Assessing genetic diversity of protected coho salmon (*Oncorhynchus kisutch*) populations in California

Katherine A. Bucklin, Michael A. Banks, and Dennis Hedgecock

Abstract: California coho salmon (*Oncorhynchus kisutch*) are under legal protection owing to significant declines in abundance over the last decades. Previously, California coho salmon were characterized as having low genetic diversity and weak population subdivision, attributable potentially to homogenization by out-of-basin hatchery releases. Here, diversity at seven highly polymorphic microsatellite DNA markers is assessed within and among 32 collections of coho salmon from 16 California watersheds. In 71% of local populations, genotypic composition deviates significantly from that expected under the assumption of random mating. We develop and apply methods to adjust for two potential causes of deviation from random mating expectations: (*i*) Wahlund effects, owing to heterogeneous collections of individuals, and (*ii*) the "Allendorf–Phelp's effect", owing to closely related juveniles in samples. Such population-level "adjustments" reduce within-region and increase among-region variance; after adjustment, we find strong concordance of genetic and geographic distances. We conclude that stock transfers have had minimal impact on population structure and that California coho salmon populations likely comprise small numbers of endemic breeders, potentially experiencing high levels of genetic drift and inbreeding.

Résumé : Les saumons coho (*Oncorhynchus kisutch*) de la Californie bénéficient d'une protection légale à cause de leur déclin significatif au cours des dernières décennies. Dans le passé, on caractérisait les saumons coho de la Californie par une faible diversité génétique et de faibles subdivisions de la population, à cause potentiellement de l'homogénéisation résultant d'empoissonnements à partir de piscicultures situées dans d'autres bassins versants. Nous évaluons ici la diversité à sept marqueurs microsatellites de l'ADN fortement polymorphes dans 32 récoltes de saumons coho de 16 bassins versants de la Californie et entre ces récoltes. Chez 71 % des populations locales, la composition génotypique diffère significativement de celle qu'on attendrait si les accouplements se faisaient au hasard. Nous mettons au point et utilisons des méthodes pour tenir compte de deux causes potentielles de déviation de l'accouplement au hasard : (*i*) les effets Wahlund par suite de collections hétérogènes d'individus et (*ii*) « l'effet Allendorf–Phelp » par suite d'échantillonnage comprenant des jeunes fortement apparentés. De tels « ajustements » au niveau de la population réduisent la variance à l'intérieur des régions et augmente celle qui existe entre les régions; après l'ajustement, il existe une forte concordance entre les distances génétiques et géographiques. Nous concluons que les transferts de stocks ont eu un impact minimal sur la structure de population et que les populations de saumons coho de la Californie contiennent vraisemblablement un petit nombre de reproducteurs endémiques et qu'elles subissent potentiellement un fort degré de dérive génétique et de consanguinité.

[Traduit par la Rédaction]

Introduction

Salmonid conservation requires identification of appropriate management units in complex, geographically structured hierarchies of populations. The challenges are to identify how biodiversity is influenced by geography (Carney et al. 1994; Waples et al. 2001), life history and ecology (Waples et al. 2001), hatchery production (Duchesne and Bernatchez 2002; Nickelson 2003; Saisa et al. 2003), and environmental degradation (Beechie et al. 1994; Nickelson and Lawson 1998; Pess et al. 2002). Under the US Endangered Species Act (ESA), the National Marine Fisheries Service grants protection to populations or "distinct population segments" called evolutionarily significant units (ESU), which are entities below the species level that can be listed under the ESA if (*i*) they are substantially reproductively isolated from other conspecific populations and (*ii*) represent an important component in the evolutionary legacy of the species (Waples 1991; Waples et al. 2001). The homing fidelity of salmon to natal spawning streams increases the potential for reproduc-

Received 6 February 2006. Accepted 15 November 2006. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 19 January 2007.
J19152

K.A. Bucklin[1] and M.A. Banks. Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365, USA.
D. Hedgecock. Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.

[1]Corresponding author (e-mail: kbucklin@sbcglobal.net).

 doi:10.1139/F06-171

tive isolation among populations and local adaptation over evolutionary time. If local adaptation is an important factor in maintaining genetic diversity among populations or groups of populations, then declining populations are at greater probability of experiencing local extinctions, which are permanent losses of diversity and evolutionary potential (Waples et al. 2001).

In the Pacific Northwest, endemic coho salmon (*Oncorhynchus kisutch*) populations have recently undergone significant reductions in population census size (Brown et al. 1994; Weitcamp et al. 1995; Frankham et al. 2002). In California, the census number of wild coho salmon spawning today is less than 6% of what it was in the 1940s (which was already smaller than historical abundance), with most spawning populations consisting of fewer than 100 individuals (Brown et al. 1994). Small numbers of breeders, combined with homing fidelity to natal spawning streams, may increase the rate of genetic drift, reducing within-population genetic variation and increasing the likelihood of inbreeding and inbreeding depression (Wright 1931; Reed et al. 2002; England et al. 2003). However, accurate sampling and identification of population units and assessment of within- and among-population diversity become problematic when spawner densities are very low. Previous allozyme studies of California and Oregon coho salmon have reported low levels of genetic variation and population subdivision (Olin 1984; Bartley et al. 1992). These findings may simply reflect small sample sizes or lower levels of protein polymorphism compared with those of other Pacific salmon (Wehrhahn and Powell 1987), high straying rates, invariant life history traits (Waples et al. 2001), or stock transfers.

Until recently, out-of-basin and even out-of-state transfers of salmon stocks were routine across much of the Pacific Northwest. In California, for example, a coastal coho salmon stock from the Noyo River was planted in at least 27 northern California watersheds (Weitcamp et al. 1995; L. Weitcamp, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112-2097, USA, unpublished data). Widespread planting of hatchery stocks can homogenize genetic differences among populations and ESUs (Reisenbichler and Phelps 1989; Krueger and May 1991; but see Utter 2004). The extent to which transfers may disrupt adaptations to local environmental conditions, especially at the southern end of the coho salmon range, is unknown.

Here, we take a novel approach in our attempt to describe genetic diversity within and among 32 collections of coho salmon from 16 California watersheds. We identify and grapple with profound problems that arise from attempting to identify and classify units of diversity in very small populations. We develop new methods for partitioning diversity within a stream into within- and among-deme components using a suite of highly polymorphic microsatellite markers. We also develop methods to utilize juvenile collections (in many cases, the only adequate population sample available), in spite of Allendorf and Phelps' (1981) cautionary advice that spawners provide more accurate estimates of among-population diversity. Our approach is an advance over previous work, which typically had the luxury of sampling adults and could identify collections only to stream and year. Our main goals were to test the efficacy of current ESU designations, using markers more polymorphic than those used previously, to determine if declining run sizes have affected population structure, or if past stock transfers have reduced genetic diversity among populations or regions. To identify the geographic scale of coho salmon population structure, we (*i*) employ environmental and biological data to partition genetic diversity within collections, (*ii*) determine relatedness in samples comprised of juveniles, (*iii*) examine temporal genetic variation among year classes, (*iv*) estimate genetic divergence among spawning runs, drainages, and regions, and finally (*v*) assess influence of hatchery plantings and reductions in abundance.

Materials and methods

Population samples

The 1483 individuals included in this analysis were collected from 32 sites in 16 watersheds or tributaries of major watersheds (Table 1) representing the southern end of the species range from the southern end of the northern California – southern Oregon (NC/SO) ESU and the central California coast (CCC) ESU, which extends south to Santa Cruz County (Weitcamp et al. 1995) (Fig. 1). Nine sites were sampled more than once in different years (Table 1), permitting study of temporal genetic variation. When breeding adults were not numerous, juvenile samples were acquired by seining or electrofishing. When high numbers of juveniles were encountered, a majority of individuals from each pool was sampled, a subset of which was later selected for genetic analyses to ensure random representation. Tissue samples for genetic analyses were obtained from juvenile caudal fins (nonlethal), caudal or operculum punches of hatchery adults, and caudal, operculum, or dorsal muscle tissue of wild adult carcasses. The data accompanying each sample varied but always included watershed, tributary, and location collected, collector, and date collected. In some cases, sex (adults), fork length, hatchery identification mark, and river mile were also included (see Table 1). Permits for tissue collection and holding were obtained from the California Department of Fish and Game (CDFG) and National Marine Fisheries Service (NMFS) for populations listed as threatened under the Endangered Species Act.

