Genetic analysis of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington reveals few Columbia River wild fish*

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Abstract—Little is known about the ocean distributions of wild juvenile coho salmon off the Oregon-Washington coast. In this study we report tag recoveries and genetic mixed-stock estimates of juvenile fish caught in coastal waters near the Columbia River plume. To support the genetic estimates, we report an allozyme-frequency baseline for 89 wild and hatchery-reared coho salmon spawning populations, extending from northern California to southern British Columbia. The products of 59 allozyme-encoding loci were examined with starch-gel electrophoresis. Of these, 56 loci were polymorphic, and 29 loci had $P_{0.95}$ levels of polymorphism. Average heterozygosities within populations ranged from 0.021 to 0.046 and averaged 0.033. Multidimensional scaling of chord genetic distances between samples resolved nine regional groups that were sufficiently distinct for genetic mixed-stock analysis. About 2.9% of the total gene diversity was due to differences among populations within these regions, and 2.6% was due to differences among the nine regions. This allele-frequency data base was used to estimate the stock proportions of 730 juvenile coho salmon in offshore samples collected from central Oregon to northern Washington in June and September-October 1998–2000. Genetic mixed-stock analysis, together with recoveries of tagged or fin-clipped fish, indicates that about one half of the juveniles came from Columbia River hatcheries. Only 22% of the ocean-caught juveniles were wild fish, originating largely from coastal Oregon and Washington rivers (about 20%). Unlike previous studies of tagged juveniles, both tag recoveries and genetic estimates indicate the presence of fish from British Columbia and Puget Sound in southern waters. The most salient feature of genetic mixed stock estimates was the paucity of wild juveniles from natural populations in the Columbia River Basin. This result reflects the large decrease in the abundances of these populations in the last few decades.

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Nearshore and riverine distributions of maturing Pacific salmon (*Oncorhynchus* spp.) are well known, largely because of the prevalence of salmon fisheries and the tremendous amount of information gathered to manage these fisheries. Out-migration timings and abundances of smolts in streams as they migrate to the sea are also well known, but information on the distributions and stock origins of wild juveniles in marine waters has been limited by two factors. The first is that a large amount of effort is needed to sample marine-phase juveniles, which generally occur at low densities (Godfrey, 1965; Hartt and Dell, 1986; Orsi et al., 2000). The second factor is that stock origins have been determined only for coded-wire–tagged (CWT) hatchery fish (e.g. Pearcy and Fisher, 1988; Orsi et al., 2000). Because only small proportions of hatchery juveniles are tagged, samples of ocean-caught salmon are largely a mixture of hatchery and wild fish of unknown stock origins. Genetic mixed-stock analysis, although used routinely to estimate the stock compositions of mature returning salmon (e.g. Milner et al., 1985; Shaklee et al., 1999; Beacham et al., 2001), has not been fully exploited to estimate stock origins of immature salmon (but see Guthrie et al., 2000). The study we describe here is the first to use genetic data to estimate the stock origins of ocean mixtures of juvenile coho salmon (*O. kisutch*).

One goal of our study was to create a baseline of allelic frequencies in spawning populations of coho salmon throughout the Pacific Northwest and California. These data were previously used by the National Marine Fisheries Service (NMFS) to define evolutionarily significant units (ESUs) for evaluation under the Endangered Species Act (Johnson et al., 1991; Weitkamp et al., 1995). In the present study, we examine levels of variability among populations and use mixed-stock simulations to assess the usefulness of such a data set as a baseline for genetic mixed-stock analysis. A second goal was to use this baseline of population data to estimate the stock compositions of juvenile coho salmon collected in the early and late summers of 1998, 1999, and 2000 off the Oregon and Washington coasts by the NMFS (Emmett and Brodeur, 2000). We use a standard approach to genetic mixed-stock analysis that yields proportional estimates of stock origin of fish in a mixed-stock sample (e.g., Pella and Milner, 1987). We compare early and

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late summer samples to detect seasonal shifts in stock compositions along the coast and make comparisons with earlier tag-recovery studies to search for decadal shifts in the marine distributions of particular stocks. Recent efforts at Pacific Northwest hatcheries to mark the majority of their releases of coho salmon with a fin clip provide new opportunities to estimate proportions of hatchery and wild coho salmon in ocean samples (Beamish et al., 2000; Lawson and Comstock, 2000). We therefore report genetic estimates for hatchery-marked and unmarked fish in ocean samples and use these estimates to identify hatchery and wild population origins of ocean juvenile coho salmon.

Materials and methods

To establish an allele-frequency baseline, populations of coho salmon were sampled from 89 hatcheries, streams, and rivers between 1984 and 1999 (Fig. 1, Table 1). Samples of skeletal muscle, eye, liver, and heart tissues were collected from adults during spawning operations in hatcheries or from whole juvenile fish in hatchery rearing ponds. All hatchery broods sampled were the progeny of al least 50 adult fish. Wild juveniles were sampled in natal streams and rivers by electroshocking. Wild adult fish were sampled in spawning areas by gaffing and dip-netting.

Samples of juvenile coho salmon in marine waters were collected during NMFS coastal pelagic trawl surveys (Emmett and Brodeur, 2000). Trawls consisted of one-half-hour long surface tows with a 264 Nordic rope trawl along nine transects perpendicular to shore ranging from La Push, Washington (47°55′N) to Cape Perpetua, Oregon (44°15′N) (Fig. 1). Sampling stations began 1–5 nautical miles offshore and continued, in about 5 nautical-mile (nmi) increments, to about 30 nmi offshore. Marine juveniles were sampled 16–24 June and 21–30 September 1998, 16–24 June and 21 September–1 October 1999, and 17–25 June and 19–24 September 2000. Fish were measured and examined for the presence of fin clips and coded wire tags (CWTs). Juveniles in their first ocean summer were separated from older coho salmon by length by using a modification of the criteria of Pearcy and Fisher (1990). Fish with fork lengths less than 330 mm (June) and 450 mm (September–October) were considered to be juveniles in their first year in the ocean. Fish with CWTs, and therefore of known brood year, provided supporting evidence for these criteria with the assumption that growth of hatchery and wild fish was similar.

