Abstract.—The blood protein transferrin is an important source for identifying genetic variation in coho salmon, *Oncorhynchus kisutch*, with starch gel electrophoresis. We present new data for transferrin allele frequencies for 48 samples of coho salmon collected at 34 different locations from Oregon to southern British Columbia. To analyze transferrin allele frequencies from a larger geographic area, data from various sources were compiled for 135 samples from sites from California to southern British Columbia. In a statistical analysis of temporal variation within locations, 26 of 63 pairwise comparisons (41%) showed significant differences between samples ($P<0.05$). An analysis of variance revealed significant between-locale variability and no significant within-locale variability due to whether or not the brood years of the samples were three years apart. Relative gene diversity values were 22.3% between geographic regions, 2.1% between stocks within geographic regions, 3.1% between temporal comparisons, and 72.5% for within-sample variation. Total genetic diversity (H_T) was 0.586, and the average genetic diversity within populations (H_s) equaled 0.425. The samples showed genetic variation that was related to geographic location. Applications of transferrin data to genetic stock identification are discussed in relation to these results.

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Transferrin polymorphism in coho salmon, *Oncorhynchus kisutch*, and its application to genetic stock identification

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The iron binding protein, transferrin (Tf), is a highly polymorphic locus in coho salmon, *Oncorhynchus kisutch*, that exhibits allelic frequency differences between populations (Utter et al., 1970; Hjort and Schreck, 1982; Olin, 1984; Bartley et al., 1992). The transferrin locus is a potentially valuable addition to the set of polymorphic loci currently used for genetic stock identification (GSI) of coho salmon (Milner, 1993). In the past, one drawback of including transferrin in multilocus GSI surveys was the necessity of acquiring a serum sample to resolve allelic variation. However, transferrin allele frequencies can now be obtained from heart tissue (Van Doornik et al., 1995), therefore it is feasible to incorporate variation at this locus into GSI analyses involving coho salmon.

This study was conducted as part of an ongoing research effort to develop a geographically extensive baseline of allele frequencies for coho salmon. Our objectives are to increase the number of polymorphic loci for which allele-frequency data are obtainable, to verify the genetic basis of previously documented and new allelic variation, and to expand the geographic range of our baseline data set. Given adequate geographic coverage and genetic differences among major contributing stocks, allele-frequency data can be used for stock identification of mixed stock fisheries (Milner et al., 1985; Milner, 1993).

The objective of this paper is to examine the potential of transferrin for use in genetic stock identification of coho salmon. Specifically, we 1) report new transferrin allele frequencies for 48 samples collected from 34 coho salmon stocks, 2) compile allele-frequency data for transferrin data from other sources to compare allele frequencies over a larger geographic area, 3) analyze the temporal stability of transferrin allele frequencies, and 4) examine levels and patterns of variation between samples using gene diversity and genetic identity analyses.

Materials and methods

Blood or tissue samples, or both, were collected, as described by Van Doornik et al. (1995), for 48 coho

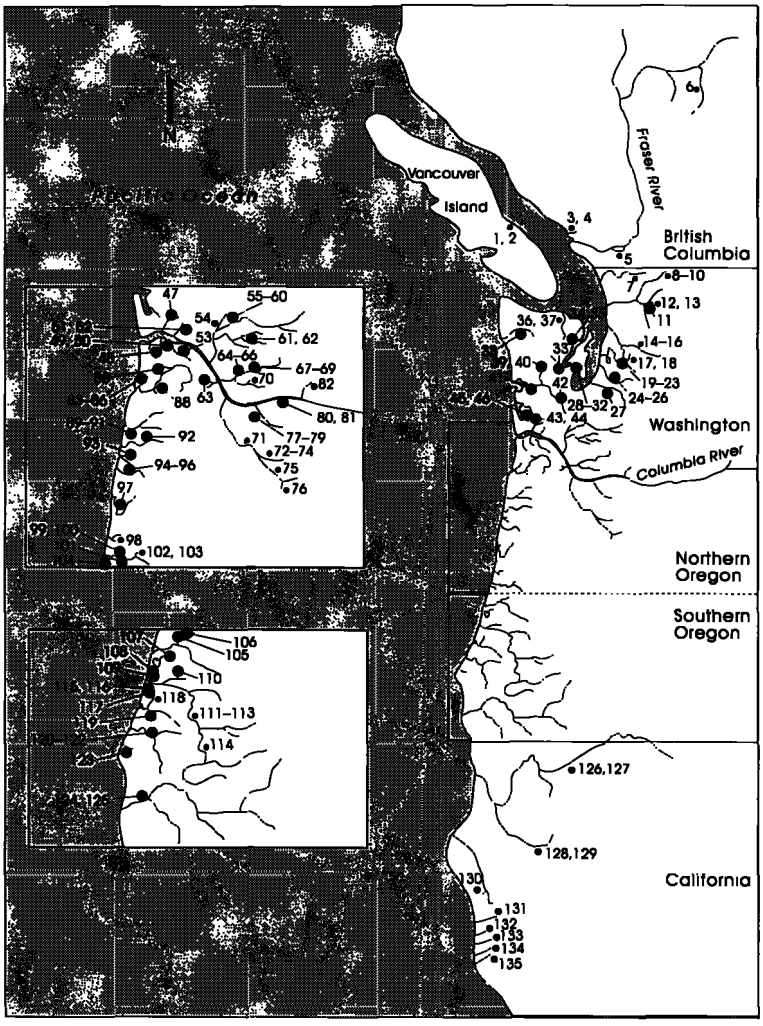

Figure 1

Locations of samples. Numbers refer to sample names identified in Table 1.