Microsatellite markers and molecular methods

Sixty-nine published salmonid microsatellite DNA markers were surveyed to determine candidate loci with high information content for California coho salmon. The screening process used samples from Scott Creek (Santa Cruz County), Noyo River (Mendocino County), Eel River (Humboldt County), and Smith River (Del Norte County) to examine variability and assess potential diagnostic power. Thirty-six markers were discarded because they did not amplify product or were not adequately polymorphic in the populations screened. Eighteen of the remaining 33 candidate markers amplified product. From these, we selected six loci with high numbers of alleles that amplified well in multiplexed reactions and achieved an assignment stringency of over 90% in the four populations screened. A preliminary analysis suggested that these six loci did not make greater than average contribution to population structure compared with the 18 variable candidate loci. Additionally, the microsatellite *iso-Ots-2*, which is known to have species-specific

Table 1. Samples of coho salmon (*Oncorhynchus kisutch*) collected from 16 California watersheds.

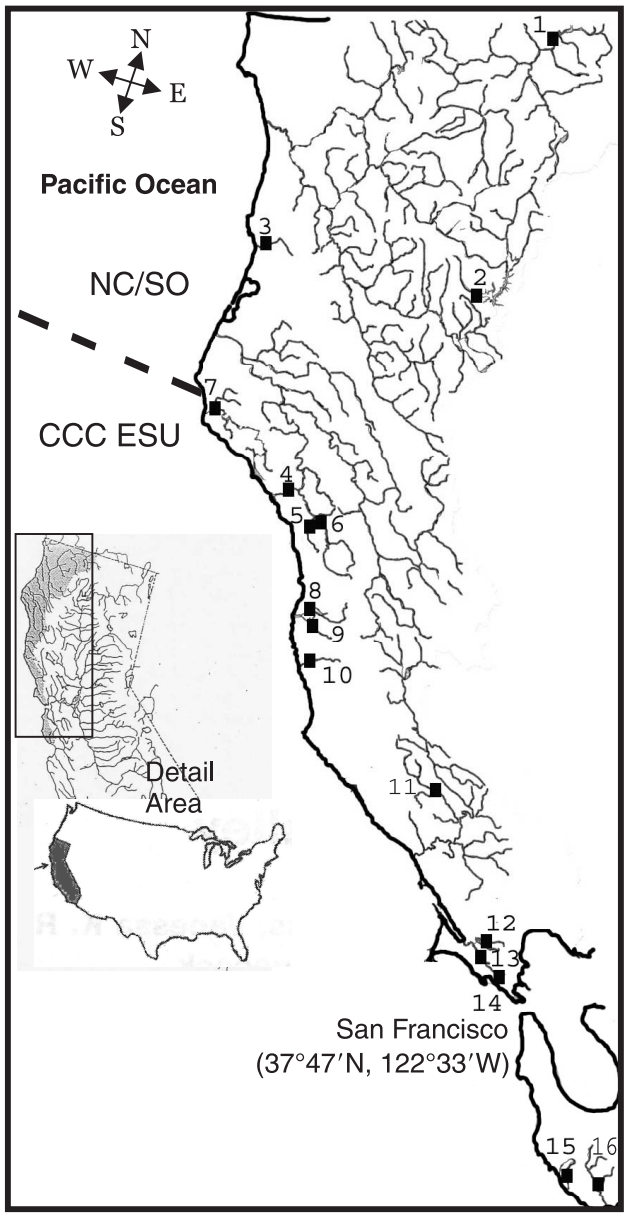
Watershed (population)	Tributary or site	*N*	Stage	Year collected	Name code	Criteria
1. Klamath River	Iron Gate Hatchery	45	A	1997/1998	KIHA97ANL	Ad, No, Left clip, FL 41–78 cm
Klamath River	Iron Gate Hatchery	36	A	1997/1998	KIHA97ML	Left clip, FL > 56 cm
2. Trinity River	Trinity River Hatchery	17	A	1997/1998	TRHA97S	FL < 45 cm
Trinity River	Trinity River Hatchery	77	A	1997/1998	TRHA97L	FL > 53 cm
3. Little River (Humboldt)	Little River Delta	72	S	2000	LRS00E	3–6 April 2000
Little River	Little River Delta	22	S	2000	LRS00L	20 April – 29 May 2000
4. SF Eel River	Hollowtree Creek	16	A	1997/1998	EHOLA97	—
5. SF Eel River	Redwood Creek	88	S	1997	EREDS97	3
6. SF Eel River	Redwood Creek	20	A	1998/1999	EREDA98	—
7. SF Eel River	SF Sproul Creek	34	S	1999	ESPRS99	—
8. Mattole River	Mattole River Delta	91	S	1998	MATS98	2, 3
9. Pudding Creek	Pudding Creek	78	Y	1998	PUDY98	3, 4
10. SF Noyo River	Egg-taking station	42	A	1997/1998	NOYA97	—
11. SF Noyo River	Egg-taking station	42	A	1999/2000	NOYA99	—
12. Albion	Albion Mainstem	20	A	1998/1999	ALBA98	2, 4
13. Albion River	Marsh Creek	18	Y	1998	ALBY98	—
14. Russian River	Warm Springs Hatchery	33	A	1995/1996	RRHA95	—
15. Russian River	Warm Springs Hatchery	25	A	1996/1997	RRHA96	—
16. Russian River	Warm Springs Hatchery	7	Y	1997	RRHY97	—
17. Russian River	Green Valley Creek	7	Y	1997	RRGV97	—
18. Russian River	Green Valley Creek	73	Y	1998	RRGV98	4
19. Russian River	Green Valley Creek	8	Y	2000	RRGV00	—
20. Lagunitas Creek	Lagunitas, San Geronimo	40	A	1996/1997	LAGA96	2, 3, 4, 5
21. Lagunitas Creek	Lagunitas, San Geronimo	71	A	1997/1998	LAGA97	2, 3, 4, 5
22. Lagunitas Creek	San Geronimo, Arroyo	30	Y	1998	LAGY98	4
23. Olema Creek	Mainstem	66	A	1996/1997	OLEA96	2, 3, 4, 5
24. Olema Creek	Mainstem	32	A	1997/1998	OLEA97	2, 3, 4, 5
25. Olema Creek	Mainstem	88	Y	1998	OLEY98	4
26. Redwood (Marin)	Mainstem	13	A	1997/1998	RWMA97	2, 3, 4, 5
27. Redwood (Marin)	Mainstem	24	Y	1998	RWMY98	4
28. Waddell Creek	Mainstem	42	Y	1999	WADY99L	RM 3.1 – 3.9
Waddell Creek	Mainstem	17	Y	1999	WADY99U	RM 4.7
29. Scott Creek	Hatchery	40	A	1995/1996	SCA95	—
30. Scott Creek	Hatchery	57	A	1997/1998	SCA97	—
31. Scott Creek	Hatchery	32	A	1998/1999	SCA98	—
32. Scott Creek	Mainstem, Big and Mill creeks	40	Y	1999	SCY99L	RM 2.55–3.55 B&M
Scott Creek	Mainstem at RM 4.9	10	Y	1999	SCY994.9	RM 4.9
Scott Creek	Upper Fork	10	Y	1999	SCY99UF	RM UF

Note: SF, South Fork. Initial populations are numbered (1–32) and designated by their name codes in subsequent tables and figures. Stages: A, adults; S, smolts; Y, young of the year. Subdividing criteria: (1) clip type (adipose (Ad.), No, Left); (2) fork length (FL); (3) date collected; (4) river mile (RM); (5) sex. When the above criteria were variable but not significant, the above numbers are shown (1–5); —, locations with no data.

differences, was included to ensure species identity (Greig et al. 2002), yielding a total of seven variable loci.