Tissue samples or whole juvenile fish were frozen on dry ice or in liquid nitrogen and stored at –80°C prior to electrophoretic analysis. We used the methods of Aebersold et al. (1987) for sample preparation and horizontal starch-gel protein electrophoresis. Electrophoretic conditions for 30 enzymes, for which we obtained reliable and interpretable data for 59 loci, are reported in an appendix that can be retrieved at the Northwest Fisheries Science Center website [http://www.nwfsc.noaa.gov]. Guidelines by Utter et al. (1987) were used to infer genotypes from banding patterns. Locus and allelic nomenclature follows Shaklee et al. (1990).

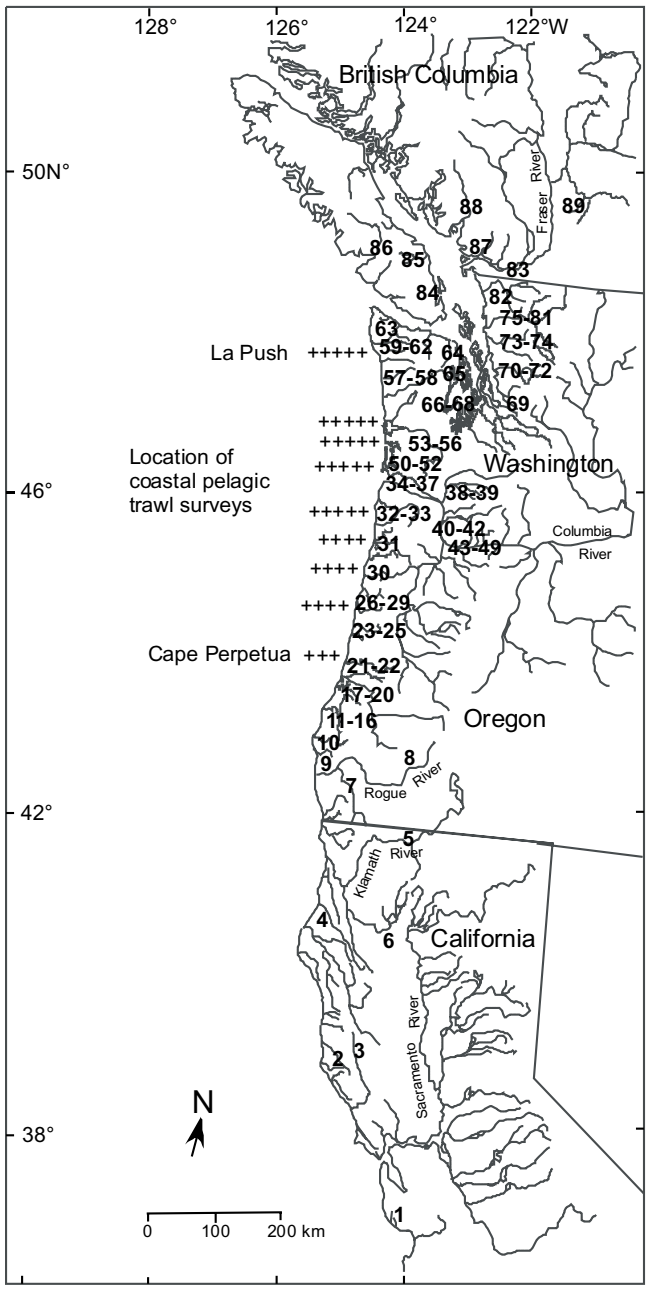

Figure 1

Locations of ocean sampling transect lines (+) and 89 coho salmon populations in California, Oregon, Washington, and British Columbia. Numbers correspond to population names in Table 1.

Genotypic frequencies of polymorphic loci for each baseline sample were examined for departures from expected Hardy-Weinberg proportions with a Fisher's exact test (Guo and Thompson, 1992) by using GENEPOP version 3.1 (Raymond and Rousset, 1995). Hardy-Weinberg tests were performed on isoloci (comigrating protein products of duplicated loci) following Waples (1988).

We estimated allelic frequencies for each sample. Allelic frequencies for isoloci were calculated as mean frequen-

Table 1

Sample information and indices of genetic variability for coho salmon from the Pacific Northwest and California. Map codes refer to Figure 1. Indices of genetic variability are $\%P_{0.95}$ = percentage of $P_{0.95}$ loci and H = heterozygosity.