salmon samples from 34 locations in northern Oregon (north of Cape Perpetua), the Columbia River, Washington coastal streams, and Puget Sound, Washington (Fig. 1). Starch gel electrophoresis was conducted as described by Aebersold et al. (1987) and Van Doornik et al. (1995).

Observed genotype frequencies were compared with expected Hardy-Weinberg proportions with a chi-square goodness-of-fit test for the 48 samples for which genotype-frequency data were available.

Allele frequencies were compiled from other studies that included 30 or more fish per sample for the transferrin locus to create a data set of 135 samples (Table 1). The log-likelihood ratio statistic (G-test) (Sokal and Rohlf, 1969) was used to compare allele frequencies of these samples that were taken in different years at the same locale. In these analyses, G-tests were performed for each polymorphic locus and the results were summed over all loci to yield an overall G-value and a standardized G-value (G/df). To test for significant differences between samples, an analysis of variance (ANOVA) of arc sine square-root transformed allele frequencies, for $n-1$ alleles, was conducted with SYSTAT statistical software (SYSTAT, 1992). Genetic diversity was partitioned into components defined by regions, samples, and within-sample (temporal variation) according to the method described by Chakraborty (1980) by using the BIOSYS-1 computer program (Swofford and Selander, 1981).

Genetic similarities among samples were estimated with Nei's (1978) unbiased genetic identity and projected as a dendrogram with the unweighted pair-group method with arithmetic averaging (UPGMA) (Sneath and Sokal, 1973) (with BIOSYS-1). Genetic similarities were also analyzed by using

a principal components analysis of the correlation matrix with NTSYS software (Rohlf, 1994).

A noteworthy characteristic of coho salmon populations south of Alaska is their unique propensity to return to spawn at three years of age (Sandercock, 1991). We classified samples from the same locale as "on cycle" if the brood years were three years apart and as "off cycle" when otherwise. We tested for a significant effect of brood-year cycle within a locale on allele frequencies by nesting the brood-year cycle within-locale in the above mentioned ANOVA.

Results

Four alleles were observed with relative mobilities of 106, 103, 100, and 97. The **106* allele was observed at only three locations (Cowlitz, Lewis, and Trinity rivers) at a low frequency ($P<0.03$). Frequencies of the other alleles varied considerably among samples: $0.116 \leq P \leq 1.000$ for the **103* allele, $0.000 \leq P \leq 0.535$ for the **100* allele, and $0.000 \leq P \leq 0.764$ for the **97* allele. Allele frequencies for the 48 new samples analyzed in our laboratory are included in Table 1 (identified as "NMFS").

Of the 48 samples, 3 (6.3%) deviated from expected Hardy-Weinberg proportions: Nehalem Hatchery, brood year 1982 ($P=0.022$); Bonneville Hatchery, brood year 1989 ($P=0.008$); and Minter Creek Hatchery, brood year 1990 ($P=0.039$). Although the total number of significant tests ($n=3$) was only slightly greater than the number expected by chance alone ($n=2.4$), we have no reason to reject the assumptions that the samples represented panmictic populations and the genetic model used to interpret the observed allozyme patterns was correct. Data from an inheritance study have confirmed that the transferrin alleles segregate according to expected Mendelian segregation proportions (Van Doornik, unpubl. data).

Transferrin data compiled from nine sources in addition to those collected for this study are shown in Table 1. The total data set included 135 samples from sites ranging from California to southern British Columbia (Fig. 1). Sample sites were grouped into the following eight geographic regions: Vancouver Island, mainland British Columbia, Puget Sound, coastal Washington, Columbia River, northern Oregon (north of Cape Perpetua), southern Oregon, and California.

Sixty-three pairwise, G-tests were made by comparing allele frequencies of samples from the same location but from different brood years. A total of 41% ($n=26$) of the temporal comparisons were statistically significant ($P<0.05$). Despite this substantial temporal variability, the ANOVA indicated significant between-sample variation ($F=21.1$, df=89, $P=0$). (The rare **106* allele was pooled with the **103* allele frequencies for this analysis.) The contribution of brood-year cycle within sample locations to the overall genetic variation was not statistically significant for the **100* allele ($F=2.1$, df=24, $P=0.06$) and the **103* allele ($F=0.9$, df=24, $P=0.60$).