DNA was extracted using the Puregene™ DNA isolation kit (Gentra Systems Inc., Minneapolis, MN 55441, USA) in a 96-well format. Using a thermocycler (PTC-100; MJ Research, Inc., Hercules, CA 94547, USA), we amplified genomic DNA in three multiplexed reactions: (*i*) *Ots-103* (Nelson and Beacham 1999), *Ots-2* (Banks et al. 1999), and *iso-Ots-2* (Greig et al. 2002); (*ii*) *Ots-3* (Banks et al. 1999) and *One-13* (Scribner et al. 1996); and (*iii*) *P-53* (Park et al. 1996) and *Oki-1* (Smith. et al. 1998), each with a total volume of 10 μL. Reaction conditions for all primer combinations were 94° for 2 min, 94° for 30 s, 60° for 30 s, and 72° for 30 s for 34 cycles. Polymerase chain reaction (PCR) products were size-fractionated by polyacrylamide gel electrophoresis (PAGE) on a 45.0 cm wide × 22.5 cm high 8% denaturing gel at 50 W for 150 min. The forward PCR primer was labeled with a fluorescent phosphoamidite (HEX, FAM, or fluorescein). DNA fragments were visualized on the FMBIO® fluorescent imaging system (Hitachi

Fig. 1. Map of northern California, USA, showing the 16 watersheds from which coho salmon (*Oncorhynchus kisutch*) were collected for population genetic analyses. Numbers correspond to the following watershed or hatchery locations, ordered from north to south based on river mouth: (1) Klamath River, Iron Gate Hatchery; (2) Trinity River, Trinity River Hatchery; (3) Little River; (4) Eel River, Hollowtree Creek; (5) Eel River, Redwood Creek; (6) Eel River, Sproul Creek; (7) Mattole River; (8) Noyo River; (9) Pudding Creek; (10) Albion River; (11) Russian River, Warm Springs Hatchery; Russian River, Green Valley Creek; (12) Lagunitas Creek; (13) Olema Creek; (14) Redwood Creek; (15) Waddell Creek; (16) Scott Creek, Scott Creek Hatchery. NC/SO, northern California – southern Oregon evolutionarily significant unit (ESU); CCC, central California coast.



Software Engineering America Ltd., South San Francisco, CA 94080, USA). The sizes of individual bands were scored relative to MapMarker® standards (70–400 bp; BioVentures, Inc., Murfreesboro, TN 37133-2561, USA) and analyzed with Bio Image software (Bio Image Systems, Inc., Jackson, MI 49203, USA), or by eye. To minimize genotype-scoring errors, individuals were genotyped twice from two independent PCR reactions, and among-tray scoring variation was controlled by co-electrophoresing eight individuals from each of the 20 trays in two sets of gels. The genotype scores were double-checked for accuracy and independently verified by at least one other researcher.

Loci were ranked by number of alleles, from most polymorphic to least, to identify the most informative loci to detect family structure within collections of individuals taken from a watershed in a single year. Individuals with "sufficient" data were those for which (*i*) separate electrophoretic analyses produced repeatable genotypes and (*ii*) the four most polymorphic loci (*Ots-103*, *iso-Ots-2*, *One-13*, *Oki-1*) amplified, or five loci successfully amplified, including three of the above most polymorphic loci. For juvenile kinship analyses, amplification of three of the four most polymorphic loci was the minimal requirement.

Statistical methods

Population genetic parameters

We tested Hardy–Weinberg genotypic proportions (HWE) for each locus in each population using GENEPOP (version 3.3; Raymond and Rousset 1995). A Markov chain method was used to determine, without bias, the exact *P* value of the test (Guo and Thompson 1992).

Allele frequencies, observed and expected proportions of heterozygotes, linkage disequilibrium (LD), and *F* statistics were calculated using GENETIX (version 4.02; Laboratoire Génome et Populations, Université de Montpellier II, http://www.univ-montp2.fr/~genetix/index.htm). Within-population (F_{IS}) and among-population (F_{ST}) significance was determined by random permutation of alleles. Homogeneity of populations was determined by testing the significance of F_{ST} between all pairs of sites within major watersheds. For each test, the data set was permuted 10 000 times, and the threshold significance ($\alpha = 0.05$) was adjusted for *n* simultaneous tests using Sidak's correction $(1 - (1 - \alpha)^{1/n})$. LD was judged to be significant when more than three pairs of 21 (19%) were significant at the 5% α level, a proportion reached only three times in 100 simulated samples of size 20 drawn from a pooled baseline population of CCC coho salmon.

The computer program PHYLIP (version 3.57c; Felsenstein 1993) was used to evaluate the spatial distribution of genetic diversity among California coho salmon populations. Cavalli-Sforza and Edwards (1967) chord measures (CSE) were calculated using GENDIST. For fitting trees to the CSE distance matrices, the program NEIGHBOR was used to construct an unrooted tree by the neighbor-joining method (Saitou and Nei 1987). To assess the significance of the nodes in the tree, bootstrap results were obtained using SEQBOOT and CONSENSE with 1000 replicates. Trees were visualized using TreeView (Page 1996).

Adjustments of samples for microgeographic structure

Heterozygote deficiency can result artificially from unwitting admixture of individuals collected from genetically differentiated demes (Wahlund effect). Adult and juvenile

collections that departed significantly from single- or multiple-locus random mating equilibria were examined for evidence of admixture. Subdivision of samples was based on genotype-independent data collected prior to analysis, such as size (fork length), clip type (hatchery release identification), collection date (day of smolt outmigration), or collection site (young of the year; river mile) (see Table 1). We tested whether partitioning of population samples produced genetically different subgroups by the significance of F_{ST}.

Partitioning of juveniles into sibships

Fifteen of the 32 collections comprised juveniles collected as young of the year or smolts. We first checked for admixture in 11 juvenile samples for which independent criteria permitted subdivision, as described above. One sample was subdivided, making 12 juvenile samples. We next applied a family adjustment procedure developed by Banks et al. (2000) to identify and remove full sibships, replacing them with their hypothetical parents. Finally, in some cases, we tested whether adjusted subpopulations could be repooled, using nonsignificance of F_{ST} as a guide.

Twelve juvenile samples with high levels of LD and (or) significant departures from HWE were adjusted for family structure. The odds of two individuals being full siblings (vs unrelated) were calculated using the program KINSHIP 1.2 (Goodnight and Queller 1999) and baseline allelic frequencies of adult samples from this study (in HWE) from the same ESU as the sample of interest. Putative family groups were partitioned by hand from the sorted KINSHIP output matrix following the rule that the majority of KINSHIP relatedness coefficients were significant at the 0.01 or 0.001 level of significance. Putative sibling groups of size 3 or greater (with the exception of the 1998 Russian River Green Valley population for which we allowed families of size 2) were examined by the computer program SIBLINGS (K.A. Bucklin, W.F. Eichert, and D. Hedgecock, unpublished data) to test for gross violations of diploid Mendelian inheritance (e.g., more than four alleles at any locus or impossible combinations of genotypes). Individuals causing such violations were discarded from the group; discards of three or more individuals were then treated as a putative sibgroup. Once Mendelian sibling groups were identified, the possible mating types for the sibship were identified (see Table 2; Banks et al. 2000). The likelihoods of all possible mating pairs were calculated and ranked. The top-ranked mating type provided the putative genotypes of the parents. After forming full-sib groups, SIBLINGS looked for families having a common parent (half siblings). All individuals in each sibling group were then removed from the data set and replaced by their putative parents.

Russian River Green Valley samples represent a special case. Samples from 70 young-of-the-year coho salmon were collected from Green Valley Creek (RRGV98), a tributary to the Russian River, on 20 July 1998. A further 58 samples were collected from the same locations on 13 October 1998. Samples taken in July could have been resampled in October. Indeed, 49 pairs of individuals (one from each of the two sampling times) had identical multilocus genotypes; we deleted one member of each pair. Twenty-four other individuals collected on either date had unique genotypes, yielding a sample of 73 unique individuals. After partitioning the sample into 16 full-sibling families, we estimated the variance effective number of breeders from the mean and variance of parent contributions following the methods of Hedrick et al. (1995).