Source Region and map code	Year sampled	Number of fish	$\%P_{0.95}$	H
California coast				
1 Scott Creek	1994	21	12.5	0.039
2 Little River	1994	27	14.3	0.040
3 Warm Springs Hatchery	1994, 1994	160	16.1	0.041
4 Mad River Hatchery	1994	120	17.9	0.040
Klamath River to Cape Blanco				
5 Iron Gate Hatchery	1994	120	9.0	0.021
6 Trinity Hatchery	1984, 1994	218	9.0	0.028
7 Rogue River (Illinois River, Greyback Creek)	1993	40	7.2	0.022
8 Cole Rivers Hatchery, stock no. 52 (Rogue River)	1993	100	9.0	0.030
9 North Fork Elk and Elk Rivers	1993	32	7.2	0.021
Oregon coast				
10 Sixes River (Crystal and Edson Creeks)	1993	44	7.2	0.026
11 New River (Bether and Morton Creeks)	1993	62	10.7	0.034
12 Butte Falls Hatchery, stock no. 44 (Coquille River)	1993	100	9.0	0.036
13 Cole Rivers Hatchery, stock no. 37 (South Fork Coos River)	1993	129	10.7	0.034
14 Coos River (Millicoma River and Marlow Creek)	1993, 1997	50	12.5	0.033
15 Butte Falls Hatchery, Eel River stock no. 63	1993	100	7.2	0.032
16 Ten Mile Lake	1992	56	7.2	0.030
17 Rock Creek Hatchery, stock no. 55 (Umpqua River)	1993	100	7.2	0.029
18 North Umpqua River (Williams Creek)	1993, 1997	67	7.2	0.025
19 Butte Falls Hatchery, stock no. 18 (Umpqua River)	1993	100	7.2	0.027
20 Smith River (Halfway Creek)	1993	40	10.7	0.034
21 Fall Creek Hatchery, stock no. 113 (Tahkenitch River)	1993	100	7.2	0.030
22 Siuslaw River	1996	51	9.0	0.029
23 Fall Creek Hatchery, stock no. 31 (Alsea River)	1993	100	14.3	0.040
24 Fall Creek Hatchery, stock no. 43 (Alsea River)	1993	95	9.0	0.037
25 Alsea River	1996	62	10.7	0.031
26 Beaver Creek	1993	62	9.0	0.035
27 Yaquina River	1996	54	12.5	0.043
28 Salmon River Hatchery, stock no. 33 (Siletz River)	1993	100	12.5	0.041
29 Siletz River (Forth of July, Sunshine, and Buck Creeks)	1993	50	10.7	0.033
30 Salmon River Hatchery, stock no. 36 (Salmon River)	1993	100	10.7	0.037
31 Trask River Hatchery, stock no. 34 (Trask River)	1992, 1993	220	16.1	0.039
32 Nehalem River Hatchery, stock no. 99 (Nehalem River)	1992	80	12.5	0.045
33 Nehalem River Hatchery, stock no. 32 (Nehalem River)	1993	100	14.3	0.044
Columbia River				
34 Lewis and Clark River	1991, 1993	36	12.5	0.038
35 Big Creek Hatchery	1991	80	12.5	0.040
36 Grays River Hatchery	1987, 1991	200	7.2	0.033
37 Clatskanie River (Carcus Creek)	1991, 1992, 1996	113	10.7	0.033
38 Cowlitz Hatchery early-run	1991	80	9.0	0.027
39 Cowlitz Hatchery late-run	1991, 1992	180	7.2	0.031
40 Scappoose River (Siercks, Raymond, and Milton Creeks)	1991	44	14.3	0.041
41 Lewis River Hatchery early-run	1991	80	5.4	0.027
42 Lewis River Hatchery late-run	1991	80	12.5	0.032
43 North Fork Clackamas River early-run	1998[a]	48	16.1	0.036
44 North Fork Clackamas River late-run	1999[a]	45	14.3	0.028
45 Eagle Creek Hatchery	1991, 1992	180	7.2	0.037

continued

Table 1 (continued)

Source Region and map code		Year sampled	Number of fish	$\%P_{0.95}$	H
46	Sandy River Hatchery	1991, 1992	180	10.7	0.046
47	Sandy River	1991, 1992, 1996	124	10.7	0.043
48	Bonneville Hatchery	1991, 1992	180	10.7	0.043
49	Willard Hatchery	1991	80	7.2	0.032
South Washington coast					
50	Naselle River Hatchery	1991	100	9.0	0.029
51	Nemah River Hatchery	1991	100	10.7	0.029
52	Willapa River Hatchery	1991	100	9.0	0.031
53	Chehalis River (Stillman Creek)	1995	71	9.0	0.026
54	Chehalis River (Satsop River, Bingham Creek)	1995	98	10.7	0.028
55	Bingham Creek Hatchery	1991,[1] 1992,[1] 1995	180	9.0	0.027
56	Chehalis River (Hope Creek)	1994, 1995, 1996	171	9.0	0.030
North Washington coast					
57	Queets River	1995	99	9.0	0.028
58	Clearwater River	1995	100	7.2	0.029
59	Bogachiel River	1987	80	10.7	0.030
60	Sol Duc Hatchery Summer Run	1994[1]	80	7.2	0.030
61	Sol Duc River Summer Run	1995	120	10.7	0.030
62	Sol Duc Hatchery Fall Run	1995[1]	80	9.0	0.032
63	Hoko River	1987	96	9.0	0.033
Puget Sound and Hood Canal					
64	Dungeness Hatchery	1987	80	12.5	0.037
65	Quilcene Hatchery	1994[1]	100	9.0	0.025
66	North Fork Skokomish River	1994,[1] 1995[1]	126	7.2	0.030
67	Dewatto River	1994,[1]1995,[1] 1996[1]	169	9.0	0.028
68	Minter Creek Hatchery	1992,[1] 1995[1]	80	9.0	0.035
69	Soos Creek Hatchery	1994,[1] 1995, 1996	680	9.0	0.034
70	Snoqualmie River (Harris Creek)	1987	120	7.2	0.034
71	Snoqualmie River (Grizzly Creek)	1994,[1] 1995,[1] 1996[1]	215	7.2	0.030
72	North Fork Skykomish River (Lewis Creek)	1995[1]	102	9.0	0.032
73	North Fork Stillaguamish River (Fortson Creek)	1987, 1989 [1]	200	9.0	0.031
74	North Fork Stillaguamish River (Mcgovern Creek)	1987	40	10.7	0.032
75	Upper Skagit River	1993	127	9.0	0.033
76	Skagit River (Carpenter Creek)	1993	139	9.0	0.032
77	Skagit River (West Fork Nookachamps Creek)	1987, 1993	220	9.0	0.035
78	Skagit River (Baker River)	1992 [1]	303	10.7	0.036
79	Skagit River (Suiattle River, All Creek)	1987, 1993	200	10.7	0.032
80	Skagit River (Upper Sauk River)	1992, 1993	200	9.0	0.034
81	Skagit River (Upper Cascade River)	1992, 1993	224	9.0	0.031
82	Samish River (Ennis Creek)	1994, [1] 1995, [1] 1996 [1]	167	9.0	0.035
British Columbia					
83	Chilliwack River Hatchery	1984	100	10.7	0.034
84	Cowichan River Hatchery	1984	80	9.0	0.036
85	Big Qualicum Hatchery	1989, [1] 1991	180	10.7	0.037
86	Robertson Creek Hatchery	1984	100	9.0	0.030
87	Capilano Hatchery	1989, [1] 1991	200	12.5	0.038
88	Squamish River Hatchery	1988[1]	98	7.2	0.035
Upper Fraser River					
89	Spius River Hatchery	1987	200	10.7	0.035
Mean				10.0	0.033

[1] Sample taken from adult fish. All other samples were from juvenile fish.

Table 2

Enzymes and study results for 59 loci in samples of 89 coho salmon populations from the Pacific Northwest and California.

