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Abstract

We genetically characterized seven species of Pacific salmonids in the Elwha River and in selected neighboring rivers prior to the impending removal of two dams. Monitoring the genetics of recolonization of the watershed by remnant native, hatchery, and/or adjacent watershed populations is a critical element to further our understanding of ecosystem restoration. By pooling data from independent studies, we assessed intraspecific diversity for pink salmon (*Oncorhynchus gorbuscha)*, chum salmon (*O. keta)*, coho salmon (*O. kisutch)*, sockeye salmon (*O. nerka),* Chinook salmon (*O. tshawytscha),* rainbow trout (*O. mykiss)* and bull trout (*Salvelinus confluentus).* Levels and patterns of genetic variability within and among collections were evaluated at 6-15 microsatellite (mSAT) loci per species. Each species had 3-8 loci with 20 or more alleles. In all species, an Elwha collection was statistically different from one or more nearest-neighbor population. In addition, the native in-river collections of Chinook salmon and steelhead (anadromous rainbow trout) were distinguishable from existing in-river hatchery stocks. In most species, Elwha populations contained similar levels of genetic diversity as observed in neighboring river systems. In *O. mykiss,* variability at an evolutionarily adaptive major histocompatibility complex (MHC) gene paralleled the mSAT variation. Given the various levels of distinctiveness of Elwha populations, we discuss the use of these data as a genetic ruler to manage and monitor the genetic aspects of recolonization of the Elwha River, and the importance of tissue archives for new genetic techniques.

Introduction

A plan for monitoring the ecological response of the Elwha River watershed to dam removal includes a genetic assessment of Pacific salmonids (McHenry and Pess 2008). We are interested in the current genetic status of the extant stocks in the river (native and hatchery) and in neighboring rivers that may be sources of recolonizing populations. The assessment of genetic variability within and among populations within the Elwha River and in neighboring populations prior to dam removal is important to our ability to evaluate and monitor the dynamics of population genetics of upriver recolonization once dams are removed. This paper outlines a preliminary characterization of intraspecific diversity in seven species of Pacific salmonids, pink salmon (*Oncorhynchus gorbuscha)*, chum salmon (*O. keta)*, coho salmon (*O. kisutch)*, sockeye salmon (*O.*

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nerka), Chinook salmon (*O. tshawytscha),* rainbow trout (*O. mykiss)* and bull trout (*Salvelinus confluentus).* Previously unpublished data from several independent studies are summarized here. Allelic variation at microsatellite (mSAT) loci is assumed to be evolutionarily neutral and is used to describe the patterns of connectivity and levels of genetic diversity among salmonid gene pools in the Elwha watershed and along the Strait of Juan de Fuca. Databases developed in this study will be used to assess the relative contributions of the populations sampled here to up-river colonization after dam removal.

Mainstem hydroelectric dams were constructed on the Elwha River in 1913 and 1925 without fish passage facilities. These actions severely impacted native salmon populations and confined anadromous salmonids to the lower 8 km of the river. An overview of the current-day status of Elwha salmonids is presented in Table 1. Each of these species has been reviewed by NOAA Fisheries or USFWS in the last ten years with regard to status under the U.S. Endangered Species Act (ESA) (see Waples et al. 2001). These comprehensive reviews compiled genetic, life history, and ecological information in order to group geographic populations into conservation units termed evolutionarily significant units (ESUs) for Pacific salmon or distinct population segments (DPSs) for bull trout. In general, Elwha River populations straddle or are close to an ESU boundary delimiting a Puget Sound ESU and a Washington Coast/Olympic Peninsula ESU, e.g., for coho and Chinook salmon, and steelhead (anadromous rainbow trout)(Weitkamp et al. 1995, Busby et al. 1996, Myers et al. 1998). Seriously reduced populations in the Elwha River include Chinook salmon, steelhead, and bull trout (Table 1). Other Elwha populations such as pink and chum salmon are critically depressed, but nested within stronger regional populations. Bull trout in the Elwha are included as part of the Puget Sound/Olympic Peninsula DPS (USFWS 1999).

We provide descriptions of preliminary genetic data sets based on 6-15 mSAT loci. Our goal is to genetically characterize populations that currently exist in the river and to describe their genetic similarity with their nearest geographic neighbors and, when relevant, with hatchery stocks used in the river or in out-planting programs. Within this framework, we were also interested in estimating relative levels of genetic diversity within population samples. We expect that additional evaluation and analyses will ensue prior to dam removal as more detailed data sets—genetic and phenetic—are developed.

Methods and Materials

Tissues were collected in a variety of independent tribal, state, and federal programs associated with population assessments, hatchery supplementation, and/or genetic surveys. Adult and juvenile collections were made during spawning surveys, smolt trapping, seining, via electrofishing, and/or at hatcheries. Laboratory analyses were conducted at six separate facilities. Microsatellite analyses followed standard protocols as previously described, e.g., for coho salmon (Van Doornik et al. 2007), chum salmon (Small et al. 2006), rainbow trout (Small et al. 2007), steelhead (Winans et al. 2004), and bull trout (Neraas and Spruell 2001, DeHaan and Ardren 2005). Procedures used for assaying

TABLE 1. Current and historical status of Pacific Salmonids and bull trout in the Elwha River (unpublished data, M. McHenry).