Pooling of temporally or spatially homogeneous subpopulations

After adjustments for admixture and family structure in collections of juveniles, we tested for temporal and spatial homogeneity among collections from the same watershed using pairwise F_{ST} with significance adjusted for n populations. Populations that did not significantly differ within the same basin were combined.

Isolation by distance

A Mantel test (Mantel 1967) implemented in GENETIX (Belkhir et al. 1998) was used to test for genetic isolation by distance between all 406 adjusted population pairs (29 populations). Genetic distance was measured as F_{ST} (Weir and Cockerham 1984). Geographic distance was measured as the shortest nearshore coastal route between river mouths (N. California Gazeteer; 1 mile = 1 inch). The genetic distance matrix was permuted 1000 times, and the correlation was quantified by Pearson's coefficient (r).

Results

Genetic diversity within coho salmon populations

Genotypes at seven microsatellite DNA markers for 1483 individuals from 32 samples, representing 16 watersheds partitioned by major tributary and year class, provide the basis for initial analyses (Table 1; Fig. 1). Nine watersheds are represented by samples from multiple years; in two cases, collections represent different tributaries of the same watershed. Ten of the 32 collections represent hatchery populations, the rest are from the wild. In 15 cases for which adults were not available or not numerous enough to provide an adequate sample, we used data from juvenile samples. Average sample size per collection is 46, although it is highly variable, with the three smallest collections having only seven or eight individuals (RRGVY97, RRGVY00, and RRHY97). However, after adjustments for family structure, small samples are pooled into larger samples (see below).

We observe widespread departures from random mating expectations in 23 of 32 sample collections (71%), as measured by tests of single-locus Hardy–Weinberg equilibria (HWE) and multiloci equilibria or LD (Table 2, values in bold). These deviations occur in 11 of the 15 juvenile population samples in which departures from random mating expectations might be expected because of relatedness. However, all nine collections of hatchery adults and three out of eight wild adult collections also depart from random mating equilibrium. Adult and juvenile populations differ in the proportion of single-locus or multiloci deviations. Of the 17 adult samples noted (Table 2), eight (47%) have significant F_{IS} and nine (52%) have more than three significant pairwise LD tests. Adult hatchery collections have significantly more deviations than do wild adult collections (12 instances of high LD or significant F_{IS} in nine hatchery collections compared with four instances in the eight wild collections). By contrast, of the 15 juvenile samples listed

Table 2. Deviations from random mating genotypic proportions for 32 samples of coho salmon (*Oncorhynchus kisutch*).

Population	*N*	H_{obs}	*Ots-103*	*Ots-2*	*iso-Ots-2*	*Ots-3*	*One-13*	*P-53*	*Oki-1*	F_{IS}	LD
KIGHA97	81	0.73			*					**0.070*** **	**7/21**
TRHA97	94	0.72	***		***			*		**0.045** **	1/21
LRS00	94	0.80	***		**	**		*	*	0.014	**12/21**
EREDA98	20	0.70	***							0.061	2/21
EREDS97	88	0.72		*						0.020	2/21
EHOLA97	16	0.68	*							0.064	3/21
ESPRS99	34	0.74	*							–0.020	**4/21**
MATS98	91	0.76								0.000	**9/21**
PUDY98	78	0.70	*							**0.057** **	**4/21**
NOYA97	42	0.70	*			**				**0.064***	2/21
NOYA99	42	0.73	*							**0.080** **	1/21
ALBA98	20	0.78					**			–0.036	**6/21**
ALBY98	18	0.78						*	*	–0.023	3/21
RRHA96	33	0.75	***							**0.057***	**4/21**
RRHA97	25	0.83								–0.046	**5/21**
RRHY97	7	0.67						*		**0.120***	**5/21**
RRGV97	7	0.71							**	–0.116	0/19
RRGV98	73	0.76	***	*	**		***	*	***	**–0.040***	**15/21**
RRGV00	8	0.78							*	**–0.257** **	1/15
LAGA96	40	0.78								0.017	1/21
LAGA97	71	0.79	*							–0.001	**4/21**
LAGY98	30	0.79						**		–0.024	**8/21**
OLEA96	66	0.66				***	*			**0.099** **	**6/21**
OLEA97	32	0.77				*		**		–0.005	3/21
OLEY98	88	0.73		*	**					0.002	**4/21**
RWMA97	13	0.69	**		**					0.099	1/18
RWMY98	24	0.66	*							–0.002	0/21
WADY99	59	0.65	*		**	***				0.011	**8/21**
SCA95	40	0.71								–0.049	**4/21**
SCA97	57	0.72	*		*	**	*			**–0.047***	**16/21**
SCA98	32	0.62	***		**	*	*	**	*	**0.097** **	**12/21**
SCY99	60	0.62								–0.008	**5/21**
No. of alleles/locus			31	9	23	18	19	15	19		

Note: Observed heterozygosity by population (H_{obs}), single-locus HWE (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, shown in bold), and over all loci (F_{IS}, P), and fraction of loci pairs showing linkage disequilibrium (LD), fractions ≥ 19% shown in bold.

Table 3. Samples of coho salmon (*Oncorhynchus kisutch*) adjusted for (*a*) subdivision and (*b*) kinship with subsequent (*c*) homogeneity testing.

(*a*) Subdividing admixed population samples.

Collection	Subpopulation	*N*	F_{ST}, *P*	F_{IS}, *P*
KIGHA97	KIGHA97ANL	45	0.0259***	0.053*
	KIGHA97ML	36		0.062*
TRHA97	TRHAL	77	0.0121*	0.053**
	TRHAS	17		–0.009
LRS00	LRS00E	72	0.0111**	0.019
	LRS00L	22		–0.015
MATS98	MATS98E	70	0.0091*	0.014
	MATS98L	21		–0.061
WADY99	WADY99L	42	0.0521***	0.011
	WADY99U	17		–0.085
SCY99	SCY99L (to UF)	40	0.0774***	–0.028
	SCY99UF (to 4.9)	10	0.0436***	–0.092
	SCY994.9 (to L)	10	0.0701**	–0.055

(*b*) Sibling groups in juvenile collections.

	No. of sibling groups of size							
Population	2	3	4	5	6	≥7	No. of FS removed	No. of parents added
LRS00E		6	2				26	18
LRS00L		1					3	2
ESPRS99		2	2				14	8
MATS98E		5	3	1			32	18
MATS98L		4					12	8
PUDY98		2	1				10	6
RRHY97	1						2	2
RRGV98	3	2	3		1	2	68	22
RRGV00			1				4	2
LAGY98			1				4	2
OLEY98		4	2			1	28	14
WADY99L		1	5	1			28	14
WADY99U			1			1	11	4
SCY99L		1		2	1		19	7
Total no. of individuals	8	84	84	20	12	53	261	127

(*c*) Pooling of homogeneous subpopulations.

Subpopulation 1	Subpopulation 2	Final population	F_{ST}	F_{IS}
LRS00E	LRS00L	LRS00	0.0045	0.0110
MATS98E	MATS98L	MATS98	0.0014	–0.0100
RRHA96	RRHA97	RRHA	0.0128	0.0249
RRHA96	RRHY97	—	–0.0033	—
RRHA97	RRHY97	—	0.0077	—
LAGA96	LAGA97	LAGA	0.0049	0.0104
RRGV97	RRGV00	RRGV9700	0.1485	–0.0118
OLEA97	OLEY98	OLEAY	0.0003	0.0224
SCA97	SCA98	SCA9798	0.0077	0.0025
SCY99L	SCY994.9	SCY99	0.0205	–0.0146

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. FS, full sibs.

(Table 2), only four (27%) have significant F_{IS}, but 10 (66%) have more than three significant pairwise LD tests.

