Major Histocompatibility Complex Differentiation in Sacramento River Chinook Salmon

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ABSTRACT

The chinook salmon of the Sacramento River, California, have been reduced to a fraction of their former abundance because of human impact and use of the river system. Here we examine the genetic variation at a major histocompatibility complex class II exon in the four Sacramento chinook salmon runs. Examination of the alleles found in these and other chinook salmon revealed nucleotide patterns consistent with selection for amino acid replacement at the putative antigen-binding sites. We found a significant amount of variation in each of the runs, including the federally endangered winter run. All of the samples were in Hardy-Weinberg proportions. A significant amount of genetic differentiation between runs was revealed by several measures of differentiation. Winter run was the most genetically divergent, while the spring, late-fall, and fall runs were less differentiated.

MOLECULAR markers have been used to study population structure and variation since the advent of allozyme electrophoresis. Currently, microsatellite loci are the nuclear marker of choice in ecological and population genetic studies because they are highly variable and are thought to be primarily influenced by nonselective mechanisms (Bruford and Wayne 1993; Ashley and Dow 1994; Queller *et al.* 1994). However, because microsatellite loci are generally selectively neutral, they may not adequately describe adaptive differences between populations. Most molecular markers that are under selection often lack the variation needed to describe population differentiation and adaptation, but the genes of the major histocompatibility complex (MHC) are an exception.

MHC genes are known to be involved in the vertebrate immune system and are believed to be the main genetic system involved in parasite resistance (Apanius *et al.* 1997; Edwards and Hedrick 1998; Hedrick and Kim 1999). Many of the genes of the MHC code for cell-surface glycoproteins that provide the mechanism of recognition of self and nonself by binding short peptides in the antigen-binding site (ABS) and presenting them to T cells, eliciting an immune response (Doherty and Zinkernagel 1975). In this way, the immune system can recognize foreign antigens and protect the body from infection.

Many MHC genes are highly polymorphic, and there are multiple lines of evidence that the polymorphism is maintained by some form of balancing selection (Hedrick and Thompson 1983; Klitz *et al.* 1986; Klitz and Thompson 1987; Hughes and Nei 1988; Hedrick *et al.* 1991; Markow *et al.* 1993; Black and Hedrick 1997). Models based on pathogen resistance, negative-assortative mating, and maternal-fetal interaction have been proposed to explain the high levels of polymorphism (Apanius *et al.* 1997). Because of the involvement of MHC in the immune response, pathogen resistance appears to be the most likely mechanism (Brown and Eklund 1994; Hedrick 1994). Recently, there have been a number of reports describing MHC variation in fishes demonstrating that fish share a number of features that are common with other vertebrate MHC genes, including high polymorphism (Klein *et al.* 1993; Ono *et al.* 1993; Grimholt *et al.* 1994; Graser *et al.* 1996; Lie and Grimholt 1996; Miller and Withler 1996; Miller *et al.* 1997; Hedrick and Parker 1998).

Sacramento River chinook salmon (*Oncorhynchus tshawytscha*) are semelparous, anadromous fishes found in the Sacramento River and connecting watersheds. Currently, four distinct runs based on migration timing are described and recognized in these salmon: winter, spring, fall, and late-fall runs (Fisher 1994). Although there is significant overlap of migration times between runs, spawning occurs at distinctly different times with the exception of a small overlap between the fall and spring runs. Over the past several decades a number of threats have reduced the Sacramento River chinook runs to a fraction of their former abundance (Fisher 1994). Because of the serious decline, the winter run chinook was federally listed as endangered in 1994, and a captive release supplementation program by the U.S. Fish and Wildlife Service (USFWS) was established in an effort to augment the natural population (Hedrick *et al.* 1995).

Here we report on the genetic variation at a MHC class

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II β chain exon in the four different runs of chinook salmon in the Sacramento River. The molecular evolution of the alleles found in these runs is discussed, and allelic frequency data are used to analyze the levels of population genetic structure within and between the runs.

