Transactions of the American Fisheries Society
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/utaf20

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Published online: 29 Aug 2013.

To cite this article: Andrew P. Kinziger, Michael Hellmair, David G. Hankin & John Carlos Garza (2013) Contemporary Population Structure in Klamath River Basin Chinook Salmon Revealed by Analysis of Microsatellite Genetic Data, Transactions of the American Fisheries Society, 142:5, 1347-1357, DOI: 10.1080/00028487.2013.806351

To link to this article: http://dx.doi.org/10.1080/00028487.2013.806351

Transactions of the American Fisheries Society 142:1347–1357, 2013

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2013.806351

ARTICLE

Contemporary Population Structure in Klamath River Basin Chinook Salmon Revealed by Analysis of Microsatellite Genetic Data

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Abstract

Chinook Salmon *Oncorhynchus tshawytscha* exhibit substantial population genetic structure at multiple scales. Although geography is generally more important than life history, particularly migration and run timing, for describing genetic structure in Chinook Salmon, there are several exceptions to this general pattern, and hatchery supplementation has altered natural genetic structure in some areas. Given that genetic structure of Chinook Salmon is often basin-specific, we assessed genetic variation of 27 microsatellite loci in geographically and temporally distinct natural populations and hatchery stocks in the Klamath River basin, California. Multiple analyses support recognition of three major genetic lineages from separate geographic regions in the Klamath River basin: the lower basin, the Klamath River, and the Trinity River. The lower basin group was sharply distinct, but populations in the Klamath and Trinity river lineages were connected by processes that can be described by a one-dimensional, linear, stepping-stone model where gene exchange occurred primarily, but not exclusively, between adjacent populations. Genetic structure by migration timing was also evident, although divergences among populations that differed by migration timing only were fewer than those observed between geographic regions. Distinct run-timing ecotypes in the Klamath River basin thus appear to have evolved independently through a process of parallel evolution. Introgressive pressure from the hatchery stocks into natural populations was attenuated by distance from the hatchery, but comparison of historical population genetic structure to contemporary patterns would be needed to fully evaluate the extent to which hatchery stocks may have altered natural genetic structure.

Chinook Salmon *Oncorhynchus tshawytscha* exhibits substantial population genetic structure across its geographic range (Waples et al. 2004; Beacham et al. 2006). Population structure in Chinook Salmon is directly related to their strong propensity to home to natal spawning grounds with high accuracy. Natal homing ultimately results in large genetic differentiation between geographically separated river drainages, presumably due to relatively low straying between basins (Waples et al. 2004; Beacham et al. 2006). Lower genetic divergence among populations from tributaries within the same drainage presumably results from straying being more common. Chinook Salmon enter freshwater during almost every month of the year in larger basins (Healey 1991) and Chinook Salmon populations often exhibit temporal genetic structure according to migration and run timing, and different run times are frequently genetically divergent from one another (Waples et al. 2004). However, Chinook Salmon stocks with temporally distinct migration timing generally exhibit less genetic differentiation than those stocks originating from separate geographic regions (Waples et al. 2004; Moran et al. 2013). These findings support the hypothesis that

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Received December 20, 2012; accepted May 14, 2013
Published online August 29, 2013

different migration times in Chinook Salmon have evolved repeatedly and independently at different geographic locations, probably due to divergent selection on run timing (Waples et al. 2004; Moran et al. 2013). However, there are exceptions to these general patterns. For example, Columbia River basin Chinook Salmon are primarily structured by life history (e.g., run timing, yearling and subyearling out-migrant types: Narum et al. 2010; Moran et al. 2013) and genetic structure of Chinook Salmon within the California Central Valley is more coincident with run timing than with geography (Banks et al. 2000; Garza et al. 2008).

Hatchery practices can also substantially alter the genetic structure of Chinook Salmon within river basins. For example, off-site release of juvenile hatchery fish in the California Central Valley has resulted in genetic homogenization and the disappearance of population structure among fall-run Chinook Salmon populations in different tributaries (Williamson and May 2005). However, the effects of hatchery stocking of salmonids can be highly variable. In some cases, genetic structure has been totally disrupted, whereas, in others, introduced stocks do not appear to substantially contribute to the recipient population (Hansen et al. 2001, 2009; Martinez et al. 2001; Hansen 2002; Williamson and May 2005; Finnegan and Stevens 2008; Matala et al. 2012). Thus, understanding the genetic structure of Chinook Salmon within a particular river basin and how it is has been affected by hatchery supplementation requires fine-scale sampling and genetic analysis of populations in that basin.