We first investigate the possibility that departures from random mating equilibrium within samples result from admixture of fish from genetically different subpopulations. Two adult (KIGHA97 and TRHA97) and four juvenile (LRS00, MATS 98, WADY98, SCY99) populations with significant F_{IS} or LD have significant pairwise F_{ST} after subdivision on the basis of independent collection data (Table 3*a*; see Table 1 for criteria).

We further investigate the possibility that departures from random mating expectations in juvenile collections with significant F_{IS} ($P < 0.05$) or LD (>19% possible pairwise loci combinations) are caused by family structure. Fifteen juve-

Table 4. Observed (Obs.) and expected (Exp.) Mendelian proportions of microsatellite genotypes in a full-sib coho salmon (*Oncorhynchus kisutch*) family from Green Valley Creek, Russian River.

Locus	Inferred P1 genotype	Inferred P2 genotype	F_1 genotype	Obs.	Exp.	Total	χ^2	*P*
Ots-103	224 236	228 232	224 228	6	7	28	1.43	0.699
			224 232	5	7			
			228 236	9	7			
			232 236	8	7			
Ots-2	180 184	180 188	180 180	6	7	28	4.86	0.183
			180 184	5	7			
			180 188	5	7			
			184 188	12	7			
iso-Ots-2	205 247	213 227	205 213	7	7	28	0.86	0.836
			205 227	5	7			
			213 247	8	7			
			227 247	8	7			
Ots-3	145 153	145 157	145 145	8	7	28	0.86	0.836
			145 157	7	7			
			145 153	8	7			
			153 157	5	7			
One-13	197 209	197 219	197 197	11	7	28	3.71	0.294
			197 209	7	7			
			197 219	6	7			
			209 219	4	7			
P-53	181 181	173 181	181 181	15	14	28	1.23	0.430
			173 181	13	14			
Oki-1	92 100	96 112	92 96	11	6.75	27	4.26	0.392
			92 112	7	6.75			
			96 100	5	6.75			
			100 112	4	6.75			

nile populations or subpopulations are tested for the presence of full-sibling relationships among all individuals (Table 3*b*). Family structure is especially strong in the RRGV98 sample, which has very high linkage disequilibrium (15 of 21 loci combinations) and a significant excess of heterozygotes (Table 2). More than 40% of pairwise tests for a full-sib relationship in this sample are below the $\alpha = 0.01$ level of significance. The largest identified sibling group contains 28 individuals, the genotypic proportions of which conform to those expected under Mendelian inheritance ratios (Table 4). From the distribution of family sizes within the Green Valley sample and equations and methods described in Hedrick et al. (1995), we estimate the effective number of breeders (N_b) in this tributary to be 14 (Table 5), suggesting that this population is propagated by few adults and is likely undergoing rapid genetic drift.

Similar adjustments to reduce family structure are made to 11 other juvenile samples besides the RRGVY98 sample (Table 3*b*). These adjustments result in a net loss of 134 individuals because of the discarding of full sibs and their replacement by reconstructed parents (261 full sibs removed, 127 parents added).

Within-watershed temporal and spatial variation

We next test for homogeneity among samples within 14 major watersheds for which multiple samples (spatial or temporal) are available (Table 3*c*). Homogeneity is tested by the significance of F_{ST} (significance threshold adjusted for *n* populations) among all samples within each drainage or site, including populations for which adjustments for admixture or family structure were made. Eight pools of homogeneous site populations are formed, including all juvenile populations (except WADY) that comprised heterogeneous subpopulations at their sites prior to adjustments for family structure. Additionally, Russian River hatchery populations are homogeneous, as are different year classes from Lagunitas and Scott creeks and two generations from Olema Creek. Pooling maximizes sample sizes within sites and reduces to 29 the number of populations for analysis of genetic distance among sites, drainages, and ESUs.

Regional variation

A Mantel test among all 29 adjusted populations revealed a highly significant association between genetic and geographic distance ($r = 0.703$, $P = 0.0009$). To visualize genetic diversity, we present two trees depicting genetic distances among samples, one for 32 unadjusted samples grouped by year and watershed (Fig. 2) and one for 29 samples formed after adjustment and homogeneity testing (Fig. 3).

The tree showing genetic distance among 32 unadjusted samples (Fig. 2) has a star-like pattern with few internal nodes having significant bootstrap replication. The tree does support the presence of at least two distinct groupings of populations of coho salmon in the regions sampled. Samples from the Mattole and Eel rivers from the SO/NC ESU are found in a cluster well supported by bootstrap analysis (93% trees). Samples from the Klamath and Trinity rivers, also from the SO/NC ESU, cluster in all of the 1000 trees but are

Table 5. Variance effective size (N_{ev}) for the 1998 Russian River Green Valley coho salmon (*Oncorhynchus kisutch*) population.

Sib group	No. per family	Contribution per parent*
1	4	1.753
2	4	1.753
3	10	4.383
4	6	2.630
5	28	12.273
6	3	1.315
7	4	1.753
8	3	1.315
9	2	0.876
10	2	0.876
11	2	0.876
12	1	0.438
13	1	0.438
14	1	0.438
15	1	0.438
16	1	0.438
	$\bar{k}_m, \bar{k}_f$	2
	$\sigma^2_{k_f}, \sigma^2_{k_m}$ †	9.0
$y = m + f\left(\frac{\sigma^2_{k_f}}{\bar{k}_f}\right), x = f + m\left(\frac{\sigma^2_{k_m}}{\bar{k}_m}\right)$ ‡		2.6
	$N_{ev} = \frac{4N_f N_m}{xN_f + yN_m}$	13.9

*Number of offspring contributed by male and female parents of each family, adjusted so that the mean numbers of offspring per parent over all 16 families is 2.0 ($\bar{k}_m = \bar{k}_f = 2$).

†Variance in adjusted number of offspring contributed by male and female parents.

‡Corrections for unequal parental contributions, where m and f are the proportions of male and female progeny at spawning age; here, we assume $m = f = 0.5$.

found on a branch that does not differ significantly from from CCC populations north of San Francisco. Samples from south of San Francisco (SSF) form a second distinct cluster (supported in 97% of trees), but other populations within the CCC ESU are not well differentiated and only several external nodes are supported. Furthermore, several of the juvenile CCC populations have branch lengths exceeding those separating regions.

The tree of the 29 samples formed after adjustments shows two regional groupings within the SO/NC ESU, those from the drainages of the Klamath, Trinity, and Little rivers (83% of trees) and those from Eel and Mattole rivers (recovered in 95% of trees), and SSF populations again cluster in 98% of trees (Fig. 3). Adjusted CCC juvenile populations have shorter branch lengths than unadjusted juvenile populations and are more likely to cluster with populations from the same watershed, producing a greater number of significant nodes among spatially and temporally proximal populations. For example, all adjusted Russian River Green Valley juvenile populations (RRGV98 and RRGV9700) form a significant cluster, whereas unadjusted RRGV97 was not associated with other RRGV populations. The population divergence with four regional groupings is as deep as the divergence when only two ESUs are recognized.

Fig. 2. An unrooted neighbor-joining tree showing chord distances (Cavalli-Sforza and Edwards 1967) among 32 California coho salmon (*Oncorhynchus kisutch*) collections formed by pooling samples within drainages by year class. Bootstrap values greater than 700 out of 1000 are shown; numbers preceding each name correspond to locations in Fig. 1.