Enzyme or protein name	Enzyme commission number	Locus abbrev.	Number of populations polymorphic	Range of common allele frequency
Aspartate aminotransferase	2.6.1.1	sAAT-1,2*	36	1.000–0.966
		sAAT-3*	1	1.000–0.956
		sAAT-4*	71	1.000–0.839
Adenosine deaminase	3.5.4.4	ADA-1*	34	1.000–0.924
		ADA-2*	15	1.000–0.929
Aconitate hydratase	4.2.1.3	mAH-1*	2	1.000–0.992
		mAH-2*	22	1.000–0.919
		mAH-3*	3	1.000–0.944
		sAH*	60	1.000–0.849
Adenylate kinase	2.7.4.3	AK*	4	1.000–0.993
Alanine aminotransferase	2.6.1.2	ALAT*	12	1.000–0.958
Creatine kinase	2.7.3.2	CK-A1*	8	1.000–0.971
		CK-A2*	22	1.000–0.919
		CK-C1*	4	1.000–0.983
		CK-C2*	9	1.000–0.972
		CK-B*	1	1.000–0.999
Esterase	3.1.-.-	EST-1*	85	1.000–0.652
Fructose-bisphosphate aldolase	4.2.1.13	FBALD-3*	1	1.000–0.996
		FBALD-4*	14	1.000–0.962
Formaldehyde dehydrogenase (glutathione)	1.2.1.1	FDHG*	33	1.000–0.954
Fumarate hydratase	4.2.1.2	FH*	43	1.000–0.835
b-N-Acetylgalactosaminidase	3.2.1.53	bGALA*	89	0.889–0.357
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH-2*	64	1.000–0.713
		GAPDH-3*	26	1.000–0.867
		GAPDH-4*	9	1.000–0.975
		GAPDH-5*	0	1.000–1.000
Glucose-6-phosphate isomerase	5.3.1.9	GPI-A*	17	1.000–0.906
		GPI-B1*	1	1.000–0.962
		GPI-B2*	47	1.000-0.815
Glutathione reductase	1.6.4.2	GR*	6	1.000–0.988

continued

cies over both loci and treated as a single tetrasomic locus. Following the recommendations of Waples (1990), allelic frequencies of samples taken in different years from the same location were combined. In general, little temporal allele-frequency variation was detected in coho salmon populations sampled over years (Van Doornik et al., 2002; present study). Levels and patterns of genetic variation within and between populations were estimated with 56 polymorphic loci (Table 2). Average expected heterozygosity per locus (isoloci excluded) for each population was calculated by using an unbiased estimator (Nei, 1978). The proportion of $P_{0.95}$ loci was computed for each population, in which a locus was considered to be polymorphic if the frequency of the most common allele was ≤0.95. Chord distances (Cavalli-Sforza and Edwards, 1967) were computed between all pairs of populations with BIOSYS (Swofford and Selander, 1981), and relationships among populations were depicted with multidimensional scaling (MDS, NTSYS-PC, Exeter Software, NY). Allele-frequency variation among baseline populations was partitioned (Chakraborty et al., 1982) into two geographic levels: 1) populations within regions; and 2) among regions (Table 1). These regions were delimited by geography and by genetic groupings in the MDS analyses.

We used the maximum likelihood procedures of Pella and Milner (1987) and the Statistical Package for Analyzing Mixtures (SPAM; Debevec et al., 2000) to estimate stock contributions to simulated and actual mixtures of coho salmon. Estimates were made by using 56 polymorphic loci (Table 2) and 89 baseline populations, except for analysis of marked (hatchery) fish where only hatchery populations were used (Table 1). Allocations to individual baseline populations were then summed to estimate contributions of regional stock groups (Pella and Milner, 1987). Average mix-

Table 2 (continued)

Enzyme or protein name	Enzyme commission number	Locus abbrev.	Number of populations polymorphic	Range of common allele frequency
Isocitrate dehydrogenase	1.1.1.42	mIDHP-1*	4	1.000–0.964
		mIDHP-2*	11	1.000–0.799
		sIDHP-1*	11	1.000–0.948
		sIDHP-2*	29	1.000–0.851
Lactate dehydrogenase	1.1.1.27	LDH-A1*	7	1.000–0.700
		LDH-A2*	2	1.000–0.995
		LDH-B1*	18	1.000–0.942
		LDH-B2*	20	1.000–0.956
		LDH-C*	0	1.000–1.000
Malate dehydrogenase	1.1.1.37	sMDH-A1,2*	35	1.000–0.976
		sMDH-B1,2*	21	1.000–0.947
Mannose-6-phosphate isomerase	5.3.1.8	MPI*	41	1.000–0.897
α-Mannosidase	3.2.1.24	MAN*	5	1.000–0.981
Dipeptidase	3.4.-.-	PEPA*	63	1.000–0.895
Tripeptide amino peptidase	3.4.-.-	PEPB-1*	10	1.000–0.979
Peptidase-C	3.4.-.-	PEPC*	89	0.903–0.391
Proline dipeptidase	3.4.-.-	PEPD-2*	56	1.000–0.798
Leucyl-L-tyrosine peptidase	3.4.-.-	PEPLT*	19	1.000–0.953
Phosphogluconate dehydrogenase	1.1.1.44	PGDH*	7	1.000–0.967
Phosphoglycerate kinase	2.7.2.3	PGK-1*	14	1.000–0.930
		PGK-2*	13	1.000–0.975
Phosphoglucomutase	5.4.2.2	PGM-1*	72	1.000–0.600
		PGM-2*	32	1.000–0.958
Purine-nucleoside phosphorylase	2.4.2.1	PNP-1*	87	1.000–0.614
Pyruvate kinase	2.7.1.40	PK-2*	14	1.000–0.980
Triose-phosphate isomerase	5.3.1.1	TPI-1*	5	1.000–0.986
		TPI-2*	0	1.000–1.000
		TPI-3*	27	1.000–0.930
		TPI-4*	2	1.000–0.994

ture estimates derived from 100 simulated mixtures were used to evaluate the accuracy of estimated contributions to each region with mixture sizes of 100, 300, and 500 fish. We analyzed mixtures composed of fish entirely from each region and also mixtures that excluded fish from regions south and north of our marine sampling area. Precisions of the stock composition estimates for the actual mixtures were estimated by bootstrapping baseline and mixture genetic data 100 times as described in Pella and Milner (1987).