Species	Prior to Dam Construction	Current Status	Estimated Run Size	% Hatchery Contribution
Spring Chinook salmon	abundant	critically low or extinct	n.a.	unknown
Summer/fall Chinook salmon	abundant	critically low, hatchery supplemented	<3,000	unknown
Coho salmon	abundant	hatchery supplemented	3-15,000	76
Chum salmon	abundant	critically low	<2,000	0
Pink salmon	abundant	critically low	<150	0
Sockeye salmon	abundant to Lake Sutherland	extinct (only kokanee in Lake Sutherland)	0	0
Winter steelhead	abundant	depressed, non-native hatchery supplemented	<500	83
Summer steelhead	abundant	depressed	<100	0
Bull trout	abundant	small native populations separated by the dams	n.a.	0

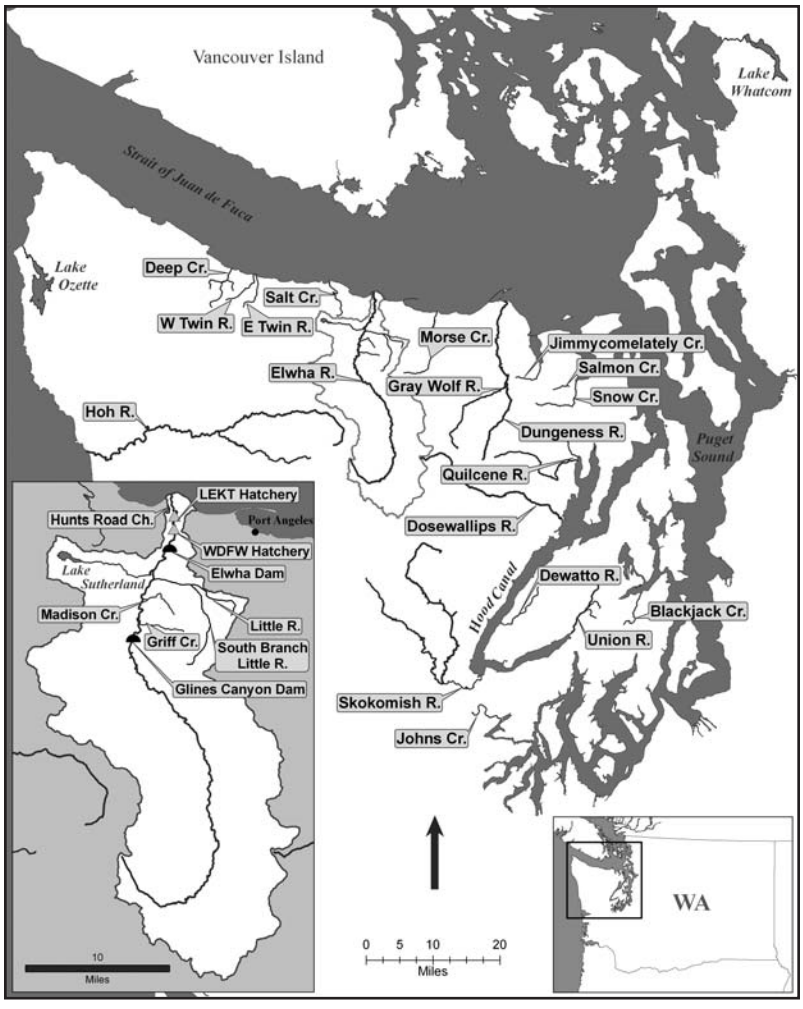

Figure 1. Location of collection sites where LEKT is Lower Elwha Klallam Tribe, Ch. is channel, and WDFW is Washington Department of Fish and Wildlife.

rainbow trout for the class II B1 locus at the major histocompatability complex (MHC) are found in Miller et al. (2001). Genetic diversity in a collection was estimated using two measures: allelic richness, which standardizes the number of alleles per locus to a common sample size (see Goudet 2001), and Nei's unbiased gene diversity (Nei 1987). To evaluate overall among-sample variability at a locus, we calculated observed and expected heterozygosity and Hardy Weinberg equilibrium (HWE) assuming all fish originated from one collection. Loci that deviate from HWE over all populations are more powerful among-collection discriminators. These basic statistical analyses were performed using GENEPOP (Raymond and Rousset 1995). Relationships among collections were estimated as neighbor-joining trees with PHYLIP (Felsenstein 1993) based on Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967). Genetically similar collections are grouped together in the tree where branch lengths reflect genetic differences. The level of among group differences was estimated with Wright's F_{ST} statistic (Wright 1978) and evaluated for significance with 1000 permutations using FSTAT (Goudet 2001). A factorial correspondence analysis was conducted on allele frequency data to provide an independent assessment of among-collection variability for Chinook salmon using GENETIX (Belkhir et al. 2001).

Results

Pink Salmon

Fall- and summer-run collections of pink salmon from the Elwha were compared to neighboring populations in the Dungeness River (fall and summer run) and Morse Creek (summer run) (Figure 1; Table 2). Moderate levels of variability were observed in 13 mSAT loci with 3 loci exhibiting > 20 alleles per locus (Table 3). There was little variability among samples in the estimates of genetic diversity (Table 2). Eight of the 13 loci were out of HWE (Table 3). Fall-run fish from the Dungeness and Elwha clustered together in the dendrogram (Figure 2); the F_{ST} value between the collections was statistically significant (Figure 2).

Chum Salmon

Chum salmon collections from Puget Sound, Hood Canal, and the Strait of Juan de Fuca were

TABLE 2. Summary of collections by species for adult samples except where noted. BY04=brood year 2004, etc; hat=hatchery; WDFW=Washington Department of Fish and Wildlife, LEKT=Lower Elwha Klallam Tribe.