MATERIALS AND METHODS

Samples: Migrating adult salmon from each run were collected by the USFWS at either the fish ladders at Red Bluff diversion dam or at Keswick dam. The winter run samples were collected for use as parents in the supplementation program and consisted of the following: 18 from 1991, 27 from 1992, 9 from 1993, 23 from 1994, and 33 from 1995. The other run samples consisted of 13 fish from the 1995 mainstem Sacramento spring run, 13 fish from the 1995 Butte Creek spring run, 19 fish from the 1993 fall run, and 20 fish from the 1995 late-fall run. Liver tissue from winter run fish sacrificed for spawning at the Coleman National Fish Hatchery (USFWS) was acquired, and genomic DNA was isolated from the tissue by lysis in proteinase K solution followed by standard phenol:chloroform extraction (Sambrook *et al.* 1989). Genomic DNA from the 1991 winter run offspring and the other three runs (spring, fall, and late fall) was extracted from 1-mm^2 finclips using Chelex (Bio-Rad, Hercules, CA) following standard manufacturing protocols.

Molecular methods: A 260-bp fragment of the MHC class II β1 domain exon containing much of the antigen-binding region was amplified by PCR using the following Atlantic salmon (*Salmon salar*) primers reported by Grimholt *et al.* (1994): (sasa-1) 5′-ATGTCTAGA TGC CGA TAC TCC TCA AAG GAC-3′ and (sasa-2) 5′-GGCAAGCTT ACC TGT CTT GTC CAG TAT GG-3′ at a final concentration of 0.5 μm each with an annealing temperature of 58° for 35 cycles. Single-stranded conformational polymorphism (SSCP) analysis (Orita *et al.* 1989) was carried out with the same PCR conditions but including 1 μCi of ^{32}[P]dATP in each reaction. Samples were electrophoresed at 4° on 6% polyacrylamide gels with 2.6 and 4% crosslinking at 50 W for 4.5 hr. The gel was transferred to 3MM Whatman paper, dried, and exposed overnight to X-ray film (Fuji RX). DNA samples with different SSCP profiles were used to amplify fragments for subcloning into the *Hin*dIII and *Xba*I sites of pUC18. Colony subclones were picked and boiled in 50 μl of HPLC grade water and 2 μl was used directly for PCR. Subclones were screened by SSCP and sequenced on both strands using an AmpliCycle Sequencing Kit (Perkin-Elmer, Foster City, CA).

Statistical analysis: Nucleotide sequences were aligned using EyeBall software (Cabot and Bechenbach 1989). MHC sequences from closely related species were acquired using the GenBank BLAST program (Altschul *et al.* 1990). Phylogenetic methods (neighbor-joining and UPGMA) were used to infer relationships among alleles and populations (Kumar *et al.* 1993). Tests for Hardy-Weinberg proportions were performed using the Hardy-Weinberg exact test (Levene 1949). The log-likelihood test (*G*-test; Sokal and Rohlf 1995) was used to determine whether the allele frequencies in the 5 winter run years were homogenous and could be pooled. The population genetic structure was examined by determining F_{ST} (Weir and Cockerham 1984), N_{ST} (Lynch and Crease 1990), genetic distance *D* (Nei 1972), and a genetic distance based on amino acid differences D_{AA} (Hedrick and Parker 1998). The F_{ST} values and the associated nonparametric *P* values for significance were calculated using the software package Arlequin (Schneider *et al.* 1996).

RESULTS

Preliminary screening of salmon samples from different runs by SSCP indicated the presence of four alleles. These alleles were isolated by PCR amplification, cloned, sequenced, and designated *Onts-wr1*, *Onts-wr2*, *Onts-wr3*, and *Onts-lf1*, where *wr* and *lf* symbolize winter and late-fall runs, respectively. The nucleotide sequences were aligned and compared to other salmon sequences obtained from GenBank (Miller and Withler 1996; Miller *et al.* 1997) as well as an allele isolated in Atlantic salmon, *S. salar* (Hordvick *et al.* 1993). *Onts-wr3* was found to be identical to alleles *Onts-Tj10b* and *Onts-N32* previously characterized in chinook salmon populations in the Fraser River drainage of British Columbia (Miller and Withler 1996; Miller *et al.* 1997). The other alleles (*Onts-wr1*, *Onts-wr2*, and *Onts-lf1*) have not been previously reported. These three sequences have been deposited in GenBank under the accession numbers AF041009-11.