The gene diversity analysis indicated that most of the genetic diversity was found within locations (72.5%). The other components of relative gene diversity were 22.3% between geographic regions, 2.1% between stocks within a geographic region, and 3.1% between temporal comparisons. Relative gene diversity (G_{st}) equaled 27.5%. Total genetic diversity (H_T) was 0.586, and the mean genetic diversity within populations (H_s) equaled 0.425.

A dendrogram of Nei's genetic identity (I) for the compiled data for 135 samples revealed three major clusters at I=0.900 that followed regional geographic patterns (Fig. 2). All samples from Vancouver Island ($n=2$), the Washington coast ($n=8$), Puget Sound ($n=32$), and northern Oregon ($n=22$) formed cluster 1. Cluster 1 is distinguished by samples with moderate frequencies of the **103* ($0.080<P<0.501$) and **97* ($0.250<P<0.77$) alleles. The 21 samples from southern Oregon appeared in all three clusters. Samples from Marlow Creek, Five Mile Creek, Elk Creek, Coos River, North Fork Siuslaw River, and two Umpqua River stock samples (brood year 1981, 1992) also grouped in cluster 1; cluster 3 contained samples from the Rogue River ($n=2$) and Morton Creek; and the remaining 11 southern Oregon samples formed the sole members of cluster 2. Cluster 2 contained samples with relatively high frequencies of the **100* allele ($0.356 <P<0.540$). All of the California ($n=10$), Columbia River ($n=36$), and mainland British Columbia ($n=4$) samples grouped together in cluster 3. Cluster 3 consisted of samples with high frequencies of the **103* allele ($0.640<P\leq 1.000$).

The principal components analysis yielded results identical to those from Nei's genetic identity values, and therefore, are not included here.

Discussion

The geographical pattern of genetic variability shown by these data supports several conclusions regarding the genetic population structure of some of these stocks. Results from cluster analysis of the total data set (Fig. 2) indicated that samples from large river systems (Fraser River, Columbia River, Rogue River, and Klamath River) are genetically similar to each other despite the geographical distances separating them. Several studies have shown that coho salmon from small coastal rivers differ from those from large

inland river systems. For example, Hjort and Schreck (1982) examined electrophoretic, morphological, and life-history characteristics of coho salmon in Washington, Oregon, and California. They found, in general, stocks from large rivers (Columbia River, Rogue River, and Klamath River) were more similar to each other than to stocks from smaller rivers. Taylor and McPhail (1985) also found significant differences in body shape