Species	Population	Sample information	No. fish	No. alleles	Alleles per locus	Allele richness	Gene diversity
Pink salmon	Elwha R.	fall run, BY01	24	178	12.71	9.81	0.744
(13 loci)	Elwha R.	sum. run, BY01	46	139	9.93	9.34	0.761
	Dungeness R.	fall run, BY01	80	175	12.50	9.29	0.748
	Dungeness R.	sum. run, BY01	94	196	14.00	9.76	0.740
	Morse Cr.	sum. run, BY01	93	184	13.14	9.16	0.751
Chum salmon	Elwha R.	fall run, BY05	93	281	21.62	14.47	0.846
(13 loci)	Dewatto R.	fall run, BY98	39	198	15.23	13.09	0.840
	Hoodsport hat.	fall run, BY98	46	203	15.62	12.89	0.839
	Quilcene R.	sum. run, BY97	42	166	12.77	11.40	0.816
	Union R.	sum. run, BY00	43	162	12.46	11.05	0.813
	Dosewallips R.	sum. run, BY00	42	183	14.08	12.19	0.830
	Johns Cr.	sum. run, BY 94	43	191	14.69	12.65	0.834
	Blackjack Cr.	sum. run, BY96	36	151	11.62	10.80	0.818
	Salmon Cr.	sum. run, BY00	48	159	12.23	10.36	0.813
	Jimmycomelately Cr.	sum. run, BY01	44	116	8.92	8.18	0.771
Coho salmon	Elwha R., LEKT hat.	BY05	96	186	16.91	7.50	0.855
(11 loci)	Deep Cr.	juv., 2006	46	164	14.91	7.60	0.863
	West Twin R.	juv., 2006	18	119	10.82	7.40	0.855
	East Twin R.	juv., 2006	24	136	12.36	7.70	0.857
	Salt Cr.	juv., 2006	49	181	16.45	7.80	0.863
	Dungeness R. hat.	juv., 2003	48	177	16.09	7.80	0.869
	Snow Cr.	BY02-04	143	202	18.36	7.60	0.865
Rainbow trout—mSATs	Griff Cr., trout	juv./ad., BY06	23	137	9.13	9.01	0.808
(15 loci)	Madison Cr., trout	juv./ad., BY06	26	140	9.33	8.87	0.801
	SB Little R., trout	juv./ad., BY04	47	94	6.27	5.66	0.685
	SB Little R., trout	juv./ad., BY05	47	94	6.27	5.53	0.669
	Elwha R. LEKT hat. steelhead	juv., BY05	47	123	8.20	7.34	0.774
	Elwha R. LEKT hat. steelhead	juv., BY06	91	141	9.40	7.38	0.818
	Elwha R., steelhead	juv., BY05	48	173	11.53	9.64	0.821
Rainbow trout—MHC	Griff Cr., trout	juv/ad., BY06	23	15	n.a.	15.00	0.920
(1 locus)	Madison Cr., trout	juv/ad., BY06	23	12	n.a.	11.64	0.819
	SB Little R., trout	juv/ad., BY04	23	9	n.a.	8.34	0.853
	LEKT hat. steelhead	juv., BY05	48	13	n.a.	10.70	0.832
	LEKT hat. steelhead	juv., BY06	47	13	n.a.	10.47	0.814
	Elwha R., steelhead	juv., BY05	48	18	n.a.	14.88	0.908
Sockeye salmon	L. Sutherland	kokanee, BY05	48	131	10.08	9.83	0.672
(13 loci)	L. Sutherland	kokanee, BY06	48	131	10.08	9.81	0.665
	L. Ozette	sockeye, BY02	48	65	5.00	4.85	0.561
	L. Whatcom hat.	kokanee, BY07	48	155	11.93	11.59	0.754
Chinook salmon	Elwha R., WDFW hat.	BY05	96	229	17.62	14.08	0.834
(13 loci)	Elwha R., Hunt's Rd. Channel	BY05	62	188	14.46	13.59	0.817
	Dungeness R.	BY04	60	194	14.92	12.86	0.824
	Elwha R. Screw Trap, juv.	Spring 2005	96	232	17.85	13.63	0.824
	Elwha R. Screw Trap, juv.	Spring 2006	96	213	16.38	13.58	0.825
Bull trout	Elwha R.	1997, above dams	40	35	5.83	n.a.	0.654
(6 loci)	Gray Wolf R.	2003-2004	27	33	8.25	n.a.	0.486
	Dungeness R.	juv., 2005	20	30	5.00	n.a.	0.446
	Hoh River R.	2002, above barriers	23	31	5.16	n.a.	0.788
	N.F. Skokomish R	2003, above dams	21	16	4.00	n.a.	0.513
	S.F. Skokomish R.	2003, upper river	22	27	5.40	n.a.	0.798

TABLE 3. Levels of genetic diversity estimated by the percentage of observed (H_o) and expected (H_e) heterozygosity, Hardy-Weinberg Equilibrium (HWE) calculated over all collections, number of observed alleles, and allele size range.