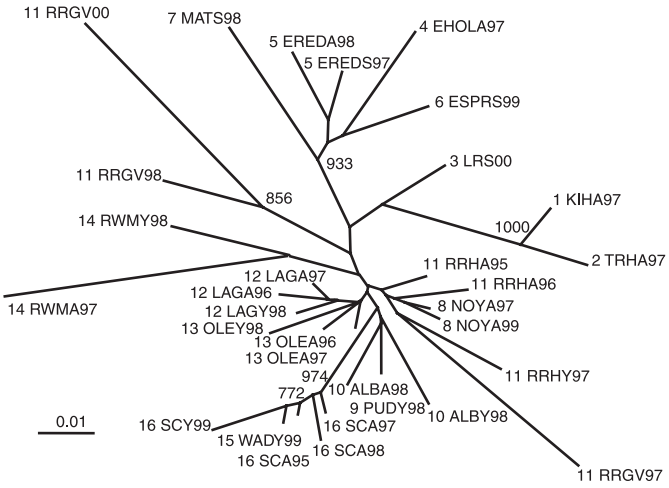
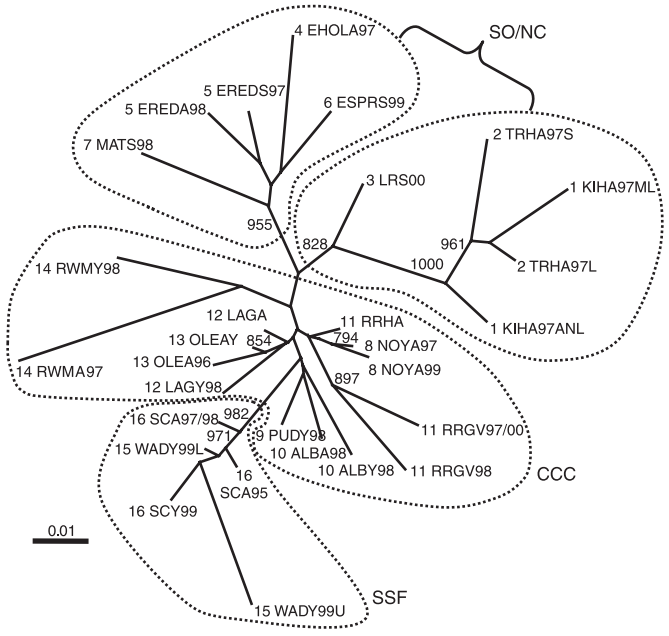


Fig. 3. An unrooted neighbor-joining tree showing chord distances (Cavalli-Sforza and Edwards 1967) among 29 California coho salmon (*Oncorhynchus kisutch*) populations formed after adjustments for admixture, family structure, and pooling of homogenous samples within drainages and sites. Bootstrap values greater than 700 out of 1000 are shown; numbers preceding each name correspond to locations in Fig. 1.



Discussion

Genetic diversity within populations

Numerous studies of Pacific salmon populations over the past 30 years show that genotypic frequencies generally con-

form to those expected under random mating (Utter et al. 1980; Beacham et al. 2003; Ford et al. 2004). Here, we report deviations from single-locus and digenic random mating equilibria in nearly three-quarters of California coho salmon populations sampled (23 of 32). This contrasts with the findings of Bartley et al. (1992) from 15 years prior (1983–1985), in which allozyme genotypes for 27 California coho salmon populations conformed to Hardy–Weinberg proportions and only 6.7% of loci pairs had significant linkage disequilibrium. However, very low levels of protein polymorphism in the Bartley et al. (1992) study significantly limited their power to detect deviations from random mating equilibria. Thus, we are unable to determine whether nonequilibrium conditions were present then and were not detected or whether they are more recent in origin, perhaps associated with reductions in population size over the last five generations. Declines of California coho salmon populations have been precipitous in that time. By 1998, we could not collect juveniles from six of Bartley et al.'s (1992) populations, and at present, four additional populations (37% overall) have declined to such levels that it would be difficult or impossible to collect a sample size of 100 juveniles (S.L. Harris, California Department of Fish and Game, P.O. Box 1690, Willits, CA 95490, personal communication, 2005).

Determining the causes of deviations from random mating equilibria in population samples is important both to our understanding of what shapes local genetic diversity and to an accurate description of genetic diversity at regional and ESU scales. Deviations of genotypic proportions from those expected under random mating can arise at microsatellite DNA markers through a variety of genotyping errors or artifacts. Genotyping errors caused by nonamplifying PCR null alleles would make estimates of F_{IS} more positive because null heterozygotes are scored as homozygotes. Our data, even for *Ots-103*, which deviates from HWE in half of our initial populations, do not show significant deficiency of all heterozygous classes, as might be expected if many nulls were present (Van Oosterhout et al. 2004); indeed, some deviations are caused by excesses rather than deficiencies of heterozygotes. We also do not observe deviations attributable to specific loci or loci interactions, or allele size dependent deficiencies of heterozygotes, which would indicate large allele dropout in competitive PCR reactions. Finally, Mendelian inheritance of all seven markers in a putative full-sib family from Green Valley supports these markers as bona fide genetic loci. Thus, it is unlikely that technical errors or artifacts are sufficient to explain the majority of departures from random mating equilibrium. We therefore turn to two biological causes of these deviations: (*i*) Wahlund effects, created by genetic subdivision of population samples collected in the traditional manner, by year class and watershed; and (*ii*) family structure within samples of juveniles, reflecting small numbers of successful spawners.

Coho salmon have a dominant 3-year age structure, suggesting that in adult collections, Wahlund effect could arise from inadvertent inclusion of genetically differentiated year classes, such as early-returning "jacks". Alternatively, geographic variation, which has been found to be greater than temporal variation (Van Doornik et al. 2002), may account for within-collection heterogeneity (e.g., Klamath hatchery returns). The Wahlund effect in juvenile collections may be the result of spatial differentiation of offspring emerging in different reaches of a stream, as was observed in Chinook salmon (Bentzen et al. 2001). Likewise, outmigrating smolts collected at a single downstream location may represent progeny from multiple subpopulations partially reproductively isolated by spatial separation of small groups of spawning adults. Within equilibrium populations, we do not observe heterogeneity among putative subgroups. For example, samples from Lagunitas and Olema creeks were tested exhaustively for spatial heterogeneity among different river reaches and tributaries and for temporal differences among year and size classes and among sexes; no heterogeneity was detected in any of these tests.

Population differentiation at very small geographic scales has been previously reported for anadromous salmon (Bernatchez et al. 1998; Hendry et al. 2000; Wofford et al. 2005), yet we know of no empirical studies that have further dissected causes of local Wahlund effects (cf. Banks et al. 2000). Here, we use genotype-independent collection data to partition collections on the basis of significant F_{ST}. Significance of departures from HWE genotypic proportions is difficult to test in the resulting subgroups because of decreased sample size and statistical power to detect non-zero LD and, to a lesser degree, F_{IS} (simulation results not shown). However, simulation results indicate that F_{ST} is not sensitive to sample size, so we conclude that biological factors are the most reasonable explanation for the observed departures from random mating.

Departure from random mating equilibria in some juvenile collections appears to stem not from admixture, per se, but from small breeding population size and resulting family structure. As a consequence, juvenile collections may exhibit the "Allendorf–Phelps effect" — many juveniles representing few adult spawners, making differences among populations or population segments appear more significant than they are (Allendorf and Phelps 1981). Whereas Allendorf and Phelps advise sampling only spawners for this reason, we were confronted in this study with very small populations that offered no alternative to juvenile sampling. Thus, to make use of the available samples and information, we attempt to resolve full-sib groups, remove these from the sample, and replace them with the inferred genotypes of their parents. We believe that such adjustments better estimate allelic frequencies in local spawning populations than do the original juveniles samples. A possible bias is introduced by our method if adults that left sampled progeny have substantially different marker allele frequencies than the spawning population as a whole. Because only those parents that have progeny contribute alleles to the next generation, the bias should be negligible.

In juvenile collections, we use a criterion of >3 (19%) significant pairwise LD ($\alpha = 0.05$) or significant F_{IS} to trigger investigation of family structure. Eleven of our initial juvenile collections exceed this threshold value. Other studies use a 10% threshold (Banks et al. 2000; Beacham et al. 2003), dismiss high LD because of multiple testing (Olsen et al. 2003), or find no LD (e.g., Smith et al. 2001). Although we do not adjust for family structure in adult collections, 10 of 17 populations exceed our 19% LD threshold as well. Results from relatedness tests (data not shown) suggest that

adult hatchery populations contain large numbers of full-sib families owing to small effective numbers of breeders, which likely contributes to the observed deviations from random mating expectations.