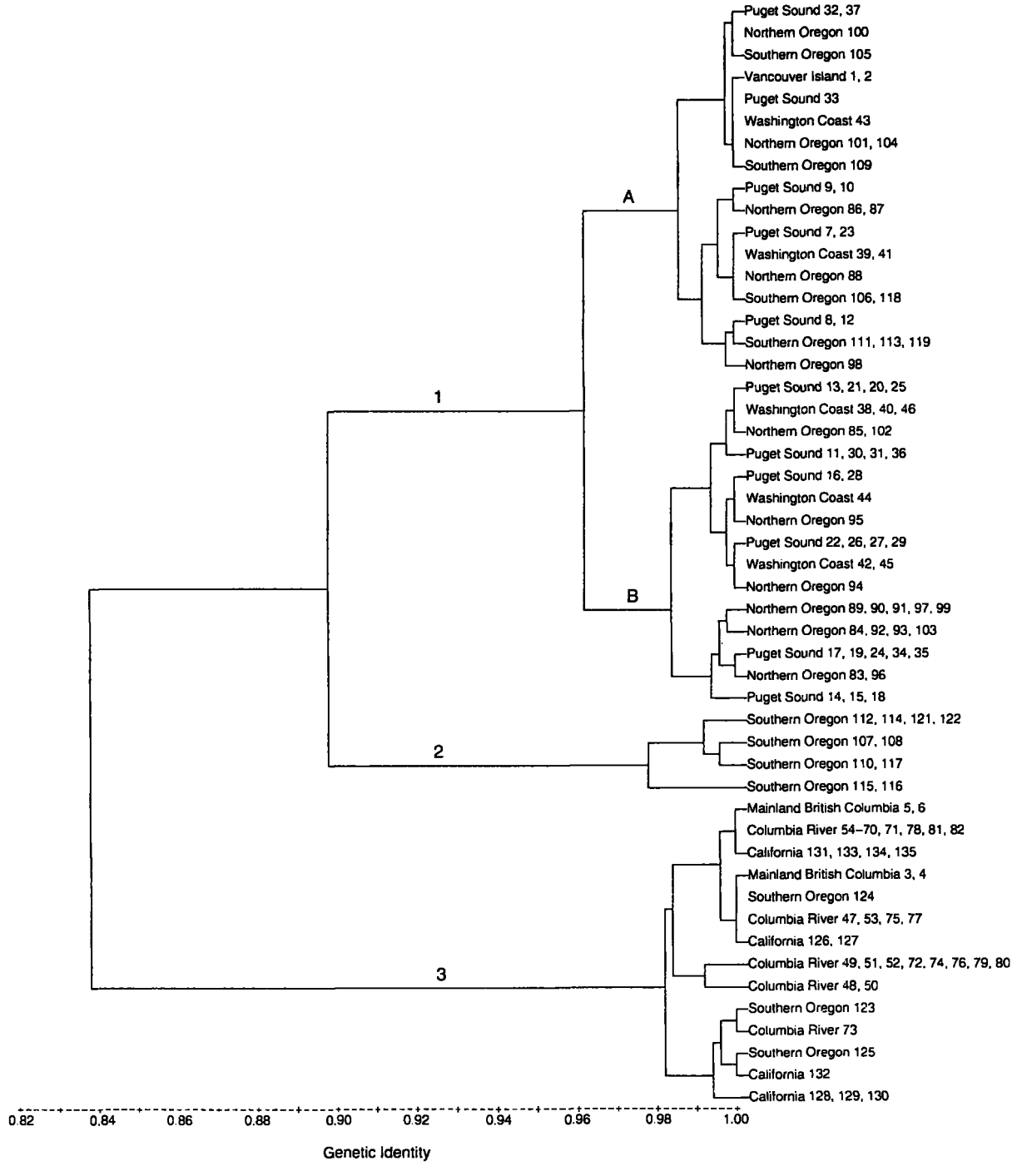

Figure 2

Dendrogram for compilation of 135 samples created with UPGMA clustering of Nei's identity values (Nei, 1978). Numbers refer to sample names identified in Table 1.

Table 1

Sample information, map codes, and allele frequencies for 135 samples of coho salmon, *Oncorhynchus kisutch*, from 81 locations.

Sample number	Name	Sample size	Brood year	Allele frequencies *103	*100	*97	*106	Source
1	Qualicum Hatchery	30	1971	0.367	0.333	0.300	0.000	Utter[1]
2	Qualicum Stock at Capillano Hatchery	91	1976	0.352	0.313	0.335	0.000	Utter[1]
3	Capillano Hatchery	45	1971	0.822	0.089	0.089	0.000	Utter[1]
4	Capillano Hatchery	85	1976	0.847	0.011	0.142	0.000	Utter[1]
5	Hope River, Fraser River	35	1972	1.000	0.000	0.000	0.000	May and Utter[2]
6	Coldwater River at Spius Hatchery	76	1985	0.961	0.039	0.000	0.000	NMFS[3]
7	North Fork Nooksack River	51	1974	0.402	0.196	0.402	0.000	May (1975)
8	Baker River	79	1989	0.544	0.146	0.310	0.000	NMFS[3]
9	Baker River at Skagit Hatchery	87	1989	0.466	0.098	0.437	0.000	NMFS[3]
10	Baker River at Skagit Hatchery	60	1991	0.483	0.125	0.392	0.000	NMFS[3]
11	Skagit Hatchery	40	1974	0.262	0.250	0.488	0.000	May (1975)
12	Clark River at Skagit Hatchery	79	1989	0.544	0.120	0.335	0.000	NMFS[3]
13	Clark River at Skagit Hatchery	57	1991	0.254	0.211	0.535	0.000	NMFS[3]
14	Skykomish River Hatchery	67	1971	0.149	0.104	0.747	0.000	Utter et al. (1973)
15	Skykomish River Hatchery	125	1972	0.116	0.120	0.764	0.000	May and Utter[2]
16	Skykomish River Hatchery	66	1974	0.356	0.053	0.591	0.000	May (1975)
17	Deep Creek, Snoqualmie River	30	1974	0.267	0.117	0.616	0.000	May (1975)
18	Harris Creek, Snoqualmie River	30	1974	0.083	0.167	0.750	0.000	May (1975)
19	Issaquah Hatchery	50	1968	0.220	0.160	0.620	0.000	Utter et al. (1970)
20	Issaquah Hatchery	100	1971	0.235	0.170	0.595	0.000	Utter et al. (1973)
21	Issaquah Hatchery	180	1972	0.278	0.172	0.550	0.000	May and Utter[2]
22	Issaquah Hatchery	88	1973	0.307	0.165	0.528	0.000	May (1975)
23	Issaquah Hatchery	80	1974	0.331	0.162	0.507	0.000	May (1975)
24	Green River Hatchery	54	1968	0.222	0.139	0.639	0.000	Utter et al. (1970)