Locus	H_o	H_e	HWE *P* value	No. alleles	Range	Reference
Pink salmon						
Oke3	0.646	0.753	0.0000	10	222-366	Buchholz et al. 2001
Omy1011	0.354	0.346	0.6949	7	180-203	Spies et al. 2005
OtsG311	0.866	0.842	0.7479	14	178-228	Williamson et al. 2002
Oki200	0.321	0.491	0.0000	7	91-107	Beacham et al. 1999
One13M	0.317	0.410	0.0167	2	181-187	Scribner et al. 1996b
Ots1	0.842	0.870	0.5709	12	217-242	Banks et al. 1999
One102	0.74	0.870	0.0000	18	228-292	Olsen et al. 2000b
One105	0.785	0.930	0.0000	13	152-196	Olsen et al. 2000b
Ots103	0.785	0.936	0.0000	29	147-264	Small et al. 1998
Str60	0.575	0.553	0.4856	6	105-117	Estoup et al. 1993
One114	0.932	0.937	0.1734	28	174-292	Olsen et al. 2000a
Ots101	0.802	0.899	0.0000	37	243-439	Nelson et al. 2001
Ssa197	0.517	0.920	0.0000	19	132-205	O'Reilly et al. 1996
Chum salmon						
Oki1	0.844	0.857	0.1245	14	174-234	Smith et al. 1998
One102	0.907	0.921	0.7295	23	217-305	Olsen et al. 2006
One114	0.882	0.912	0.0013	30	176-292	Olsen et al. 2006
One18	0.679	0.686	0.1066	6	162-176	Scribner et al. 1996
Otsg311	0.909	0.961	0.0023	54	241-489	Williamson et al. 2002
Omy1011	0.843	0.876	0.0725	15	194-250	Paul Bentzen, McGill Univ., pers. comm.
One108	0.879	0.951	0.0000	46	137-350	Olsen et al. 2006
One111	0.921	0.931	0.1697	59	137-334	Olsen et al. 2006
Ots2m	0.466	0.537	0.0000	6	146-160	Banks et al. 1999
Ots3m	0.708	0.720	0.0341	13	135-161	Banks et al. 1999
One101	0.908	0.936	0.3943	38	128-268	Olsen et al. 2006
One106	0.944	0.952	0.4385	34	165-317	Olsen et al. 2006
Ssa419	0.788	0.813	0.1631	12	262-306	Cairney et al. 2000
Coho salmon						
Ocl8	0.893	0.911	0.2678	18	98-138	Condrey and Bentzen 1998
Oki1	0.749	0.799	0.0028	14	86-146	Smith et al. 1998
Oki10	0.928	0.953	0.0000	36	91-243	Smith et al. 1998
Oki23	0.836	0.889	0.0035	23	116-204	Spidle et al. 2000
One13	0.886	0.904	0.0004	19	151-189	Scribner et al. 1996b
Ots3	0.744	0.778	0.1249	14	63-116	Banks et al. 1999
Ots103	0.835	0.953	0.0006	43	65-325	Small et al. 1998
Ots213	0.776	0.786	0.4762	29	155-315	Greig et al. 2003
Ots505	0.848	0.832	0.6636	13	230-254	Naish and Park 2002
OtsG422	0.940	0.965	0.0000	44	232-456	Williamson et al. 2002
P53	0.838	0.856	0.0103	11	163-187	de Fromentel et al. 1992
Rainbow trout						
Ocll	0.757	0.870	0.0000	16	150-220	Condrey and Bentzen 1998
Ogo4	0.767	0.760	0.0047	10	115-133	Olsen et al. 1998b
Omy1001	0.784	0.906	0.0000	24	172-258	Spies et al. 2005
Omy7	0.705	0.820	0.0000	18	178-278	Karim Gharbi, Univ. of Guelph, pers. comm.
One14	0.636	0.762	0.0000	9	150-204	Scribner et al. 1996b
Ots100	0.807	0.854	0.0002	22	164-220	Nelson & Beacham 1999
Ots3	0.676	0.725	0.0016	9	74-90	Banks et al. 1999
Ots4	0.731	0.798	0.0004	8	105-127	Banks et al. 1999
Oke4	0.739	0.823	0.0000	14	234-272	Buckholz et al. 2001
Oki23	0.871	0.905	0.0000	20	116-200	Spidle et al. 2000
Omy1011	0.833	0.893	0.0000	20	134-222	Spies et al. 2005

continued next page

TABLE 3. Continued.

Locus	H_o	H_e	HWE *P* value	No. alleles	Range	Reference
Omy77	0.771	0.831	0.0000	18	95-139	Morris et al. 1996
Ssa289	0.587	0.757	0.0000	8	105-119	McConnell et al. 1995
Ssa407	0.749	0.852	0.0000	21	163-243	Cairney et al. 2000
Ssa408	0.847	0.893	0.0152	18	173-245	Cairney et al. 2000
MHC class II B1	0.812	0.858	0.0010	30	n.a.	Miller et al. 2001
Sockeye salmon						
Oke2	0.571	0.690	0.0000	9	87-103	Bucholz et al. 2001
One110M	0.857	0.943	0.0000	35	143-225	Olsen et al. 2000b
One18	0.511	0.710	0.0000	9	168-212	Scribner et al. 1996b
Ots10M	0.691	0.861	0.0000	20	105-149	Greig and Banks 1999
Ots100	0.649	0.819	0.0000	20	155-207	Nelson and Beacham 1999
Ssa85	0.728	0.875	0.0000	22	123-185	O'Reilly et al. 1996
One13	0.534	0.765	0.0000	9	151-171	Scribner et al. 1996a
Omm1159	0.464	0.647	0.0000	9	185-223	Rexroad et al. 2002b
Omy77	0.573	0.768	0.0000	12	90-120	Morris et al. 1996
Ots103	0.891	0.908	0.0270	19	146-218	Beacham et al. 1998
One21	0.722	0.859	0.0000	16	127-159	Scribner et al. 1996b
Omm1068	0.635	0.703	0.0250	7	133-154	Rexroad et al. 2002a
Oki29	0.882	0.930	0.0000	28	204-312	Nelson et al. 2003
Chinook salmon						
Ogo2	0.691	0.688	0.4614	11	214-240	Olsen et al. 1998a
Ogo4	0.740	0.807	0.0103	12	132-164	Olsen et al. 1998a
Oki100	0.923	0.954	0.0405	34	164-329	Unpublished data, Pacific Biological Station, British Columbia
Omm1080	0.925	0.933	0.3069	39	162-362	Rexroad et al. 2001
Ots201b	0.882	0.916	0.0134	30	137-326	Michael Banks, Oregon State University, pers.comm.
Ots208b	0.902	0.890	0.2142	31	154-298	Greig et al. 2003
Ots211	0.911	0.929	0.3461	24	200-296	Greig et al. 2003
Ots212	0.855	0.870	0.3051	22	135-231	Greig et al. 2003
Ots213	0.936	0.937	0.1070	28	202-342	Greig et al. 2003
Ots3M	0.792	0.779	0.9096	12	132-160	Greig and Banks 1999
Ots9	0.616	0.625	0.0958	5	101-113	Banks et al. 1999
OtsG474	0.521	0.560	0.3955	11	152-212	Williamson et al. 2002
Ssa408UOS	0.864	0.872	0.8235	21	184-280	Cairney et al. 2000
Bull trout						
Omm1128	0.606	0.772	0.0000	19	276-362	Rexroad and Palti 2003
Sco105	0.230	0.349	0.0000	8	130-178	Sewall Young, Washington Department Fish and Wildlife, pers. comm.
Sco200	0.603	0.735	0.0000	8	141-190	DeHaan and Ardren 2005
Sco212	0.728	0.818	0.0000	12	184-300	DeHaan and Ardren 2005
Sco220	0.700	0.892	0.0000	18	208-352	DeHaan and Ardren 2005
Smm22	0.660	0.887	0.0090	18	147-315	Crane et al. 2004