Sequence analysis: A total of six chinook salmon MHC class II β1 alleles have been reported, three alleles found exclusively in Sacramento River chinook, two alleles (*Onts-HN2* and *Onts-H1*) found exclusively in northern chinook reported from the Fraser River (Miller *et al.* 1997), and one allele common to both regions (*Onts-wr3*). The four Sacramento chinook sequences were 196 bp in length, and eight nucleotide positions (4.1%) were polymorphic. These alleles exhibited a mean pairwise nucleotide similarity of 97.7%. The mean pairwise nucleotide similarity among all six chinook alleles was 96.9% (range 95–99%), and there was only one silent substitution.

The amino acid sequences for the variable positions for all of the chinook salmon alleles and *Sasa-c144* are shown in Table 1. Codon numbers and the putative antigen-binding sites (pABS) correspond with those for human class II sequences (Brown *et al.* 1993). (The term putative is used here because the actual antigen binding sites have not been determined in fish.) Six of the 65 amino acid positions (9.2%) were variable among the Sacramento chinook sequences (positions 28, 37, 53, 55, 77, and 85) with a mean pairwise amino acid similarity of 95% (range 92–98%). Seven amino acid positions (10.8%) were variable among all the chinook salmon sequences with a mean pairwise amino acid similarity of 92.9% (range 88–98%). A total of 21 amino acid positions (32.3%) were variable when compared to the Atlantic salmon allele, *Sasa-c144* (Hordvick *et al.* 1993). The two conserved amino acids on the mammalian beta chain (W at position 61 and N at position 82) were also conserved, consistent with other reports of chinook alleles (Miller *et al.* 1997).

Among the chinook sequences, 15% (3 out of 20 codons) of pABS are variable and 8.8% (4 out of 45 codons) on the nonbinding sites (non-pABS) are variable. Within the Sacramento chinook, 15% of the bind-

TABLE 1

The amino acid sequences for the variable amino acid sites

Position:	26	28	34	35	37	46	47	53	55	66	68	69	71	72	73	77	80	83	84	85	87
Consensus:	F	D[c]	K	V	H[c]	R	Y[c]	L	V	Q	G[c]	Q	Q[c]	A	E	F	P	A	L	H	R
Alleles																					
Onts-wr1	—	—	—	—	N	—	—	H	—	—	—	—	—	—	—	V	—	—	—	—	—
Onts-wr2	—	H	—	—	—	—	—	—	L	—	—	—	—	—	—	V	—	—	—	—	—
Onts-wr3	—	H	—	—	—	—	—	—	L	—	—	—	—	—	—	V	—	—	—	E	—
Onts-lf1	—	—	—	—	—	—	—	—	L	—	—	—	—	—	—	—	—	—	—	—	—
Onts-H1[a]	—	—	—	A	Y	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Onts-HN2[a]	—	—	—	A	Y	—	—	H	—	—	—	—	—	—	—	—	—	—	—	—	—
Sasa-c144[b]	L	T	Q	A	N	K	F	H	—	E	A	G	L	G	V	Y	F	P	I	D	S

[a] Chinook alleles from Miller *et al.* (1997).
[b] Atlantic salmon (*Salmo salar*) allele from Hordvick *et al.* (1993).
[c] Putative antigen binding sites (pABS) following Brown *et al.* (1993).

ing sites compared to 6.7% of the nonbinding sites are variable. There is only one silent substitution located in the non-pABS region among the chinook sequences (GAG to GAA in codon 52 of *Onts-wr1* and *Onts-HN2*). The numbers of synonymous (d_S) and nonsynonymous (d_N) substitutions per nucleotide in the chinook alleles are given in Table 2. The ratio of nonsynonymous substitutions to synonymous substitutions (d_N/d_S) in the pABS (∞) compared to the ratio in the non-pABS (0.75) indicates selection for amino acid replacement at the antigen-binding region.