25	Green River Hatchery	87	1971	0.282	0.155	0.563	0.000	Utter et al. (1973)
26	Green River Hatchery	58	1974	0.353	0.190	0.457	0.000	May (1975)
27	Puyallup Hatchery	60	1974	0.358	0.150	0.492	0.000	May (1975)
28	Minter Creek Hatchery	50	1968	0.410	0.060	0.530	0.000	Utter et al. (1970)
29	Minter Creek Hatchery	84	1971	0.357	0.140	0.503	0.000	Utter et al. (1973)
30	Minter Creek Hatchery	175	1972	0.289	0.200	0.510	0.000	May and Utter[2]
31	Minter Creek Hatchery	79	1974	0.215	0.259	0.526	0.000	May (1975)
32	Minter Creek Hatchery	34	1990	0.338	0.235	0.426	0.000	NMFS[3]
33	George Adams Hatchery	88	1974	0.295	0.341	0.364	0.000	May (1975)
34	Quilcene River Hatchery	60	1968	0.200	0.125	0.675	0.000	Utter et al. (1970)
35	Quilcene River Hatchery	60	1971	0.200	0.125	0.675	0.000	Utter et al. (1973)
36	Dungeness River Hatchery	47	1971	0.287	0.266	0.447	0.000	Utter et al. (1973)
37	Dungeness River Hatchery	80	1985	0.331	0.313	0.356	0.000	NMFS[3]
38	Soleduck River Hatchery	50	1971	0.190	0.220	0.590	0.000	Utter et al. (1973)
39	Simpson Hatchery	127	1972	0.378	0.224	0.398	0.000	May and Utter[2]
40	Simpson Hatchery	40	1992	0.275	0.212	0.513	0.000	NMFS[3]
41	Chehalis Hatchery	51	1971	0.373	0.236	0.411	0.000	Utter et al. (1973)
42	Newaukem River at Green River Hatchery	42	1974	0.393	0.119	0.488	0.000	May (1975)
43	Willapa River Hatchery	50	1971	0.280	0.350	0.370	0.000	Utter et al. (1973)
44	Willapa River Hatchery	37	1990	0.338	0.054	0.608	0.000	NMFS[3]
45	Nemah Hatchery	50	1971	0.300	0.180	0.520	0.000	Utter et al. (1973)
46	Nemah Hatchery	74	1974	0.223	0.216	0.561	0.000	May (1975)
47	Grays River Hatchery	39	1989	0.872	0.038	0.090	0.000	NMFS[3]
48	Klaskanine Hatchery	85	1990	0.653	0.029	0.318	0.000	NMFS[3]
49	Big Creek Hatchery	77	1989	0.812	0.013	0.175	0.000	NMFS[3]
50	Big Creek Hatchery	100	1990	0.675	0.015	0.310	0.000	NMFS[3]
51	Elokomin Hatchery	50	1968	0.800	0.000	0.200	0.000	Utter et al. (1970)
52	Elokomin Hatchery	98	1971	0.786	0.000	0.214	0.000	Utter et al. (1973)
53	Clatskanie River	31	1989	0.855	0.097	0.048	0.000	NMFS[3]
54	Lacamas River at Cowlitz Hatchery	32	1974	0.906	0.063	0.031	0.000	May (1975)