compared to an Elwha River collection (Figure 1; Table 2). Seven of the 13 loci contained >20 alleles (Table 3). The highest levels of diversity were found in the Elwha collection. Allelic richness at Jimmycomelately Creek was approximately 50% the value of the Elwha collection (Table 2). Five of the 13 loci were out of HWE and F_{ST} values were generally low (Figure 3). Populations grouped by locale and by run timing (Figure 3); the fall-run fish from the Elwha River formed a branch off the Puget Sound fall-run group (Figure 3).

Coho Salmon

Coho salmon were collected from six sites along the Strait of Juan de Fuca and compared to an Elwha River collection site at 11 mSAT loci (Figure 1; Table 2). Five loci had >20 alleles and 6 loci were out of HWE. Allelic and gene diversity

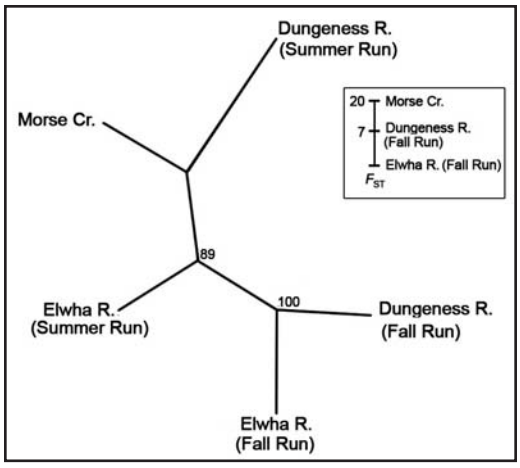

Figure 2. Consensus neighbor-joining tree of CSE chord distances between pink salmon collections based on 13 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant.

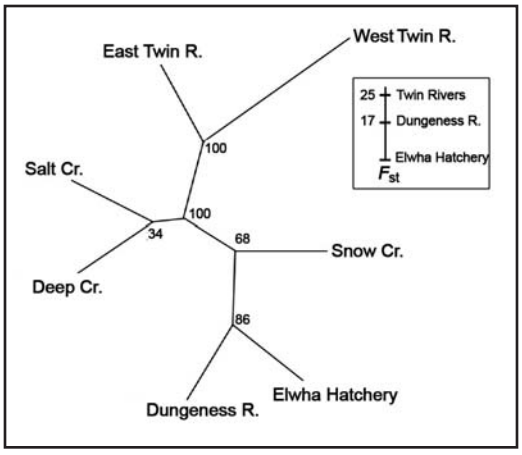

Figure 4. Consensus neighbor-joining tree of CSE chord distances between coho salmon collections based on 11 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant.

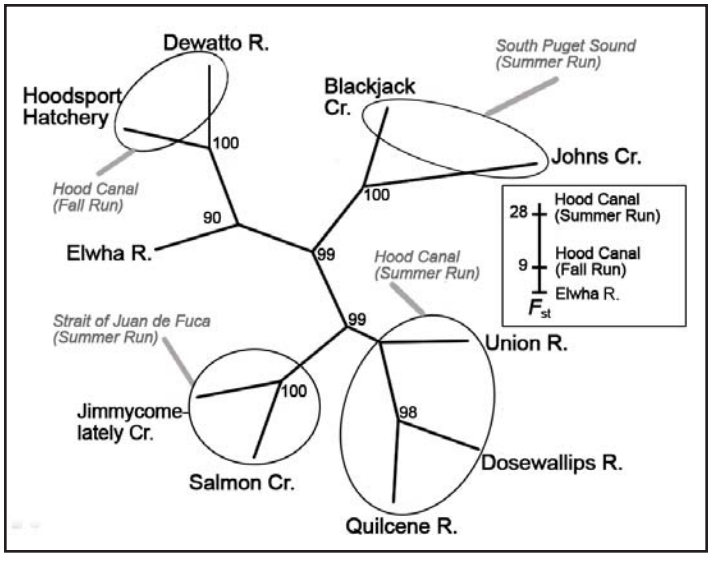

Figure 3. Consensus neighbor-joining tree of CSE chord distances between chum salmon collections based on 13 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant. Ellipses enclose collections by group for illustrative purposes.

measures were similar over all sites (Table 2). The relationships among samples did not follow a geographic pattern (Figure 4). For example, Deep and Salt creeks, which are found on either side of East and West Twin Creeks, grouped together. Elwha grouped with the Dungeness collection even though the two collections have statistically different allele frequencies (overall chi-square P=0.000; F_{ST}=0.173). As a group these collections clustered with coho salmon from the northern Washington coast (as opposed to exhibiting a Puget Sound affinity) (VanDoornik et al. 2007).