Molecular evolution of alleles: Results of the BLAST search found a number of alleles from other salmonids that exhibit a high level of similarity to the Sacramento chinook alleles. A number of these alleles are included in a neighbor-joining tree showing the relationships among the amino acid sequences (see Figure 1). The topology of the tree is supported by bootstrapping with only the major branch between Pacific and Atlantic salmon being significant. Differing by only one amino acid, *Onts-wr3* and *Onts-wr2* cluster together. *Onts-wr1* is divergent from the other Sacramento alleles, differing from *Onts-wr2* at four sites (28, 37, 53, and 55) and *Onts-wr3* at five sites (28, 37, 53, 55, and 85). Four of the coho salmon (*O. kisutch*) alleles (*Onki*) cluster together closely to the winter run alleles. Interestingly, a coho salmon allele (*Onki-87f*) was quite divergent from the other coho alleles and was more similar to the chinook alleles. This transspecies allelic similarity is not unusual for MHC genes, and it has been proposed that MHC allelic lineages are maintained by selection and are often older than the species themselves. All of the Pacific salmonid alleles were divergent from the Atlantic salmon (*S. salar*) alleles.

Population structure: We characterized the MHC variation in each of the runs using SSCP analysis. To ensure that we were amplifying from a single locus and that the MHC locus exhibited Mendelian segregation, we examined the progeny from a number of crosses. We scored 25 progeny from one family cross and 5 progeny from three family crosses. In all cases, all of the expected genotypes were observed. Salmonids are generally tetraploid and are in the process of rediploidization (Allendorf and Thorgaard 1984). Results from the crosses indicate that this locus is segregating as a diploid, which is consistent with earlier reports in chinook salmon (Miller *et al.* 1997) and inconsistent with some of the results found in Atlantic salmon (Grimholt *et al.* 1994).

There were a total of four alleles in the samples (Table 3). Winter run was segregating for all four alleles with *Onts-wr1* in high frequency and *Onts-lf1* in very low frequency found in only three heterozygous individuals (2.7%). Spring run was also segregating for all four alleles with *Onts-wr3* present in high frequency and the

TABLE 2

Synonymous (d_S) and nonsynonymous (d_N) substitutions in the putative antigen (pABS) and nonantigen binding sites (non-pABS) among chinook alleles

Region	No. of codons	d_S	d_N	d_N/d_S
pABS	20	0.0000 (0.0000)	0.0719 (0.0269)	∞
Non-pABS	45	0.0256 (0.0210)	0.0192 (0.0099)	0.75
Total	65	0.0182 (0.0149)	0.0352 (0.0105)	1.93

Standard error given in parentheses.