Stock compositions were estimated for June and September–October. We also combined samples over surveys and made separate estimates from samples of marked (fin-clipped and tagged hatchery fish) and unmarked fish to examine hatchery and wild stock compositions. However, because not all hatchery fish are marked, unmarked fish are a mixture of wild and hatchery fish. We therefore estimated the proportion of hatchery fish for a region in the sample of unmarked fish (P_{UH}) by

$$P_{UH} = (P_{MH}(R_U/R_M))/(S_U/S_M), \quad (1)$$

where P_{MH} = the proportion of hatchery fish from a particular region in the sample of marked fish;
R_U/R_M = the ratio of unmarked to marked releases in a region; and
S_U/S_M = the ratio of unmarked to marked fish in our ocean samples.

The R_U/R_M for 1997 and 1998 brood years varied considerably among regions: California coast 1.0, Klamath River to Cape Blanco 0.01, Oregon coast 0.12, Columbia River 0.12, southern Washington coast 0.03, northern Washington coast 0.69, Puget Sound 0.43, southern British Columbia 0.09, and Upper Fraser River 0.80 (Lavoy[1]; PSMFC[2]). We

[1] Lavoy, L. 2001. Personal commun. Washington Department of Fish and Wildlife, Olympia, WA. 98501.

[2] PSMFC (Pacific States Marine Fisheries Commission). 2001. Regional Mark Information System (RMIS) coded-wire tag on-line database. [Available from Pacific States Marine Fisheries Commission, 45 SE 82nd Dr., Suite 100, Gladstone, OR 97027-2522.]

then subtracted P_{UH} for each region from the genetic estimate of the region's contribution to the sample of unmarked fish. The sum of the remaining values estimated the proportion of wild fish in the sample of unmarked fish. When P_{UH} for a region was greater than the genetic estimate of the region's contribution to the sample of unmarked fish, the percentage of wild fish from that region was considered to be zero.

We estimated regional proportions of hatchery and wild coho salmon in the all-fish marine sample that included both marked and unmarked coho salmon. Regional hatchery contributions to the all-fish sample were made by summing each region's estimated contribution to the sampled marked and unmarked fish, weighted by the proportion of each of these sample types in the total sample. Regional proportions of wild coho salmon in the all-fish sample were made by multiplying a region's estimated proportion of wild coho salmon in the unmarked sample by the proportion of unmarked fish in the total sample.

Results

Baseline genetic data and population structure

Although coho salmon generally have low levels of genetic variability in relation to other Pacific salmon, a sufficient number of polymorphic loci were detected to distinguish many populations and regional population groups. Of 59 loci screened in all 89 populations, 56 were polymorphic, and 29 of these were at the $P_{0.95}$ level of polymorphism in at least one population (Table 2). Allelic frequencies are reported in an appendix that can be retrieved at the Northwest Fisheries Science Center website [http://www.nwfsc.noaa.gov]. Twenty of the 56 polymorphic loci had two alleles per locus, 24 had three alleles per locus, nine had four alleles, two had five alleles, and one had six alleles. Two loci (*B*GALA* and PEPC*) varied in all populations studied. Three loci (GAPDH-5*, LDH-C*, and TPI-2*) were monomorphic in all populations. Observed genotypic proportions for polymorphic loci in 128 samples departed significantly (P<0.05) from expected Hardy-Weinberg proportions in 75 of 1476 tests (5.1%). There were no consistent trends by population or locus. Because the number of significant tests is close to the number expected by chance for this rejection level, we did not attach any biological significance to these departures.

The percentages of $P_{0.95}$ loci and average heterozygosities over 56 loci for each population appear in Table 1. The percentage of $P_{0.95}$ loci ranged from only 5.4% in Lewis River hatchery early run (population 41) to 17.9% in the Mad River hatchery (4). Average heterozygosities ranged from 0.021 in Iron Gate hatchery (5) and Elk River (9) to 0.046 in Sandy River hatchery (46). Gene diversity analysis of the 89 populations resulted in a total gene diversity (H_T) of 0.035 and an average sample diversity (H_S) of 0.033. Thus, 94.5% of the total genetic diversity was attributable to within-sample variability and 5.5% was attributable to variability among samples. About 2.9% of the total gene diversity was due to variability among populations within regions, and 2.6% was due to variability among the nine regions.

Genetic relationships among populations of coho salmon as revealed by two-dimensional MDS analysis showed that genetic differences among populations were geographically structured (Fig. 2). The first axis in the plot separated populations in coastal Oregon and California from northern populations. Several populations, including two from the Rogue River in southern Oregon (numbers 7 and 8) and Big Qualicum hatchery (85) on Vancouver Island, were positioned near the convergence of the southern and northern population groups. The Iron Gate hatchery sample (5) from the Klamath River, California, clustered with the northern population group. Several genetically discrete groups appeared on smaller geographical scales. However, samples from Iron Gate hatchery (5), Yaquina River (27), Nehalem hatchery (33), Willapa Bay area (50, 51, and 52), Dungeness hatchery (64), McGovern Creek (74), upper Cascade River (81), and Ennis Creek (82) did not cluster with nearby populations. The single population in our study from the upper Fraser River region—Spius hatchery (89) of the Thompson River–was the most genetically distinct in the MDS analysis (x=–2.3, y=–0.9) and was positioned beyond the scaling shown in Figure 2. The Little River (2) population also fell outside the area of the plot (x=5.1, y=2.0), but was genetically most similar to other California coastal populations (1, 3, and 4).

Genetic estimates of simulated stock mixtures

One demonstration of discreteness among regional groups is the correct allocation in a mixed-stock analysis of simulated samples from baseline populations to their stock of origin. We used simulated sample sizes of 100, 300, and 500 taken from one region at a time; therefore the results represent the accuracy of reallocation back to the region of origin. Table 3 presents the average values of 100 bootstrap resamplings of both the baseline and the mixture samples. For simulated sample sizes of 100, reallocation accuracy ranged from 81% (coastal northern Washington) to 98% (upper Fraser River population) and averaged 88.7% over the nine regions. Average accuracy increased to 92.9% with an increase in the size of the simulated sample to 300. Only marginal improvement (93.6% accuracy) was achieved by increasing the simulated sample size to 500.