Rainbow Trout

Three collections of resident rainbow trout above the Elwha Dam were compared to a wild and a hatchery collection of steelhead from the lower river (Figure 1; Table 2). Five of 15 mSAT loci contained 20 or more alleles (Table 3). There was considerable variability in the estimates of genetic diversity. The wild steelhead collection from the lower Elwha River had the largest values of allelic richness and gene diversity, whereas a collection of rainbow trout from the South Branch Little River had 40-50% less at both estimates of allelic richness and

gene diversity (Table 2). Allelic richness and gene diversity were higher in two resident trout collections (Griff and Madison creeks) than in the hatchery steelhead collections. All the loci departed significantly from HWE (Table 3) and F_{ST} values were moderate, e.g., F_{ST} between the wild and the brood year 2006 hatchery collection was 0.035 (P<0.001) (Figure 5a). It is worth noting that the wild fish were significantly different from the hatchery fish at all loci. The resident rainbow trout formed a separate branch from the wild and hatchery steelhead collections on the mSAT tree (Figure 5a).

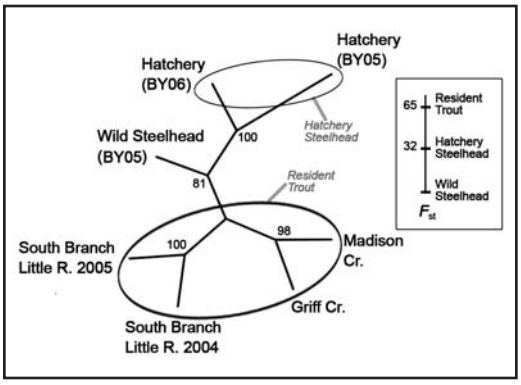

Figure 5a. Consensus neighbor-joining tree of CSE chord distances between rainbow trout and steelhead collections based on 14 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant. Ellipses enclose collections by group for illustrative purposes.

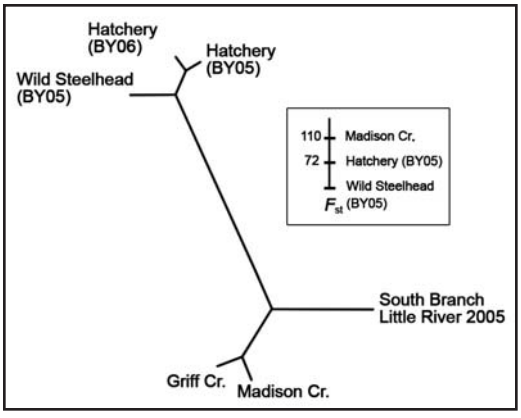

Figure 5b. Consensus neighbor-joining tree of CSE chord distances between rainbow trout and steelhead collections based on variability at the class II B1 locus at the major histocompatability complex (MHC). F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant.

Similar patterns of genetic variability were seen at the MHC locus for which 9-18 alleles were seen in the various collections (Table 2). The largest values of genetic variability were seen in the wild steelhead collection, the smallest values were seen in the rainbow trout from South Branch Little River, and reduced levels were seen in the hatchery collections (Table 2). The level of differentiation at this locus as estimated by F_{ST} was roughly twice that of the mSATs (Figures 5a and 5b).

Sockeye salmon

The Elwha watershed has a population of landlocked sockeye salmon (kokanee) in Lake Sutherland (Figure 1), although occasional adult sockeye strays have been observed in the lower Elwha River. Two kokanee collections from Lake Sutherland were compared with Lake Ozette sockeye salmon (the nearest sockeye salmon stock in Washington) and with Lake Whatcom kokanee (historically, the most frequently outplanted kokanee in western Washington; Figure 1, Table 2). Over 13 loci, there were moderate levels of variability, with 5 loci containing 20 or more alleles (Table 3). The Ozette sockeye salmon collection had low values of genetic diversity compared to the other samples (Table 2). None of the loci were in HWE over all collections. The three collections were strongly differentiated (Figure 6).

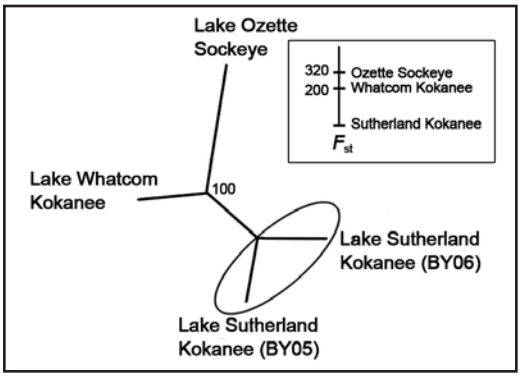

Figure 6. Consensus neighbor-joining tree of CSE chord distances between sockeye salmon/kokanee collections based on 13 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant. Ellipses enclose collections by group for illustrative purposes.

Chinook Salmon

Tissues of Elwha River Chinook salmon were analyzed from collections taken from the Washington Department of Fish and Wildlife hatchery, a spawning site at Hunt's Road Channel, and a screw trap (rkm 0.5) sampling of 0-age non-hatchery Chinook salmon (Figure 1; Table 2). These were compared to a collection from the Dungeness River. Eight of the 13 mSAT loci screened for the chinook collections had 20 or more alleles. Allelic and gene diversity measures were similar over all sites (Table 2). Ten of the 13 loci were in HWE (Table 3), indicating a low level of differentiation among collections. The Hunt's Road Channel fish were distinctive in the mSAT dendrogram (Figure 7a). The same relationship was seen using Nei's genetic distance (Nei 1978). However, these results were not consistent with F_{ST} values among the collections, e.g., the F_{ST} value between Hunt's Road Channel and the hatchery was not significantly different from zero, whereas all other pair-wise comparisons with the Dungeness collection were statistically significant. For an independent view of collection variability, we viewed the data in a factorial correspondence analysis. The first three axes explained 85.3% of the variance (Figure 7b). Dungeness River was set apart from the other collections on Axis 1, the screw trap collections were distinctive on Axis 2, and the hatchery was distinctive on Axis 3. The disparity in results between the dendrogram and the factorial correspondence analysis plot is partially explained by the low level of divergence among the collections.