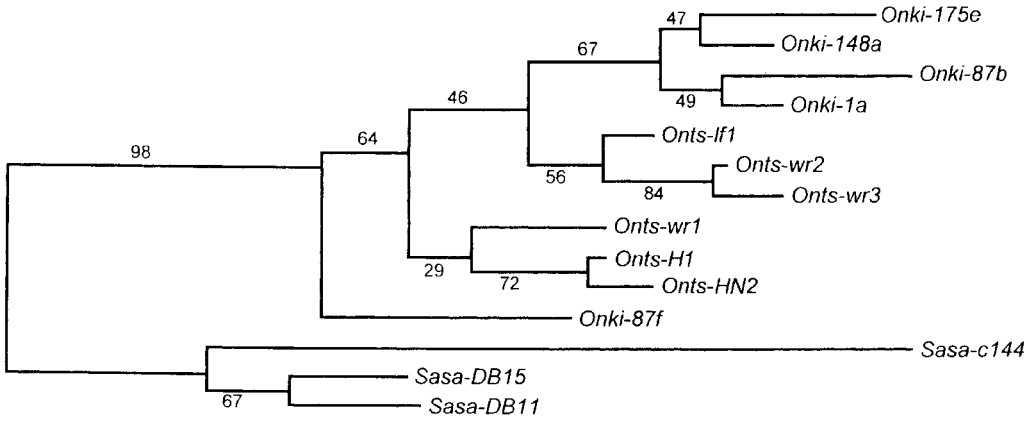

Figure 1.—Neighbor-joining tree based on the MHC class II β1 amino acid sequences. The topology of the tree is supported by bootstrap *P* values (500 iterations). Alleles from the chinook (*Onts*), coho (*Onki*), and Atlantic (*Sasa*) salmon are included.

other three alleles in lower frequencies. The fall run was also segregating for all four alleles with *Onts-wr3* in high frequency, but *Onts-wr1* and *Onts-wr2* were in low frequencies and *Onts-lf1* was in moderate frequency. The late-fall run segregated for only three alleles with *Onts-wr3* and *Onts-lf1* in relatively equal and high frequencies and *Onts-wr2* in low frequency.

The small Butte Creek spring run sample ($N = 13$) deviated significantly from Hardy-Weinberg expectations ($P = 0.016$) because of an excess of *Onts-wr3* homozygotes. However, after correcting for multiple comparisons using the Dunn-Sidak method (Sokal and Rohlf 1995), this is also not statistically significant. Interestingly, the most common allele in the winter run, *Onts-wr1*, is not present in late fall and is in low frequencies in the spring and fall runs. Furthermore, three of the alleles (*Onts-wr1*, *Onts-wr2*, and *Onts-lf1*) were not detected in previous reports of chinook salmon populations in the Fraser River (Miller *et al.* 1997).

Results from the log-likelihood tests indicated that the 1991, 1992, 1993, and 1994 winter run years were homogenous, and we have pooled them in the subsequent analyses. These combined samples are referred to as winter run henceforth. The pooled 1991–1994 frequencies of *Onts-wr1*, *Onts-wr2*, *Onts-wr3*, and *Onts-lf1* are 0.839, 0.097, 0.058, and 0.007, respectively. The 1995 winter run sample was not homogenous with the other samples and is referred to as winter 95. The mainstem Sacramento River and Butte Creek spring run samples could also be pooled and are referred to as spring run. The pooled spring run frequencies are 0.103, 0.241, 0.500, and 0.155 for the four alleles. Both winter and spring run pooled samples were in Hardy-Weinberg proportions ($P = 1.000$ and 0.144, respectively).

Table 4 includes pairwise F_{ST} values showing the relative population differentiation between runs. The significance of the F_{ST} values was determined using a nonparametric permutation test (Excoffier *et al.* 1992). The overall F_{ST} value of 0.129 indicates a fairly high level of differentiation. All pairwise comparisons were significant except for the comparison between fall and late fall. The winter run shows the highest F_{ST} values when compared to the fall run and the least F_{ST} value when compared to the spring run. The F_{ST} values in the other run comparisons demonstrate much lower levels of population subdivision. Apparently the winter run appears to be more isolated genetically from the other runs, while the other runs have higher levels of gene flow. N_{ST} takes into account the number of nucleotide differences between the alleles. The N_{ST} values show the

TABLE 3

Allelic frequencies, sample sizes (*N*), observed (H_O) and expected (H_E) heterozygosities of the four MHC class II β1 alleles