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Table 1 (continued)

Sample number	Name	Sample size	Brood year	Allele frequencies *103	*100	*97	*106	Source
55	Cowlitz Hatchery, Early	70	1989	0.908	0.021	0.043	0.028	NMFS[3]
56	Cowlitz Hatchery, Late	39	1989	0.961	0.013	0.000	0.026	NMFS[3]
57	Cowlitz Hatchery	50	1971	1.000	0.000	0.000	0.000	Utter et al. (1973)
58	Cowlitz Hatchery	38	1983	0.974	0.013	0.013	0.000	Gall[4]
59	Cowlitz Hatchery	52	1983	0.894	0.077	0.190	0.010	Gall[4]
60	Cowlitz Hatchery	100	1990	0.955	0.025	0.020	0.000	NMFS[3]
61	Toutle River Hatchery	50	1968	1.000	0.000	0.000	0.000	Utter et al. (1970)
62	Toutle River Hatchery	100	1971	0.980	0.000	0.020	0.000	Utter et al. (1973)
63	Scappoose Creek	40	1989	0.938	0.038	0.025	0.000	NMFS[3]
64	Lewis Hatchery, Early	78	1989	0.923	0.071	0.006	0.000	NMFS[3]
65	Lewis Hatchery, Late	79	1989	0.962	0.025	0.000	0.013	NMFS[3]
66	Lewis Hatchery, Late	86	1990	0.913	0.029	0.058	0.000	NMFS[3]
67	Speelyai Creek Hatchery	50	1968	0.990	0.000	0.010	0.000	Utter et al. (1970)
68	Speelyai Creek Hatchery	99	1971	1.000	0.000	0.000	0.000	Utter et al. (1973)
69	Speelyai Creek Hatchery	96	1990	0.938	0.010	0.052	0.000	NMFS[3]
70	Rock Creek, East Fork Lewis River	31	1974	0.935	0.000	0.065	0.000	May (1975)
71	Clackamas River	30	1989	1.000	0.000	0.000	0.000	NMFS[3]
72	Eagle Creek at Clackamas Hatchery	50	1983	0.800	0.010	0.190	0.000	Gall[4]
73	Eagle Creek Hatchery	75	1989	0.713	0.167	0.120	0.000	NMFS[3]
74	Eagle Creek Hatchery	96	1990	0.792	0.005	0.203	0.000	NMFS[3]
75	North Fork Clackamas Dam	40	1990	0.900	0.013	0.087	0.000	NMFS[3]
76	Fish Creek, Clackamas River	42	1983	0.774	0.000	0.226	0.000	Gall[4]
77	Sandy River Hatchery	50	1983	0.880	0.000	0.120	0.000	Gall[4]
78	Sandy River Hatchery	75	1989	0.947	0.013	0.040	0.000	NMFS[3]
79	Sandy River Hatchery	100	1990	0.770	0.055	0.175	0.000	NMFS[3]
80	Bonneville Hatchery	49	1983	0.806	0.031	0.163	0.000	Gall[4]
81	Bonneville Hatchery	79	1989	0.949	0.013	0.038	0.000	NMFS[3]
82	Willard Hatchery	80	1989	0.944	0.038	0.019	0.000	NMFS[3]
83	Nehalem River at Nehalem Hatchery	87	1981	0.213	0.109	0.678	0.000	Olin (1984)
84	Nehalem River at Nehalem Hatchery	79	1992	0.209	0.019	0.772	0.000	NMFS[3]
85	Nehalem Hatchery	84	1982	0.226	0.185	0.589	0.000	NMFS[3]
86	Nehalem Hatchery	73	1990	0.445	0.130	0.425	0.000	NMFS[3]
87	Cronin Creek, Nehalem River	36	1981	0.486	0.028	0.486	0.000	Olin (1984)
88	Fishhawk Creek at Nehalem Hatchery	63	1981	0.413	0.198	0.389	0.000	Olin (1984)
89	Trask Hatchery	79	1981	0.285	0.025	0.690	0.000	Olin (1984)
90	Trask Hatchery	117	1991	0.265	0.021	0.714	0.000	NMFS[3]
91	Trask River at Trask Hatchery	79	1992	0.291	0.032	0.677	0.000	NMFS[3]
92	Bogus Creek, Nestucca River	79	1983	0.215	0.017	0.766	0.000	Gall[4]
93	Hawk Creek, Neskowin River	47	1981	0.181	0.053	0.766	0.000	Olin (1984)
94	Salmon River Hatchery	71	1981	0.317	0.141	0.542	0.000	Olin (1984)
95	Salmon River Hatchery	86	1982	0.343	0.116	0.541	0.000	NMFS[3]
96	Salmon River at Salmon River Hatchery	80	1992	0.237	0.094	0.669	0.000	NMFS[3]
97	Siletz River at Salmon River Hatchery	77	1992	0.227	0.026	0.747	0.000	NMFS[3]
98	Beaver Creek	46	1981	0.500	0.250	0.250	0.000	Olin (1984)
99	Alsea River at Fall Creek Hatchery	85	1981	0.259	0.041	0.700	0.000	Olin (1984)
100	Alsea River at Fall Creek Hatchery	44	1992	0.386	0.273	0.341	0.000	NMFS[3]
101	Meadow Creek, Alsea River	41	1981	0.341	0.354	0.305	0.000	Olin (1984)
102	Fall Creek at Fall Creek Hatchery	50	1983	0.240	0.170	0.590	0.000	Gall[4]
103	Fall Creek at Fall Creek Hatchery	68	1992	0.176	0.059	0.765	0.000	NMFS[3]
104	Yachats River	35	1981	0.271	0.314	0.415	0.000	Olin (1984)
105	North Fork Siuslaw River	70	1983	0.371	0.236	0.393	0.000	Gall[4]
106	Elk Creek, Siuslaw River	47	1981	0.457	0.181	0.362	0.000	Olin (1984)
107	Maple Creek	41	1983	0.427	0.402	0.171	0.000	Gall[4]
108	Tahkenitch Lake at Fall Creek Hatchery	77	1992	0.377	0.409	0.214	0.000	NMFS[3]
109	Five Mile Creek	41	1981	0.329	0.341	0.330	0.000	Olin (1984)
110	Johnson Creek	49	1981	0.400	0.500	0.100	0.000	Olin (1984)

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Table 1 (continued)