Bull Trout

Bull trout in the Elwha River were compared with collections ranging from the west coast of Washington to the Hood Canal in Puget Sound (Figure 1; Table 2). The number of alleles per locus ranged from 8-19 (Table 2). There was considerable variability in gene diversity estimates between collections within watersheds, and among collections overall (Table 2). Estimates of gene diversity were smallest in two collections from the Dungeness watershed. None of the loci were in HWE over all collections. The Elwha collection was clearly differentiated from all other neighboring populations (F_{ST}=0.168; Figure 8), consistent with bull trout variability throughout its range (Spruell et al. 2003).

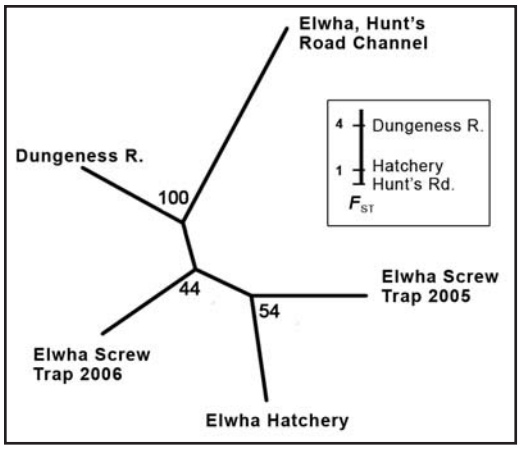

Figure 7a. Consensus neighbor-joining tree of CSE chord distances between Chinook salmon collections based on 13 mSAT loci. Numbers at the nodes indicate the percentage of 1000 trees in which collections grouped together. F_{ST} values x 1000 are provided for select comparisons against the principal Elwha collection and are not statistically different from zero.

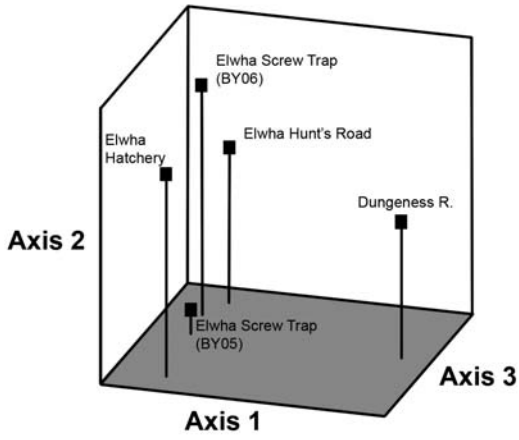

Figure 7b. Plot of average scores of five collections of Chinook salmon along the first three axes of a factorial correspondence analysis based on 13 mSAT loci.

Discussion

A basic tenant of conservation genetics is that natural genetic diversity, as estimated in some cases by molecular markers, enhances the probability of a population's survival over ecological and evolutionary time (Avise 1994). In our synopsis of several independent and preliminary studies, collections within the Elwha river system generally exhibited moderate levels of genetic

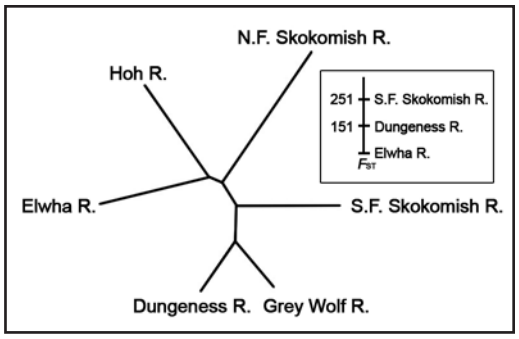

Figure 8. Consensus neighbor-joining tree of CSE chord distances between bull trout collections based on 6 mSAT variability. Bootstrap values at the nodes are not available. F_{ST} values (x 1000) for select comparisons against the principal Elwha collection are statistically significant.

diversity, levels that were similar with those from neighboring sites (Table 2). These estimates do not guarantee rates or levels of recolonization success; rather, they indicate an absence of genetically impoverished groups of populations in the set of collections noted here. Two findings deserve extra comment. Among the three resident rainbow trout collections, the South Branch Little River had substantially reduced values of allelic richness and heterozygosity at both mSAT and MHC loci (Table 2). This population differs from the other two collections because it is isolated above a barrier in the upper Little River. A simple explanation therefore is that it has experienced periodic fluctuations in population numbers. Based on protein genetic data, Phelps et al. (2001) concluded that the South Branch Little River may be closely related to coastal winter steelhead, a finding not corroborated with our preliminary data. We (G. Winans, J. Baker, and K. Miller) are also investigating whether genetic introgression with non-native hatchery trout has occurred in the above-dam resident trout populations, including the South Branch Little River. The Elwha hatchery steelhead and the Lake Ozette sockeye salmon collection also exhibited a moderate reduction in genetic diversity (Table 2). The latter result was previously observed in a protein genetic survey (Winans et al. 1996). It is also worth noting that the Elwha chum salmon collection contained relatively higher levels of genetic diversity. This may be due in part to the hatchery transfers of chum salmon from Hood Canal (the Quilcene National Fish Hatchery) in the 1970's and 1980's to the Lower Elwha Klallam Tribal Hatchery (Nick Lampsakis, The Point No Point Treaty Council, personal communication).