We also used mixed-stock analysis of simulated samples to examine the accuracy of composition estimates for California, Puget Sound, and British Columbia regions when fish from these areas were not present in mixtures. Average values for sample sizes of 100 ranged from 0% (California coast, upper Fraser River) to 4% (Oregon coast) and averaged 1.8% over the five regions (Table 4). Increased sample sizes of 300 and 500 resulted in small improvements in average accuracy (1.4% and 1.0 %).

Stock compositions of ocean-caught coho salmon

Genotypes for 56 loci were scored for 730 juvenile coho salmon captured in ocean trawls in 1998–2000 (Table 5). About 65% of the 455 fish in June trawls were sampled

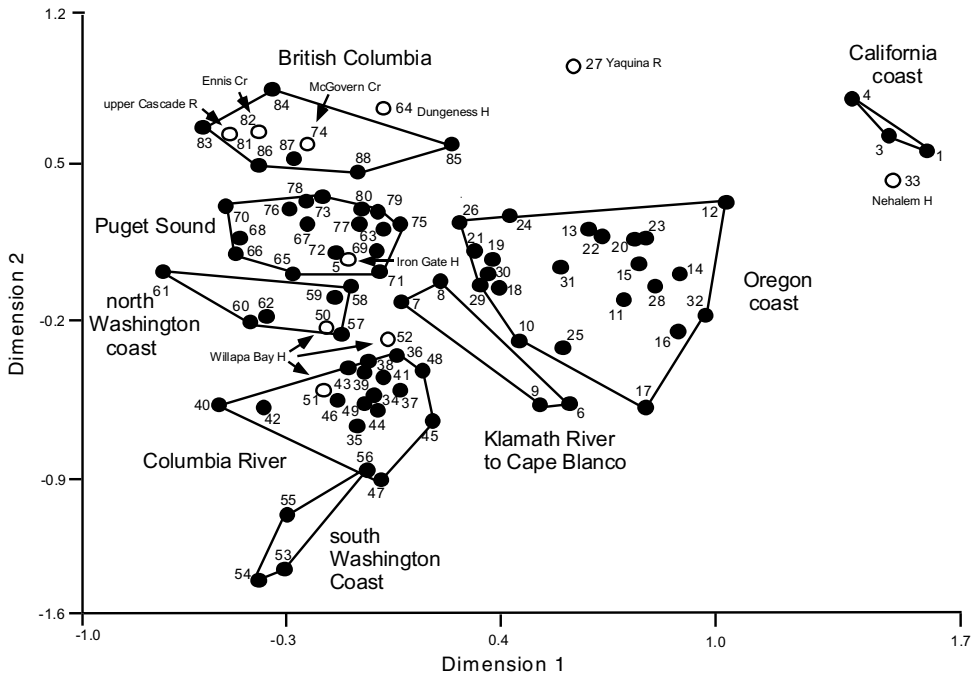

Figure 2

Multidimensional scaling (MDS) of Cavalli-Sforza and Edwards (1967) chord distances based on 56 allozyme loci between samples of 89 populations of coho salmon extending from northern California to southern British Columbia. Location numbers are given in Table 1 and Figure 1. Populations within regions are identified with polygons where possible. Open circles indicate populations that did not cluster closely with nearby populations. Populations 2 from the California coast and 89 from the upper Fraser River fall beyond the scale of the plot.

Table 3

Mean estimated percentage contributions (± standard deviations) of 100 bootstrap resamplings of mixtures composed of fish from only one region. Population numbers are explained in Table 1.

Region (populations)	n=100 Estimate	n=300 Estimate	n=500 Estimate	Region of largest misallocation
California coast (1–4)	95 ±4	97 ±3	97 ±3	Oregon coast
Klamath River to Cape Blanco (5–9)	94 ±5	96 ±3	96 ±3	Columbia River
Oregon coast (10–33)	86 ±7	91 ±4	92 ±3	Klamath River to Cape Blanco
Columbia River (34–49)	84 ±8	92 ±3	93 ±3	Oregon coast
South Washington coast (50–56)	88 ±8	95 ±3	95 ±3	North Washington coast
North Washington coast (57–63)	81 ±10	88 ±5	90 ±4	Puget Sound
Puget Sound (64–82)	85 ±7	90 ±5	90 ±4	British Columbia
British Columbia (83–88)	83 ±9	88 ±6	90 ±5	Puget Sound
Upper Fraser River (89)	98 ±2	99 ±1	99 ±1	Columbia River

in the two northern most transects along the Washington coast, 24% in three transects closest to the Columbia River, and 10% in the four most southern transects along the Oregon coast. Samples from these three areas comprised 43%, 23%, and 33%, respectively, of the 275 fish caught in September trawls. The numbers of offshore juveniles caught in 1998 were too small to provide accurate mixed-stock estimates; therefore the ocean samples collected in 1999 and 2000, and a sample pooled over 1998–2000, were analyzed separately. In the 1998–2000 pooled sample, Columbia River populations were estimated to be the major contributing regional group in June (47%, SD=6%)

Table 4

Actual percentage composition and mean estimated percentage contributions (± standard deviations) of 100 bootstrap resamplings of mixtures composed of 100, 300, and 500 fish. Population numbers are explained in Table 1.

Region (populations)	Actual	*n*=100 Estimate	*n*=300 Estimate	*n*=500 Estimate
California coast (1–4)	0	0 ±1	0 ±0	0 ±0
Klamath River to Cape Blanco (5–9)	0	3 ±4	2 ±2	1 ±2
Oregon coast (10–33)	20	21 ±8	20 ±5	20 ±4
Columbia River (34–49)	50	44 ±11	46 ±6	48 ±5
South Washington coast (50–56)	15	14 ±8	14 ±4	14 ±3
North Washington coast (57–63)	15	10 ±7	12 ±5	12 ±4
Puget Sound (64–82)	0	4 ±5	4 ±3	3 ±2
British Columbia (83–88)	0	2 ±3	1 ±2	1 ±1
Upper Fraser River (89)	0	0 ±1	0 ±1	0 ±0

and September (32%, SD=9%). The Oregon coastal region contributed about 18% (SD=5%) to the June mixture and 21% (SD=7%) to the September sample. The estimated contribution of Puget Sound fish to the pooled ocean samples was much greater in September (17%, SD=7%) than it was in June (3%, SD=2%).