The Klamath River basin in California is one of the largest producers of Chinook Salmon in western North America. Two large-scale hatchery programs were initiated in the basin in the early 1960s to mitigate for habitat loss caused by construction of impassable dams. Iron Gate Hatchery is at the current upstream limit of anadromy on the main-stem Klamath River, and Trinity River Hatchery is at the current upstream limit of its largest tributary (Figure 1). Iron Gate Hatchery raises fall-run Chinook Salmon only, whereas Trinity River Hatchery raises both fall-run and spring-run fish. The spring-run salmon that historically spawned upstream from Iron Gate Hatchery are presumed to have been extirpated (Snyder 1931; Hamilton et al. 2005). Currently, hatchery releases number about 10 million juvenile Chinook Salmon annually (both hatcheries combined; Hamilton et al. 2011) and hatchery fish contribute substantially

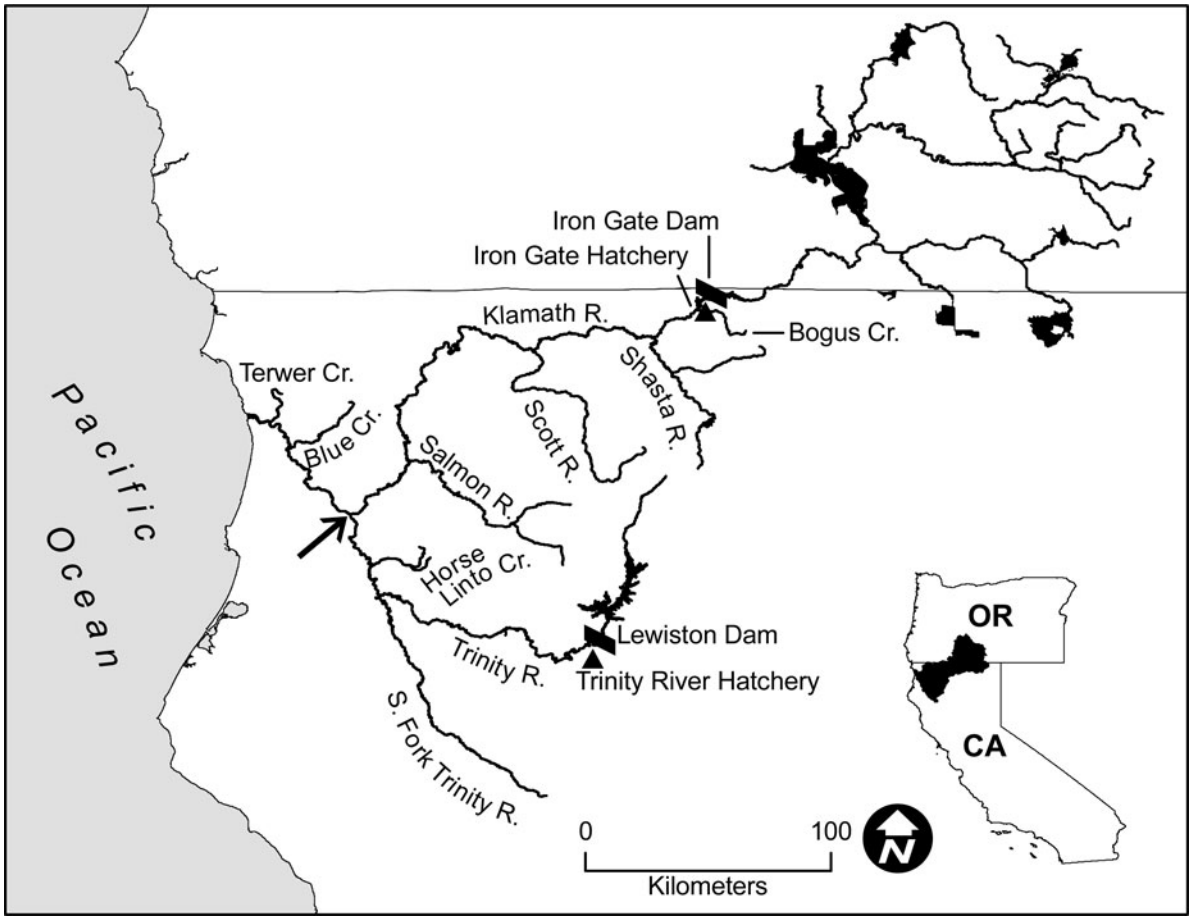

FIGURE 1. The Klamath River basin, California–Oregon, depicting major tributaries, hatcheries, and dams. Iron Gate and Lewiston dams are barriers to anadromy and represent the extent of upstream migration in the Klamath and Trinity rivers, respectively. The arrow indicates the confluence of the Klamath and Trinity rivers and the division between the Upper Klamath and Trinity Rivers ESU (above) and the Southern Oregon and Northern California Coast ESU (below).