The increased potential for inbreeding from routine artificial supplementation has been well documented (reviewed in Wang et al. 2002; but see Hedrick et al. 2000). Estimates for populations, such as Russian River Green Valley, further suggest that very few spawners may propagate most wild California coho salmon populations. Supplementation efforts have already been initiated in Green Valley and Scott creeks. In 2000, a program involving a consortium of stakeholder groups and federal and state agencies began using the Green Valley remnant population as a source to reseed extirpated portions of the Russian River. Approximately 300 juveniles collected in-stream per year are raised in captivity to serve as broodstock. Although adult broodstock are genotyped to reduce potential matings between full and half sibs, the consequence of increasing the census size to many thousands of individuals from a stock that in at least one year had 14 estimated breeders is uncertain at best.

Genetic diversity among populations

Bartley et al. (1992), using protein markers, found little concordance between genetic and geographic distances among coastal California populations of coho salmon, although Olin (1984) did find evidence of divergence on a larger scale between Oregon and California stocks. In contrast, we find that genetic distances among coho salmon samples are significantly correlated with geographic distances among populations. After adjustments for admixture and family structure, all populations show high regional fidelity. The major effect of family adjustments is to make populations within and among watersheds of the same region more homogeneous. However, this appears not simply to be the result of our having used regional baseline data to drive selection of mating types. We note that the tree made from adjusted data has three significant nodes within the CCC, where none was found using unadjusted data, and a Mantel test of within-CCC adjusted populations was highly significant ($r = 0.300$, $P = 0.0039$). Furthermore, pairwise F_{ST} of all adjusted populations to the relevant regional baseline population are significant, indicating that use of baseline data for parent reconstruction does not predetermine homogeneity of the adjusted sample.

Finally, concordance of genetic and geographic distances among populations suggests that out-of-basin and out-of-state stocking of hatchery fish have had little impact on the structure of extant California populations. Given the long history of stock transfers within California and between California and other Pacific Northwest states, this finding suggests that stock transfers have not "taken" owing to reduced fitness of salmon introduced via stocking. Two Oregon stocks (Alsea and Klaskanine) have been planted extensively in California watersheds, including the Klamath, Eel, and Russian rivers and Lagunitas and Scott creeks (Weitcamp et al. 1995; L. Weitcamp, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112–2097, USA, unpublished data). California's Noyo River stock has been repeatedly planted in at least seven of the watersheds sampled in this study (L. Weitcamp, unpublished data). In addition, Klamath River stock, which has been extensively stocked with out-of-basin (and state) coho salmon, maintains more similarity to the geographically proximal Little River coho population than to those of other systems that have received similar plantings. Isolation by distance among California coho salmon populations is inconsistent with recent stocking events and has likely developed over longer time scales.

Other examples of indigenous populations persisting in the face of intensive stocking efforts over long time periods have been reported (e.g., Utter 2000; Nielsen et al. 2001; Spidle et al. 2001). Furthermore, similarly high levels of diversity among small, genetically isolated populations of native coho salmon have been reported recently (Miller et al. 1996; Gharrett et al. 2001; Olsen et al. 2003), though not all studies support a model of isolation by distance. In the Olsen et al. (2003) study, the lack of strong agreement between genetic and geographic distance in some regions (e.g., Kodiak archipelago) may reflect historical colonization events, complex ocean currents, or large spatial distances among populations within regions. Small et al. (1998) provide evidence for the existence of at least three primary components of genetic diversity among British Columbia coho salmon stocks (coastal islands, mainland, and the Thompson drainage). Overall, a cline of decreasing mtDNA diversity is observed from south to north across the Pacific Northwest, indicating that a substantial portion of coho salmon genetic diversity is concentrated in the southerly region (Smith et al. 2001).

Our study implicates population fragmentation, genetic drift, and isolation by distance, owing to very low levels of migration, as the major evolutionary forces shaping genetic diversity within and among extant California coho salmon populations. In reaching these conclusions, we have had to overcome significant challenges, posed by low abundance itself, to sampling and deducing the units of population structure. These challenges are unfortunately likely to arise in other species or regions with similarly declining abundance, and we hope that the methods developed and applied here or improvements upon them will prove useful in such cases. The implications of correctly identifying the units of population structure for conservation can be substantial. In the case of California coho salmon, resolution of smaller population units suggests that they are experiencing rapid genetic drift, inbreeding, and the associated deleterious effects of inbreeding depression. Accordingly, management and rehabilitation of these populations is needed at much smaller scales than current ESU designations.

Acknowledgements

We thank Carolyn Greig for technical assistance in testing and evaluating microsatellite markers, Cheryl Dean for genotyping, and A. I. Pudovkin and W. Eichert for developing the computer applications. We also thank the many dedicated collectors and volunteers who provided samples and insight into the intricacies of the different watersheds. We are especially indebted to Paul Siri, Bob Chamberlain, Mitch Farro, Michael Fawcett, Ken Fox, Scott Harris, David Hines,

Wendy Jones, Dave Manning, Jerry Smith, Dave Streig, David Teel, Harry Vaughn, Jerry Wall, Jim Waldvogel, John West, Bob Will, and the lumber companies for access to their property. We thank Robin Waples whose comments substantially improved this paper. This study was supported by contracts TW 96/97-10 and TW 99/00-110 with the Sonoma County Water Agency.

References

Allendorf, F.W., and Phelps, S.R. 1981. Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci. **38**: 1507–1514.

Banks, M.A., Blouin, M.S., Baldwin, B.A., Rashbrook, V.K., Fitzgerald, H.A., Blankenship, S.M., and Hedgecock, D. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). J. Hered. **90**: 281–288.

Banks, M.A., Rashbrook, V.K., Calavetta, M.J., Dean, C.A., and Hedgecock, D. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. Can. J. Fish. Aquat. Sci. **57**: 915–927.

Bartley, D.M., Bentley, B., Olin, P.G., and Gall, G.A.E. 1992. Population genetic structure of coho salmon (*Onchorhynchus kisutch*) in California. Calif. Fish Game, **78**: 88–104.

Beacham, T.D., Supernault, J.K., Wetklo, M., Deagle, B., and Labaree, K., Irvine, J.R., Candy, J.R., Miller, K.M., Nelson, J.R., and Withler, R.E. 2003. The geographic basis for population structure in Fraser River chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull. **101**(2): 229–242.

Beechie, T.J., Breamer, E., and Wasserman, L. 1994. Estimating coho salmon rearing habitat and smolt production losses in a large river basin, and implications for habitat restoration. N. Am. J. Fish. Manag. **14**: 797–811.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L., and Bonhomme, F. 1998. GENETIX, logiciel sous Windows^{TM} pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Bentzen, P., Olsen, J.B., McLean, J.E., Seamons, T.R., and Quinn, T.P. 2001. Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. J. Hered. **92**: 127–136.

Bernatchez, L., Dempson, B., and Martin, S. 1998. Microsatellite gene diversity analysis in anadromous Arctic char, *Salvelinus alpinus*, from Labrador, Canada. Can. J. Fish. Aquat. Sci. **55**: 1264–1272.

Brown, L.R., Moyle, P.B., and Yoshiyama, R.M. 1994. Historical decline and current status of coho salmon in California. N. Am. J. Fish. Manag. **14**: 237–261.

Carney, B.L., Gray, A.K., and Garrett, A.J. 1994. Mitochondrial DNA restriction site variation within and among five populations of Alaskan coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **54**: 940–949.

Cavalli-Sforza, L.L., and Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. Evolution, **32**: 550–570.

Duchesne, P.,and Bernatchez, L. 2002. An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. Conserv. Genet. **3**: 47–60.

England, P.R., Osler, G.H., Woodworth, L.M., Montgomery, M.E., Briscoe, D.A., and Frankham, R. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. Conserv. Genet. **4**: 595–604.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package). Version 3.57c. Department of Genetics, University of Washington, Seattle, Wash.