Sample number	Name	Sample size	Brood year	Allele frequencies *103	*100	*97	*106	Source
111	Umpqua River at Rock Creek Hatchery	74	1981	0.478	0.179	0.343	0.000	Olin (1984)
112	Umpqua River at Rock Creek Hatchery	50	1983	0.570	0.410	0.020	0.000	Gall[4]
113	Umpqua River at Rock Creek Hatchery	69	1992	0.522	0.188	0.290	0.000	NMFS[3]
114	South Fork Umpqua River at Butte Falls Hatchery	70	1992	0.529	0.357	0.114	0.000	NMFS[3]
115	Eel Lake at Butte Falls Hatchery	120	1981	0.280	0.535	0.185	0.000	Olin (1984)
116	Eel Lake at Butte Falls Hatchery	63	1992	0.286	0.492	0.222	0.000	NMFS[3]
117	Tenmile Lake	50	1981	0.466	0.489	0.045	0.000	Olin (1984)
118	Marlow Creek	50	1981	0.375	0.182	0.443	0.000	Olin (1984)
119	Coos River at Cole River Hatchery	65	1992	0.546	0.169	0.285	0.000	NMFS[3]
120	Coquille River	84	1983	0.280	0.535	0.185	0.000	Gall[4]
121	Coquille River at Butte Falls Hatchery	57	1992	0.509	0.412	0.079	0.000	NMFS[3]
122	Coquille River at Butte Falls Hatchery	73	1981	0.570	0.410	0.020	0.000	Olin (1984)
123	Morton Creek	48	1981	0.643	0.204	0.153	0.000	Olin (1984)
124	Rogue River	65	1983	0.869	0.077	0.054	0.000	Gall[4]
125	Rogue River at Cole River Hatchery	57	1992	0.702	0.281	0.018	0.000	NMFS[3]
126	Klamath River at Irongate Hatchery	92	1981	0.852	0.031	0.117	0.000	Olin (1984)
127	Klamath River at Irongate Hatchery	81	1983	0.852	0.031	0.117	0.000	Gall[4]
128	Trinity River Hatchery	83	1983	0.813	0.181	0.000	0.006	Gall[4]
129	Trinity River Hatchery	111	1985	0.831	0.164	0.000	0.005	Bartley (1987)
130	Huckleberry Creek, South Fork Eel River	52	1985	0.837	0.163	0.000	0.000	Bartley (1987)
131	Pudding Creek	47	1985	0.914	0.057	0.029	0.000	Bartley (1987)
132	Caspar Creek	82	1985	0.730	0.230	0.040	0.000	Bartley (1987)
133	Russian Gulch	31	1985	1.000	0.000	0.000	0.000	Bartley (1987)
134	Little River	51	1985	0.929	0.071	0.000	0.000	Bartley (1987)
135	Albion River	30	1985	0.944	0.056	0.000	0.000	Bartley (1987)

[1] Utter, F. M. 1979. Memorandum dated January 19, 1979 to Dr. Keith Sandercock, Canadian Dep. of the Environment, Vancouver, B.C. Subject: Transferrin frequencies of coho salmon from Qualicum and Capillano Hatcheries, 3 p.
[2] May, B., and F. M. Utter. 1974. Biochemical genetic variation of the genus *Oncorhynchus* in Pacific Northwest populations: a progress report and program summary, 12 p. Available: Washington Dep. Fisheries, P.O. Box 43151, Olympia, WA 98504-3151.
[3] Samples analyzed in our laboratory.
[4] Gall, G. A. E. 1991. Allele frequencies of selected stocks of California, coastal Oregon, and Columbia River coho salmon, 59 p. Available: Dep. Animal Sci., Univ. California, Davis, CA 95616-8521.

of juvenile coho salmon between fish from coastal rivers and those from large inland river systems. They concluded that these differences are coastwide and are at least partially genetically determined.

It is possible that there is some selective pressure on the transferrin alleles occurring in large rivers, because all the samples from the Fraser, Columbia, Rogue, and Klamath rivers have high frequencies of the **103* allele, and low frequencies of the **97* allele.

Samples from California clustered with Columbia River samples. California stocks may have been affected by imported stocks: most of California's hatchery stocks originated from eggs imported from Oregon (Bartley et al., 1992), primarily from hatcheries on the Columbia River (Brown et al., 1994).

In other areas, however, stocks appear to follow a regional pattern of genetic differences, despite transfers of fish between geographical areas. For example, the Bonneville and Klaskanine Hatcheries on the Columbia River have received hatchery stocks from northern and southern Oregon (Oregon Dep. Fish and Wildlife[1]), yet still remain most similar genetically to other Columbia River samples (Fig. 2). Again, this could be due to selective factors unique to a given area.

Our findings are similar to results from related genetic studies of coho salmon. Noting allele frequencies at 26 enzyme loci, Wehrhahn and Powell (1987) also found significant differences between samples from Vancouver Island and the southern coastal region of mainland British Columbia. In a study of mitochondrial DNA variation in coho salmon from Oregon, Currens and Farnsworth[2] found that Colum-

[1] Oregon Department of Fish and Wildlife. 1991. Lower Columbia River coho salmon, evaluation of stock status, causes of decline, and critical habitat—Part 2. Report to ESA Administrative Record, 43 p., plus appendices. Available: Oregon Dep. Fish Wildl., P.O. Box 59, Portland, OR 97207.

[2] Currens, K. P., and D. Farnsworth. 1993. Mitochondrial DNA variation in Oregon coho salmon. Report to Oregon Department of Fish and Wildlife, 12 p. Available: Oregon Dep. Fish Wildl., P.O. Box 59, Portland, OR 97207.

bia River samples clustered separately from northern Oregon coastal samples. However, the two southern Oregon coastal samples that they examined (Rogue River and Coquille River) clustered with the Columbia River samples, rather than with samples from the Oregon coast. This result is similar to ours, except that the Coquille River did not cluster with Columbia River samples in our analysis.

Another important discovery was the relatively high level of temporal variation for transferrin allele frequencies. Accurate stock identification from allele-frequency data requires that allele frequencies remain relatively stable over time. Because most coho salmon south of Alaska return to spawn at 3 years of age (Sandercock, 1991), it is possible that allele frequencies for a particular stock could show marked differences between brood years. Waples and Teel (1990) found that for salmon populations where 90% of a brood year returned to spawn in the same year, the probability of finding a significant test for temporal differences increased dramatically.