Genetic mixed-stock analysis of ocean-caught hatchery fish with CWTs provided a direct comparison of genetic estimates and a mixed-stock sample of known origins (Brodziak et al. 1992). Only 41 fish had CWTs (Table 6). No fish with CWTs appeared in the 1998 sample. Most of the fish with CWTs in 1999 and 2000 originated from Columbia River (68%, *n*=28) and Oregon coastal (12%, *n*=5) hatcheries. In the genetic analysis of the 41 fish, Columbia River hatcheries were estimated to contribute about 22 fish (53%, SD=21%). Approximately 7 fish (16%, SD=17%) were estimated to originate from Oregon coastal hatcheries.

Of the 730 juveniles sampled during the study, 501 (69%) bore hatchery marks (clipped adipose fins). The percentage of unmarked fish in the September sample (35%) was greater than that in June (29%). Genetic mixed-stock estimates for hatchery-marked fish alone indicated that 69% (SD=6%) originated from the Columbia River and 14% (SD=4%) from Oregon coastal hatcheries (Table 7). The sample of unmarked fish, which contained a mixture of wild and unmarked hatchery fish, was estimated to have a much smaller proportion of Columbia River fish (20%, SD=8%) but a larger proportion of coastal Oregon (36%, SD=9%) and northern Washington (25%, SD=7%) fish (Table 7). About 30% of unmarked fish in the pooled ocean sample originated from hatcheries (Eq. 1) and 70% from wild populations. Estimated contributions from hatchery and wild populations of all ocean juveniles sampled (marked and unmarked) were 78% and 22%, respectively. Coho salmon originating in the Columbia River were estimated to comprise 54% of the total sample, but only 1% consisted of wild fish. Oregon coastal rivers contributed 21% to the total ocean sample, and nearly equal proportions were contributed from hatcheries and wild populations.

Discussion

Usefulness of coho salmon allozyme data for mixed-stock analysis

Although the gene diversity analysis indicated that the level of allele-frequency differentiation among populations within regions was similar to that between regions, further analyses showed that the magnitude of regional differentiation in the baseline was sufficient to provide accurate mixed-stock estimates. First, we found several genetically discrete population groups of coho salmon over an area extending from California to southern British Columbia. Most of the samples in the MDS plot clustered with nearby samples, and the north-south arrangement of neighboring population groups indicated that isolation by distance is an important component of genetic population structure on this geographic scale. As with other species of Pacific salmon, natal homing to spawning areas is an important isolating mechanism between populations of coho salmon.

Second, the analysis of simulated stock mixtures also demonstrated that regional differences were sufficient to provide reliable estimates of coho salmon stock compositions. Accurate estimates were obtained from simulated sample sets composed of 100% contributions from each region (Table 3). Third, a more rigorous test of the adequacy of the baseline was made by comparing genetic estimates with direct determinations based on CWTs. These estimates were reasonably accurate, especially for the largest contributing regions (Table 6), given the small sample of only 41 fish bearing CWTs. Both the simulation and CWT mixture results are consistent with the findings of Wood et al. (1987) that estimation accuracy decreases substantially when mixture sample sizes are small and when genetic separation among stocks is limited. Lastly, the analyses of ocean-caught mixture samples themselves appeared to provide reasonable composition estimates (Table 5). Additionally, estimates for samples pooled over years tended to be intermediate between the two annual estimates, as would be expected from pooling.

Table 5

Estimated percentage stock compositions (standard deviations), sample sizes (n), and recoveries of coded wire tags (CWT) for coho salmon sampled in trawl surveys along the Oregon and Washington coasts in June and September 1998, 1999, and 2000. Stock compositions were not estimated for June (n=43) and September (n=18) 1998 because of small sample sizes. None of the 1998 samples contained coded wire tags.

	June		September	
Region	Est.	CWT	Est.	CWT
1999				
n	278		152	
California coast	0 ±1	0	0 ±0	0
Klamath River to Cape Blanco	6 ±6	0	0 ±0	0
Oregon coast	25 ±7	5	25 ±8	0
Columbia River	46 ±9	8	20 ±14	4
South Washington coast	11 ±4	2	9 ±5	0
North Washington coast	10 ±5	2	18 ±15	0
Puget Sound	3 ±4	0	25 ±9	1
British Columbia	0 ±1	0	3 ±3	0
Upper Fraser River	0 ±0	0	0 ±0	0
2000				
n	134		105	
California coast	0 ±0	0	1 ±3	0
Klamath River to Cape Blanco	1 ±7	0	0 ±0	0
Oregon coast	11 ±7	0	17 ±8	0
Columbia River	40 ±11	11	48 ±16	5
South Washington coast	17 ±7	0	6 ±9	0
North Washington coast	21 ±11	0	10 ±16	0
Puget Sound	11 ±8	0	14 ±7	1
British Columbia	0 ±0	0	3 ±8	2
Upper Fraser River	0 ±0	0	0 ±0	0
1998, 1999, and 2000 combined				
n	455		275	
California coast	0 ±0	0	0 ±0	0
Klamath River to Cape Blanco	7 ±4	0	0 ±0	0
Oregon coast	18 ±5	5	21 ±7	0
Columbia River	47 ±6	19	32 ±9	9
South Washington coast	11 ±3	2	9 ±4	0
North Washington coast	13 ±4	2	19 ±11	0
Puget Sound	3 ±2	0	17 ±7	2
British Columbia	0 ±0	0	2 ±2	2
Upper Fraser River	0 ±0	0	0 ±0	0