In general, patterns of genetic similarity (Figures 2-8) follow patterns of differentiation that have been seen in more far-ranging investigations for each species. For example, nearest neighbors of chum salmon are genetically similar, whereas the pattern of differentiation in sockeye and coho salmon is more mosaic-like (see Waples et al. 2001). Two particular findings are worth emphasizing however. The collection of wild steelhead was clearly different from the hatchery stock (Figure 5a and 5b). This result was anticipated as the hatchery fish have an out-of-basin origin and spawn in December and January, whereas the wild Elwha population spawns from May to July. Less clear are the results for the Chinook salmon. The hatchery stock of Chinook salmon is based on local fish returning to the hatchery, although fish are 'mined' from the local stretch of the river in some years to meet egg-take goals (Michael L. McHenry, personal observation). In spite of this periodic mining and the proximity of the two sites, there is some indication that the hatchery stock may be dissimilar from the Hunt's Road Channel (Figure 7b). Further, the screw trap data indicate the possible presence of more populations in the river (Figure 7b). These preliminary results will be reevaluated as we collect more temporal and spatial data.

These baselines are all work-in-progress data sets requiring additional sampling to broaden the geographic coverage and to statistically confirm relationships. For example, Fraser River and southern Vancouver Island populations are needed for both the pink and sockeye salmon baselines, as recolonization to the Elwha River may involve strays from these large Northwest stocks. We (G. Winans, J. Baker, and K. Miller) are in the process of analyzing upper Elwha resident rainbow trout, as well as non native hatchery rainbow trout stocks (which were planted into the upper Elwha; Brenkman et al. 2008), to complete our genetic and phenetic survey of *O. mykiss* in the basin. We recognize that collections of chum salmon and steelhead are needed from the Dungeness River; and the late fall-run chum salmon from adjacent populations along the Strait of Juan de Fuca are missing in the baseline.

We also recognize that other character sets are needed to more completely understand local

adaptation and, therefore, the characteristics of fish stocks that may successfully recolonize a reopened river. For rainbow trout, MHC variability is being used in conjunction with neutral markers (mSATs) to help dissect levels of adaptability with respect to disease challenges (Miller et al. 2001). We are also evaluating multivariate morphometrics for kokanee, coloration of juvenile rainbow trout, and morphometrics for juvenile and adult rainbow trout (see Winans et al. 2003). Other data that are useful for defining populations but are difficult to collect in a large river system such as the Elwha River include run timing, spawn timing, and ocean age.

As these genetic data sets are expanded for each species, we will be in a position to monitor the population composition of recolonization of the upper Elwha River. Mixture analysis of outmigrating juveniles and/or returning adults is essentially a genetic stock identification (GSI) procedure, where mixture genotypes are compared against a population baseline to estimate population proportions (Shaklee et al. 1999). We describe a range in the levels of population differentiation, from low F_{ST} values in pink and Chinook salmon to high values for sockeye salmon and bull trout. These values are well within the range of F_{ST} estimates that simulation studies have used to demonstrate accurate GSI applications (Beacham et al. 2000, Winans et al. 2004, Van Doornik et al. 2007).

Just as valuable as these descriptions of population differences using mSATs are the tissues used to collect the data. As pointed out by Shaklee et al. (1999), DNA-based techniques represent an "evolving technology." As mSAT baselines have supplemented and then replaced protein genetic baselines for salmon population genetic work (Beacham et al. 1998b, Small et al. 1998), so to will other genetic markers be developed that will be as (or more) discriminating, cheaper, or more objective to score than mSATs. For example, laboratory techniques to score large numbers of single nucleotide polymorphisms are being developed for salmon that meet the above desired attributes of a new technology (Smith et al. 2005, Elfstrom et al. 2006). Thus, an important challenge for collaborators in the field is the proper archiving of baseline tissue samples. When mSAT data are no longer the technology of choice and incorporated into a newer, more advanced molecular technique (see Avise 1994), archived tissues from our studies will be extremely valuable to maintain pertinent genetic baselines and to extend our gene inventory/GSI work started here on salmon recolonization in the Elwha River.

There are several other difficult management and conservation issues that can be addressed with these data. Hatchery Chinook salmon are slated for outplanting into various reaches of the Elwha River (Ward et al. in press). Our data suggest that Elwha Chinook salmon may not consist of one interbreeding population. The early-returning fish at Hunt's Road Channel may represent a unique population. The screw trap data suggest there may be other unrecognized populations in the river. If either of these findings is true, after additional sampling and analyses, how will hatchery outplanting effect these populations? There have also been suggestions to move steelhead above the dams prior to dam removal. Here we show high levels of differentiation at the mSAT and MHC loci between resident trout and below-dam steelhead. Resident trout are different from steelhead, but we know little about the role of rainbow trout in steelhead adaptability and evolution, and vice versa. For example, in sympatry, low but steady genetic contributions of trout to steelhead populations may be important in the population genetic stability of steelhead (Araki et al. 2007). And in one case, rainbow trout crosses made from a population isolated in allopatry for about 80 years were able to produce smolts (in a hatchery environment), but the adults returned in very small percentages (Thrower et al. 2004). If steelhead are not passed upstream prior to dam removal, it is feasible that these data can be used to monitor the interactions of the various gene pools and study the characteristics of recolonization. We recognize that preserving natural processes associated with recolonization may be as important as recognizing and protecting natural biological diversity (Avise 1994).

The success of reestablishing sustainable populations will vary by species (Pess et al. 2008) and dam sites. It will vary due to factors such as species composition, habitat quality and quantity, the number and biological fitness of available gene pools, the number and strength of neighboring populations and hatcheries, and, to some degree, the extent of management involvement. Some of us (G. Winans, J. Baker, and K. Miller) are presently involved in monitoring *O. mykiss* in five dam recolonization efforts in the Pacific Northwest. Our goal is to provide a "genetic ruler" that assesses

relevant gene pools prior to dam removal. This ruler will be used in the future as part of a multivariable evaluation of recolonization success. As more dam sites are reconsidered for removal (McHenry and Pess 2008), our accumulated information on the genetics of recolonization will help guide these events. The genetic data provided here are the first such data that will be used as a genetic ruler in an attempt to manage and conserve Pacific salmonids in the Elwha River recolonization program.

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