	Winter					Spring[b]		Fall	Late fall
	1991	1992	1993	1994	1995	1995 (M)	1995 (B)	1993	1995
Onts-wr1	0.750	0.833	0.889	0.891	0.576	0.094	0.115	0.026	—
Onts-wr2	0.167	0.093	—	0.087	0.121	0.250	0.231	0.026	0.075
Onts-wr3	0.056	0.074	0.111	0.022	0.273	0.469	0.539	0.632	0.425
Onts-lf1	0.028	—	—	—	0.030	0.188	0.115	0.316	0.500
N	18	27	9	23	33	16	13	19	20
H_O	0.500	0.300	0.220	0.220	0.450	0.710	0.460	0.530	0.750
H_E	0.410	0.290	0.200	0.200	0.580	0.730	0.630	0.500	0.560
P[a]	1.000	0.540	1.000	1.000	0.346	0.484	0.016	1.000	0.346

[a] *P* values from Hardy-Weinberg exact tests are given.
[b] Spring runs (M) and (B) denote the mainstem Sacramento and Butte Creek samples.

TABLE 4

Measures of population differentiation

Comparison	D	F_{ST}	N_{ST}	D_{AA}
Winter-winter 95	0.069	0.125***	0.0026	0.0048
Winter-spring	1.270	0.486***	0.0183	0.0336
Winter-fall	2.155	0.619***	0.0227	0.0408
Winter-late fall	2.691	0.607***	0.0217	0.0399
Winter 95-spring	0.522	0.180***	0.0072	0.0133
Winter 95-fall	0.817	0.308***	0.0101	0.0181
Winter 95-late fall	1.132	0.322***	0.0101	0.0191
Spring-fall	0.090	0.052*	0.0005	0.0009
Spring-late fall	0.220	0.078**	0.0012	0.0032
Fall-late fall	0.106	0.045	0.0009	0.0020
Overall	0.907	0.129	0.0073	0.0078

Pairwise differences based on allelic frequencies (D and F_{ST}), allelic frequencies and nucleotide composition (N_{ST}), and allelic frequencies and amino acid composition (D_{AA}). The significance of the nonparametric permutation test for F_{ST} values is as follows: *** for $P < 0.001$; ** for $P < 0.01$; and * for $P < 0.05$.

same trend as the F_{ST} values. The genetic distance values based on amino acid differences, D_{AA}, show the same trends and are very similar to the N_{ST} values. This is to be expected because there were very few silent substitutions as reflected in the high d_N/d_S ratio discussed above.

The standard genetic distance values (D) of Nei (1972) were used to construct a phenogram (Figure 2) demonstrating the relationships of the runs. The 1991, 1992, 1993, and 1994 winter runs cluster closely and apart from the 1995 winter run, which is closer to the other runs. The fall and late-fall runs cluster together and are the most divergent from the winter run samples. The spring run samples are intermediate between the winter run and the other two runs.

DISCUSSION

We characterized class II MHC variation in Sacramento River chinook salmon and found significant frequency differences between all runs except fall and late fall. In particular, the endangered winter run is quite distinct from the other runs and homogeneous over different years. There have been very few studies in which significant genetic differences have been detected between sympatric populations (or runs in this case) that are separated temporally.

Molecular evolution of the MHC class II gene: The lack of different alleles with identical amino acid sequences (only silent substitutions) suggests that effects due to finite population size (*e.g.*, founder effects or population bottlenecks) have reduced the amount of synonymous variation. This observed lack of neutral variation is supported by other studies of genetic variation in chinook populations (Bartley and Gall 1990; Miller and Withler 1996; Miller *et al.* 1997).

The high d_N/d_S ratio in the pABS indicates that some sort of selection for replacement has occurred. All of the substitutions in the pABS are nonsynonymous. This excess of nonsynonymous substitutions in the pABS is consistent with observations in mammals (Nei and Hughes 1991) and was also reported in other salmonids (Grimholt *et al.* 1994; Miller and Withler 1996; Miller *et al.* 1997). Models of balancing selection based on the notion that antigen binding is increased by carrying alleles differing at the pABS could explain this high ratio.