Nonetheless, the usefulness of the allozyme baseline that we compiled for coho salmon is limited by two factors. First, few samples in the baseline are from California and British Columbia populations. Although the baseline appears to be adequate to analyze stock mixtures of juvenile coho salmon off Oregon and Washington, mixed stock analyses of samples from other marine areas, particularly to the north, requires the sampling of additional populations. Second, our study demonstrated that estimates of stock compositions are not sufficiently accurate to effectively identify stock groups that are absent from mixtures or present in small proportions (Tables 4 and 6). Estimation accuracy can be improved by using additional gene markers. These markers will likely be based on DNA variability because coho salmon minisatellite (Miller et al., 1996; Beacham et al., 1996) and microsatellite (Small et al., 1998a; 1998b; Beacham et al., 2001) loci show much higher levels of polymorphism than do allozyme loci. Recently, variation at eight microsatellite DNA loci and one Mhc locus in coho salmon populations in British Columbia and Washington was used to estimate the stock compositions of fisheries off the west coast of Vancouver Island (Shaklee et al., 1999; Beacham et al., 2001). However, the use of highly polymorphic microsatellite loci may not provide increased discrimination among populations on large geographical scales because of allelic convergence from multiple mutations (Nauta and Weiss-

ing, 1996). Nonetheless, the extension of a DNA baseline to include populations in Oregon and California, may resolve fine-scale (geographic and temporal) differences between coho salmon populations in southern coastal areas.

Stock compositions of ocean-caught juvenile coho salmon

Studies using large purse seines conducted in 1981–85 revealed that juvenile coho salmon were the most abundant of the *Oncorhynchus* species in the nearshore areas along the Oregon and Washington coasts (Pearcy and Fisher, 1988; 1990). Pearcy and Fisher (1988; 1990) captured hatchery-tagged juvenile coho salmon and concluded they were not highly migratory, often remaining close to their point of sea entry for several months. Our genetic results corroborate that finding. Genetic estimates indicate that about 89% of ocean juveniles caught in June and 81% in September originated from the Columbia River and adjacent coastal rivers. Recoveries of hatchery-tagged fish (n=41) also indicate that juveniles remain near river mouths in their first few months after ocean entry; only three of these CWT-marked fish came from hatcheries in other regions.

However, our genetic results indicate that a change has occurred in the distribution of Washington coastal and Puget Sound juvenile coho salmon. In the 1980s, juvenile coho salmon from Washington coastal hatcheries were not recovered along the Washington and Oregon coasts after mid summer, apparently having migrated northward (Pearcy and Fisher, 1988). Pearcy and Fisher (1990) also found that Puget Sound coho salmon did not migrate along the Washington and Oregon coast until sometime between their first and second summer at sea. However, our genetic results showed that in 1998–2000 fish from Washington coastal streams and hatcheries comprised substantial proportions of the juveniles in nearshore areas along the Washington and Oregon coast in both early and late summer (24% and 28%). We also found that juvenile coho salmon from Puget Sound are present in late summer. Our finding that coho salmon from northern stocks move south along the coast during their first summer was substantiated by the catch of CWT-marked fish originating from Puget Sound (n=2) and southern British Columbia (n=2).

Recent reductions in the number of coho salmon smolts released from the region's hatcheries have not resulted in a decrease in the proportion of hatchery juveniles along the Oregon and Washington coasts. Annual releases of hatch-

Table 6

Actual composition and estimated contributions (± standard deviations) of a mixture of 41-CWT fish.

	Actual		Genetic estimate	
Region	Number	%	Number	%
California coast	0	0	1	3 ±4
Klamath River to Cape Blanco	0	0	0	0 ±0
Oregon coast	5	12	7	16 ±17
Columbia River	28	68	22	53 ±21
South Washington coast	2	5	0	0 ±0
North Washington coast	3	7	5	11 ±9
Puget Sound	1	2	5	11 ±18
British Columbia	2	5	2	6 ±11
Upper Fraser River	0	0	0	0 ±0

Table 7

Estimated percentage stock compositions and sample sizes for populations of marked (fish with clipped adipose fins) and unmarked coho salmon sampled in trawl surveys along the Oregon and Washington coasts in 1998, 1999, and 2000. Samples from June and September were combined. Separate estimates for the contributions of hatchery and wild stocks were made by using estimates of hatchery marking rates for each region.

	Marked fish (hatchery fish)	Unmarked fish (hatchery and Wild fish)			All fish		
Region	Genetic estimate (n=501) (%)	Genetic estimate (n=229) (%)	Hatchery (%)	Wild (%)	Hatchery (%)	Wild (%)	Total (%)
California coast	0 ±0	1 ±2	0	1	0	0	0
Klamath River to Cape Blanco	1 ±2	1 ±7	0	1	1	0	1
Oregon coast	14 ±4	36 ±9	4	32	11	10	21
Columbia River	69 ±6	20 ±8	18	2	53	1	54
South Washington coast	4 ±4	9 ±5	0	9	3	3	6
North Washington coast	1 ±7	25 ±7	2	23	1	7	8
Puget Sound	6 ±5	8 ±5	6	2	6	1	7
British Columbia	5 ±2	0 ±0	0	0	3	0	3
Upper Fraser River	0 ±0	0 ±0	0	0	0	0	0
Total	100	100	30	70	78	22	100

ery smolts exceeded 64 million fish during the early 1980s but have decreased to about 39 million in recent years, a 40% reduction (PSMFC[2]; NRC[3]). Nonetheless, the proportion of hatchery coho salmon in nearshore marine waters has remained high, averaging 74% in 1981–85 (Pearcy and Fisher, 1990) and 78% in 1998–2000 (present study). This result, therefore, leads to the conclusion that the number of naturally produced juveniles in Oregon and Washington coastal waters has also decreased proportionately during this period. If so, wild populations of coho salmon may also have experienced a decline in abundance on the order of 40%.

Steep declines in Columbia River wild populations are particularly evident. At the beginning of the 20th century, populations in the Columbia River are thought to have been the largest producers of coho salmon in the region (Chapman, 1986; Lichatowich, 1989) and likely contributed a substantial proportion to the nearshore population of juvenile salmon. At present, Columbia River juveniles predominate along the coast. However, these fish are almost entirely releases from hatchery facilities and Columbia River wild coho salmon are conspicuously absent.

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