Ford, M.J., Teel, D.J., Van Doornik, D.M., Kuligowski, D.R., and Lawson, P.W. 2004. Genetic population structure of central Oregon Coast coho salmon (*Oncorhynchus kisutch*). Conserv. Genet. **5**: 797–812.

Frankham, R., Ballou, J.D., and Briscoe, D.A. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK.

Gharrett, A.J., Gray, A.K., and Brykov, V. 2001. Phylogeographic analysis of mitochondrial DNA variation in Alaskan coho salmon, *O. kisutch*. Fish. Bull. **99**: 528–544.

Goodnight, K.F., and Queller, D.C. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Mol. Ecol. **8**: 1231–1234.

Greig, C., Robertson, J.M., and Banks, M.A. 2002. Rapid PCR-based species tests for threatened sympatric salmonids. Conserv. Genet. **3**: 83–86.

Guo, S.W., and Thompson, E.A. 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics, **48**: 361–372.

Hedrick, P.W., Hedgecock, D., and Hamelberg, S. 1995. Effective population size in winter-run Chinook salmon. Conserv. Biol. **9**: 615–624.

Hedrick, P.W., Hedgecock, D., Hamelberg, S., and Croci, S.J. 2000. The impact of supplementation in winter run Chinook salmon on effective population size. J. Hered. **91**(2): 112–116.

Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C., and Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science (Washington, D.C.), **290**(5491): 516–518.

Krueger, C.C., and May, B.P. 1991. Ecological and genetic effects of salmonid introductions in North America. Can. J. Fish. Aquat. Sci. **48**: 66–77.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. **27**: 209–220.

Miller, K.M., Whithler, R.E., and Beacham, T.D. 1996. Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation. Can. J. Fish. Aquat. Sci. **53**: 181–195.

Nelson, R.J., and Beacham, T.D. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Anim. Genet. **30**: 228–229.

Nickelson, T. 2003. The influence of hatchery coho salmon (*Oncorhynchus kisutch*) on the productivity of wild coho salmon populations in Oregon coastal basins. Can. J. Fish. Aquat. Sci. **60**: 1050–1056.

Nickelson, T.E., and Lawson, P.E. 1998. Population viability of coho salmon, *Oncorhynchus kisutch*, in Oregon coastal basins: application of a habitat-based life cycle model. Can. J. Fish. Aquat. Sci. **55**: 2383–2392.

Nielsen, E.E., Hansen, M.M., and Bach, L.A. 2001. Looking for a needle in a haystack: discovery of indigenous Atlantic salmon (*Salmo salar*) in stocked populations. Conserv. Genet. **2**: 219–232.

Olin, P.G. 1984. Genetic variability in hatchery and wild populations of coho salmon *Oncorhynchus kisutch* in Oregon. M.S. thesis, University of California–Davis, Davis, California.

Olsen, J.B., Miller, S.J., Spearman, W.J., and Wenburg, J.K. 2003. Patterns of intra- and inter-population genetic diversity in Alaskan coho salmon: implications for conservation. Conserv. Genet. **4**: 557–569.

Page, R.D.M. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. **12**: 357–358.

Park, L.K., Moran, P., and Dightman, D.A. 1996. A Chinook salmon PCR-RFLP marker in the p53 locus. Anim. Genet. **27**: 127–128.

Pess, G.R., Montgomery, D.R., Steel, E.A., Bilby, R.E., Feist, B.E., and Greenberg, H.M. 2002. Landscape characteristics, land use, and coho salmon (*Oncorhynchus kisutch*) abundance, Snohomish River, Wash., U.S.A. Can. J. Fish. Aquat. Sci. **59**: 613–623.

Raymond, M., and Rousset, F. 1995. GENEPOP. Version 3.3. Population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Reed, D.H., Lowe, E.H., Briscoe, D.A., and Frankham, R. 2002. Inbreeding and extinction: effects of rate of inbreeding. Conserv. Genet. **4**: 405–410.

Reisenbichler, R.R., and Phelps, R. 1989. Genetic variation in steelhead (*Salmo gairdneri*) from the north coast of Washington. Can. J. Fish. Aquat. Sci. **46**: 66–73.

Saisa, M., Koljonen, M.L., and Tahtinen, J. 2003. Genetic changes in Atlantic salmon stocks since historical times and the effective population size of a long-term captive breeding programme. Conserv. Genet. **4**: 613–627.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**(4): 406–25.

Scribner, K.T., Gust, J.R., and Fields, R.L. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can. J. Fish. Aquat. Sci. **53**: 833–841.

Small M.P., Withler, R.E., and Beacham, T.D. 1998. Population structure and stock identification of British Columbia coho salmon, *Oncorhynchus kisutch*, based on microsatellite DNA variation. Fish. Bull. (Washington, D.C.), **96**: 843–858.

Smith, C.T., Koop, B.F., and Nelson, R.J. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol. Ecol. **7**: 1614–1616.

Smith, C.T., Nelson, R.J., Wood, C.C., and Koop, B.F. 2001. Glacial biogeography of North American coho salmon (*Oncorhynchus kisutch*). Mol. Ecol. **10**: 2775–2785.

Spidle, A.P., Bane, D., Schill, W., Lubinski, B.A., and King, T.L. 2001. Fine-scale population structure in Atlantic salmon from Maine's Penobscot River drainage. Conserv. Genet. **2**: 11–24.

Utter, F.M. 2000. Patterns of subspecific anthropogenic introgression in two salmonid genera. Rev. Fish Biol. Fish. **10**: 265–279.

Utter, F.M. 2004. Population genetics, conservation and evolution in salmonids and other widely cultured fishes: some perspectives over six decades. Rev. Fish Biol. Fish. **14**: 125–144.

Utter, F.M., Campton, D., and Grant, S., Milner, G., Seeb, J., and Wishard, L. 1980. Population structures of indigenous salmonid species of the Pacific Northwest. *In* Salmonid ecosystems of the Pacific Northwest. *Edited by* W.J. McNeil and D.C. Himsworth. Oregon State University Press and Oregon State University Sea Grant College Program, Corvallis, Oregon. pp. 285–304.

Van Doornik, D.M., Ford, M.J., and Teel, D.J. 2002. Patterns of temporal genetic variation in coho salmon: estimates of the proportion of 2-year-olds in natural and hatchery production. Trans. Am. Fish. Soc. **131**: 1007–1019.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**(3): 535–538.

Wang, S., Hard, J.J., and Utter, F. 2002. Salmonid inbreeding: a review. Rev. Fish Biol. Fish. **11**: 301–319.

Waples, R.S. 1991. Definition of "species" under the Endangered Species Act: application to Pacific Salmon. NOAA Tech. Memo. No. NMFS-F/NWC-19.

Waples, R.S., Gustafson, R.G., Weitkamp, L.A., Myers, J.M., Johnson, O.W., Busby, P.J, Hard, J.J., and Bryant, G.J., Waknitz, F.W., Neely, K., Teel, D., Grant, W.S., Winans, G.A., Phelps, S., Marshall, A., and Baker, B.M. 2001. Characterizing diversity in salmon from the Pacific Northwest. J. Fish Biol. **59**(Suppl. A): 1–41.

Wehrhahn, C.F., and Powell, R. 1987. Electrophoretic variation, regional differences and gene flow in the coho salmon (*Oncorhynchus kisutch*) of southern British Columbia. Can. J. Fish. Aquat. Sci. **44**: 822–831.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Weitcamp, L.A., Wainwright, T.C., Bryant, G.J., Milner, G.B., Teel, D.J., Koop, R.G., and Waples, R.S. 1995. Status review of coho salmon from Washington, Oregon and California. US Department of Commerce, NOAA Tech. Memo. No. NMFS-NWFSC-24.

Wofford, J.E.B., Gresswell, R.E., and Banks, M.A. 2005. Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecol. Appl. **15**(2): 628–637.

Wright, S. 1931. Evolution in Mendelian populations. Genetics, **16**: 97–159.