Our test for temporal allele stability assumed there was no influx of nonnative fish into the gene pool; however, it is unlikely that this assumption was valid. All comparisons were made between samples obtained from hatcheries, and many of these hatcheries have imported and released fish that were not native to the hatchery location. For example, from 1952 to 1991, 20.2% of the coho salmon released at the Minter Creek Hatchery were not native to Minter Creek (Washington Dep. Fish and Wildlife[3]).

Another assumption that we made when testing temporal stability was that no natural selection occurred. Previous studies have found some evidence that transferrin alleles are influenced by natural selection. Suzumoto et al. (1977) observed that fish that have the **97* transferrin allele are less susceptible to bacterial kidney disease (BKD). Pratschner (1978) found that the **103/*103* phenotype had greater resistance to furunculosis, whereas the **97/*97* phenotype was most resistant to vibriosis. A study by McIntyre and Johnson (1977) indicated that fish with a **103/*103* phenotype had a faster growth rate than those with a **103/*97* phenotype, but the escapement rate for the two different phenotypes was equal. However, results of a study by Winter et al. (1980) conflicted with both Suzumoto et al. (1977) and Pratschner (1978). Their results indicated that only certain stocks show a genetically influenced resistance to BKD, furunculosis, and vibriosis. They concluded that "the importance of transferrin genotypes in resistance to disease is stock specific." The extent to which these factors affect transferrin allele frequencies and how they relate to GSI analyses are uncertain. If certain alleles are being selected for, according to location-specific factors, then allele frequencies could be more stable over time for a given location, even after nonnative stocks are introduced into an area. However, if selection factor(s) were to change, allele frequencies would change significantly over time, which would help to explain the large percentage of significant temporal comparisons that we found.

We found at least as much temporal variation between samples analyzed in our own lab as we did between samples analyzed in two different laboratories. Therefore, we ruled out the possibility that temporal variation was due to differing techniques or to differing interpretations of data, or both, in two different laboratories.

These results suggest that before the transferrin locus is used in GSI analysis, temporal variation of allele frequencies between brood years will need to be considered. However, temporal variation does not necessarily preclude the use of the transferrin locus in GSI analysis. In a study of temporal variation in lake trout, *Salvelinus namaycush*, Grewe et al. (1994) found that although there were significant differences in allele frequencies between year classes, the accuracy of their mixed-stock contribution estimates was not substantially affected. Waples (1990) suggested that to obtain maximum precision of estimates, temporally spaced samples should be pooled, unless the temporal differences are too large to be attributable to sampling error and to genetic drift. Of course, if the baseline data include only the brood years known to be contributing to the mixed-stock, temporal variation will not be a problem.

The mean heterozygosity value we calculated (0.425) was indicative of a high level of heterozygosity for the transferrin locus. This was one of the highest mean heterozygosity values we have observed for any polymorphic locus in coho salmon (Van Doornik, unpubl. data). It is also one of the highest mean heterozygosity values in comparison with other studies of salmonids. For example, the highest mean heterozygosity found by Wehrhahn and Powell (1987) in their study of 26 coho salmon loci from 95 sites in British Columbia was 0.0099 for the locus *LDH-B2** (transferrin was not included in this study). The largest mean heterozygosity value, in a study of 25 loci of 86 populations of chinook salmon, was 0.420 for the locus *PGK-2** (Utter et al., 1989).

As shown by the results from the ANOVA, there were significant differences between samples. The relative gene diversity values showed that there was

[3] Washington Department of Fish and Wildlife. 1994. Historical releases of juvenile salmon into Washington waters, coho salmon. Available: Washington Dep. Fish Wildl., 600 Capitol Way N., Olympia, WA 98501-1091. (Interactive database).

a high degree of genetic differentiation between samples from different geographic regions (22.3%). However, the level of differentiation between samples within regions was actually less than the level of temporal variation. This could be due to the extensive number of stock transfers within regions that have taken place for coho salmon. Therefore, we concluded that although the transferrin locus may be used to discriminate between samples from different geographic regions, caution must be used when attempting to use this locus to discriminate between samples from within a region.

It is important to realize that these results and conclusions are based on data from only one locus. Accurate GSI procedures rely upon baseline data from many polymorphic loci. Our current protocol for obtaining baseline genetic data includes using more than 60 loci that have been shown to be polymorphic for coho salmon. A large baseline of genetic information, from potential source populations and from enough loci with significant allele-frequency differences between stocks, to identify the contributing stocks is generally required for GSI methods to be successful (Milner et al., 1985).

Our results provide further evidence that identifiable regional genetic differences can be detected among coho salmon populations. GSI investigations of coho salmon will be more precise when data from the transferrin locus is used in conjunction with allozyme data and perhaps DNA-type variation (Park et al., 1993). As these data show, the transferrin locus exhibits significant allele-frequency differences between stocks from different geographical areas and has the potential to increase the discriminating power of GSI analyses for coho salmon.

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