Population structure: The F_{ST} analysis shows a significant amount of differentiation between the winter run and the other three runs, while the other three runs have very low F_{ST} values between them. The overall F_{ST} value (0.129) shows a relatively high level of differentiation and subdivision. Estimates of the amount of gene flow (*Nm*, number of migrants per generation) can be determined using F_{ST} (Wright 1943; Slatkin 1995). The F_{ST} values in the winter run comparisons give *Nm* values between 0.31 (fall) and 0.53 (spring). The F_{ST} values in the other run comparisons give much higher *Nm* values ranging from 5.91 (late fall and spring) to 10.59 (fall and late fall). Previous allozyme studies for nonsympatric populations of chinook in the Sacramento-San Joaquin drainage have found relatively high levels of gene flow with *Nm* values of 2.96 (Bartley and

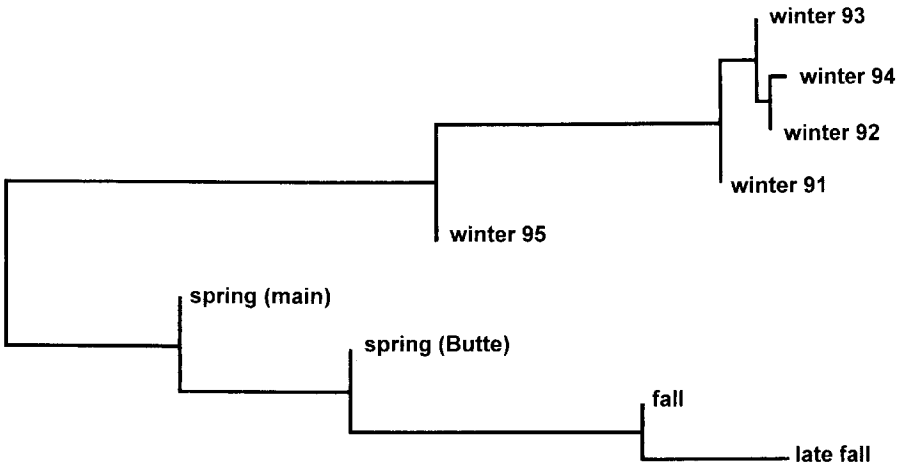

Figure 2.—Phenogram (neighbor-joining) based on Nei's genetic distance (D) demonstrating the relationships of the runs.

Gall 1990). Divergence measures such as F_{ST} and N_{ST} are weighted by within-population variation and can sometimes give surprising results. For example, high within-population variation tends to cause lowered values in populations that have had little gene flow (Hedrick and Parker 1998; Hedrick 1999).

The observed heterozygosities are relatively uniform (Table 3) among runs, and the late-fall run has one less allele (*Onts-wr1* was not detected). Interestingly, the late-fall run had the lowest estimated population size in the 1960s and has lost a large portion of historic spawning grounds because of construction of the Friant and Shasta Dams (Fisher 1994).

There has been evidence for admixture using microsatellite loci in the 1995 winter run brood year (D. Hedgecock, unpublished results). On the basis of homogeneity tests, our MHC results also conclude some sort of admixture in this brood year. It seems likely that the spring run is mixed into the 1995 winter run because the run is most similar to spring, and migration timing overlaps from March to July when the sample fish were captured. Thorough analysis with larger sample sizes known to be from each of the runs could possibly determine which run is responsible for the admixture. Techniques such as stock composition analysis (Pella and Milner 1987) could be used to determine the amount of contributions from the source populations.

Sacramento River chinook runs: The distinctness of the winter run from the other runs in the Sacramento is evident in the different measures of population differentiation. These results are due to the high frequency of the *Onts-wr1* allele and low frequency of the *Onts-lf1* allele in the winter run samples. The spring run is the only other run that has an appreciable frequency of *Onts-wr1* (0.103). Consequently, the winter run is most similar to the spring run as indicated by the various measures in Table 4. Interestingly, the other three runs are more similar to each other than to the winter run. The winter run has suffered the most population reduction over the past few decades, and genetic drift may be a much stronger force in the winter run.

