is available at their natal sites. The isolation of Squaxin Pass may be similar to Esquimalt Harbor on the Southern Coast of Vancouver Island (Beacham et al. 2002) or Bras d'Or Lake along Nova Scotia (McPherson et al. 2004), where geographic isolation promoted genetic divergence.

Comparison with Pacific Herring Structure in Alaska

O'Connell et al. (1998b) examined population structure among herring in Alaska over a scale similar to this study and larger ones. Over similar scales, genetic distance depended on spawn timing; over a larger scale, genetic distance was explained by geographic distance. Within Prince William Sound (a geographic scale similar to that of PS), three of four comparisons among late and early spawning groups indicated genetic differences. Over a larger scale, Gulf of Alaska versus Bering Sea, all comparisons were significant, similar to the findings of Grant and Utter (1984). However, when Bentzen et al. (1998) examined population structure in Alaska herring, temporal variation within location equaled or exceeded spatial variation within basins, suggesting temporal variance in spatial patterns.

Comparison with Atlantic Herring

The population structure of Atlantic herring has been investigated over small and large scales with a variety of molecular markers (Shaw et al. 1999; McPherson et al. 2001; Hauser et al. 2001; McPherson et al. 2004). Similar to Pacific herring in Alaska (O'Connell et al. 1998b) and in PS and SOG (this study), fine-scale population structure in Atlantic herring was defined more by spawn timing than geographic proximity. Over large scales, geography dictated genetic structure.

Microsatellite DNA and F_{ST}

The high polymorphism of microsatellite DNA makes it a useful marker for investigating fine-scale population structure (Balloux and Lugon-Moulin 2002). However, microsatellite DNA also challenges the resolving power of genetic tests. The pairwise tests we used address related components of the same question: Are the populations different (G-test and F_{ST} test), and if so, what is the magnitude of the differences (F_{ST} test)? The difference we observed between the pairwise genotypic and pairwise F_{ST} tests underscores the conservative nature of the pairwise F_{ST} test: Departures from heterozygosity are calculated without identifying specific alleles and the upper bounds for F_{ST} values are set by homozygosity (Hedrick 1999). Although Kalinowski (2005b) showed that coefficients of variation for F_{ST} values were lower with high polymorphism, the extreme heterozygosity displayed by some microsatellite DNA loci may limit the utility of F_{ST} estimations (Balloux and Lugon-Moulin 2002; Estoup et al. 2002). Another concern about F_{ST} values and herring: with population sizes generally in the millions, mutation is probably more important than genetic drift, yet mutational constraints on microsatellite DNA

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will cause much homoplasy (Nauta and Wiessing 1996; Estoup et al. 2002), further limiting the resolving power of F_{ST} estimations (Shaw et al. 1999).

In view of these considerations about F_{ST} tests, Goudet et al. (1996), Hedrick (1999), and McPherson et al. (2001) advocated the use of pairwise exact tests of genetic differentiation for microsatellite DNA. However, with the high heterozygosity in microsatellite DNA and power of these pairwise tests, type I errors become more of a concern: Pairwise tests may be statistically significant although differences may not be not biologically significant (Waples 1998; Hedrick 1999; Balloux and Lougon-Moulin 2002). Thus, population biologists seek corroborating evidence for significant genetic differences indicated by genotypic distributions (Waples 1998). At Cherry Point, environmental parameters may require late spawning and thus promote temporal isolation and the genetic divergence detected in pairwise tests. Isotope ratios in otoliths (Gao et al. 2001) indicate that young Cherry Point herring remain in an estuarine environment, possibly feeding on copepods in the Fraser River estuary and then recruiting to their local population. Hexochlorobenzene measures in adult tissues (Jim West, Washington Department of Fish and Wildlife [WDFW], personal communication) indicate that the feeding area for adult Cherry Point herring differs from that where other herring in PS and SOG feed. Thus, Cherry Point herring remain associated throughout juvenile and adult stages and then return to spawn at Cherry Point. For Squaxin Pass, geographic isolation from other spawning areas in south PS, rather than a unique physical environment, is a probable mechanism for promoting its reproductive isolation. In both Cherry Point and Squaxin Pass, genetic patterns persisted over collection years, which suggests that patterns were systematic rather than random (Waples 1998), further supporting biologically meaningful differences.

Metapopulation Structure

McQuinn (1997) hypothesized that Atlantic herring population structure follows a metapopulation model wherein local populations persist as relatively independent groups. Recruits are from the local population or strays, and they learn migration patterns from mature adults (McQuinn 1997). Although Pacific herring from a single site tend to remain associated (Hay and McKinnell 2002), herring in BC stray at various rates and most genetic structure appears to conform to a metapopulation model (Hay et al. 2001; Ware and Schweigert 2001, 2002). However, relatively few migrants could lead to the low differentiation among BC populations, the straying levels staying below an effective recolonization rate on a management timescale but exceeding a rate that allows genetic differences to accumulate (Waples 1998). In the Gulf of Alaska and Bering Sea, annual variation also exceeded variation among locations within basins (Bentzen et al. 1998). Although McQuinn (1997) references Atlantic herring studies that show temporal heterogeneity, McPherson et al. (2004) found that replicate samples of Atlantic herring were temporally homogeneous. Straying in some PS and SOG herring may be similar to that in BC herring. In BC herring, Beacham et al. (2002) found more divergence among collection years and little differentiation within and between regions, except for groups with different spawn timings or in isolated sites. Our analysis showed greater differentiation among spawning sites than among collection years. However, our data set was weighted by the two divergent groups, Cherry Point and Squaxin Pass. Except for the Squaxin Pass herring, PS and SOG populations with similar run timing were genetically more connected and may follow a metapopulation model, similar to BC herring. Genetic patterns among the other PS and SOG sites (excluding Cherry Point and Squaxin Pass) were somewhat mixed, with some differences and some overlap. Inconsistencies may reflect that migration rates are higher during warmer years (Ware and Schweigert 2002). Inconsistencies may also indicate a ''sweepstakes'' style of recruitment where, although breeding numbers are large, chance events prevent most offspring from surviving to adulthood—which results in low numbers of effective spawners and high variance in genetic structure (Hedgecock 1994). However, our data lack patterns of homozygote excess one might expect to find for samples from a limited mix of family groups. Additional years of collection would help clarify temporal and spatial genetic patterns.

Conservation Implications

The Cherry Point population has declined 10-fold within the past 30 years, stimulating a petition in 2004 to list Cherry Point herring under the Endangered Species Act. In addition to decreased population size, the age structure has compressed to mostly 1- and 2-year-olds, where formerly the mix of age classes included up to 7-year-olds (Stout et al. 2001). Because younger fish are less

fecund, recruitment will continue to decline. Under a metapopulation model, other populations would support the Cherry Point population or recolonize the Cherry Point spawning ground. However, although adjacent herring populations are healthy and should thus export individuals (Ware and Schweigert 2001), the Cherry Point population has continued to decline. Further, other populations lack the late spawn timing required to utilize the Cherry Point spawning area, which is available only after winter storms abate. The physically isolated Squaxin Pass herring, while not endangered, are similarly genetically distinct and may thus be vulnerable to loss of genetic variation. Although other PS and SOG populations appear to conform to a metapopulation structure, Cherry Point and Squaxin Pass herring are genetically and behaviorally unique and may be repositories of irreplaceable variation, which should be protected through careful management.

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