Behaviorally, there is evidence that the winter run chinook of the Sacramento River is unique. All chinook salmon exhibit one of two basic life-history characteristics, designated stream- or ocean-type behavior (Healey 1991). The spring run exhibits typical stream-type chinook salmon behavior, while the fall and late-fall runs exhibit the typical ocean-type behavior. The winter run of the Sacramento appears to be unique among all chinook salmon in that they have characteristics of both stream- and ocean-type races (Healey 1991). They enter the river green and migrate far upstream. Spawning is delayed for some time after river entry. Furthermore, young winter-run chinook migrate to sea after only 4 to 7 mo of river life.

Conservation genetics: Molecular markers have been used to address issues in population genetics and conservation biology. Because of their high variability and assumed selective neutrality, microsatellite loci are often used to describe intraspecific population structure and differentiation. However, because of their neutrality, these loci may not reflect selective adaptations of populations. On the other hand, MHC loci are thought to be under balancing selection because of their involvement in the immune response. MHC loci may have patterns of variation indicating adaptive differences between populations that reflect past selective events. This variation at loci under selection may be very useful in determining the unit of conservation (Boyce *et al.* 1997).

The drastic reduction in the Sacramento chinook is mostly due to loss of historical spawning habitat because of human use of the Sacramento River system. Water degradation and diversion are apparently the major cause of chinook salmon decline (Moyle 1994), with all runs having incurred permanent habitat loss (Fisher 1994). Of particular concern is the winter-run chinook salmon, which have lost nearly all of their historic spawning grounds because of hydroelectric development. These unique chinook salmon had estimated annual runs of nearly 60,000 in the late 1960s. Within the past two decades, the winter run has declined severely to estimates as low as 191 adult spawners in 1991 (Fisher 1994). Currently, the effective population size of the winter-run salmon is estimated to range between 200 and 600 (Hedrick *et al.* 1995).

Evidence for hybridization between runs because of forced coexistence in spawning grounds and current hatchery practices has been reported and poses a major threat to the genetic integrity of each of the runs (Fisher 1994; P. W. Hedrick, D. Hedgecock and S. Hamelberg, unpublished results; D. Hedgecock, unpublished results). Criteria for authenticating winter-run salmon sampled are essential to prevent artificially induced hybridization of the runs. Work on run discrimination using microsatellite loci is currently under way (Banks *et al.* 1996). Our results suggest MHC variation could be used to help authenticate winter-run salmon because the frequency differences of *Onts-wr1* between the winter run and all the other runs are substantial.

A major issue in conservation is determining the unit of conservation. It has been suggested that the species is the appropriate unit (*e.g.*, Caughley and Gunn 1995), but others have suggested that the units of conservation should be evolutionary significant units (ESUs), which are on independent evolutionary trajectories (*e.g.*, Waples 1995) and could be species, subspecies, populations, or stocks. Defining units of conservation in Pacific salmonids is particularly difficult. The concept of ESUs has been used as a basis for units in conservation in Pacific salmonids (Waples 1995). Not only do the Sacramento River chinook differ from other more northern chinook in geographic distribution and life history traits, they also differ genetically at the MHC.

This genetic divergence is possibly due to historical isolation and differing selective pressures affecting the MHC, such as disease. Furthermore, the differences between the Sacramento River chinook salmon runs indicate that the winter run is distinct and should be a separate ESU. The other runs also appear to be significantly different and could be considered ESUs or separate management units.

Variation in antigen binding by alleles in the MHC appears to be a basis for selective pressure by parasitic or pathogen resistance (Davenport *et al.* 1995). It has been suggested that populations with low MHC variation, such as endangered populations, are particularly susceptible to infectious disease and parasites (for review, see Edwards and Potts 1996; Hedrick and Kim 1999). Thus, variation at the MHC may be important for population persistence. Hughes (1991) has argued that preservation of MHC variability should be the first priority of those designing captive breeding programs for endangered species of vertebrates. Therefore, it seems worthwhile to learn as much as possible about the MHC of endangered vertebrates by determining the levels of natural variation and the mechanisms that might maintain the natural levels. Once this information is available, it could then help managers better understand how to preserve endangered species.

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