to ocean and in-river tribal, commercial, and recreational harvest. While the hatchery stocks are derived from local sources, eggs have been exchanged between the hatcheries and hatchery fish have been released off-site in various Klamath River basin tributaries. The data in Supplementary Tables 1–3 (in the online version of this article) are illustrative of the types of hatchery releases that have occurred in the Klamath River basin, but are not comprehensive. Overall, the extent of these activities for Klamath River Chinook Salmon is less than for most other basins with substantial hatchery production (Bjorkstedt et al. 2005; see also Spence et al. 2011)

Several Klamath River tributaries contain naturally spawning Chinook Salmon stocks that are presumably outside of the influence of hatchery stocking, due to large geographic separation from both Iron Gate and Trinity River hatcheries. These include the Scott, Salmon, and South Fork Trinity rivers and smaller tributaries in the lower basin (Figure 1). Two stocks from these natural areas, spring-run fish from the South Fork Trinity River and Salmon River, occur in low abundance and are of conservation concern. The South Fork Trinity River Chinook Salmon population currently consists of a few hundred returning adults, down from >11,000 in 1964, and the Salmon River population is also at low abundance, estimated at 250–1,400 returning adults per year.

Klamath River Chinook Salmon have been divided into two evolutionarily significant units (ESU) separated by the confluence of the Klamath and Trinity rivers, based upon analysis of genetic and other data (Figure 1; Gall et al. 1992; Myers et al. 1998; Waples et al. 2004; Beacham et al. 2006; Seeb et al. 2007). Populations downstream from the confluence are part of the Southern Oregon and Northern California Coast ESU, which includes additional stocks north of the Klamath River basin, including the Rogue River in Oregon. Populations upstream from the confluence are assigned to the Upper Klamath and Trinity Rivers ESU, which includes both spring- and fall-run stocks. The extent of genetic differentiation between spring- and fall-run stocks from the Trinity River Hatchery has previously been shown to be low (Kinziger et al. 2008).

The objective of the current study was to determine within-basin genetic population structure of Chinook Salmon from the Klamath River basin and evaluate the effects of decades of large-scale hatchery production on this population structure. We analyzed genotypic data from 27 highly informative microsatellite loci for 790 fish from 10 natural populations and all three hatchery stocks (Table 1). Our study includes representatives of all major Chinook Salmon populations in the Klamath River basin comprising the vast majority of all natural Chinook Salmon production in the basin, including spring-run populations at very low levels of abundance. We provide the first comprehensive evaluation of Chinook Salmon population structure in the Klamath–Trinity River and establish a baseline for evaluation of future trends in the basin.

METHODS

Sample collections.—Tissues (fins, scales, or both) were collected during carcass surveys, operation of weirs, hatchery spawning, or by electrofishing and placed into 95% ethanol, dried on blotter paper, or frozen until DNA extraction. All collections were from adults except those from Iron Gate Hatchery (IGH), Terwer Creek (TC), and Blue Creek (BC) (Table 1), which were from juveniles. Juvenile collections were spaced out temporally to minimize problems associated with family sampling (e.g., Allendorf and Phelps 1981; Hansen et al. 1997). To assess the extent of family sampling in our juvenile collections, we estimated pairwise relatedness among individuals within

TABLE 1. Population, run time, population abbreviation (ID), sample size (n), year of field collections, rarified private alleles (A_p), rarified allelic richness (A_R), allelic richness (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and proportion of membership in each of three Chinook Salmon population clusters (lower basin, Klamath, and Trinity) inferred using STRUCTURE.

Population	Run time	ID	n	Year	A_p	A_R	A	H_o	H_e	Lower basin	Klamath	Trinity
Iron Gate Hatchery	Fall	IGH	104	2006	0.3	9.7	15.0	0.74	0.74	0.06	0.88	0.06
Bogus Creek	Fall	BOG	32	2006	0.3	9.4	10.3	0.72	0.74	0.08	0.84	0.08
Shasta River	Fall	SHST	31	2002	0.3	9.8	11.3	0.73	0.74	0.08	0.85	0.07
Scott River	Fall	SCOT	64	2002	0.4	10.7	15.1	0.75	0.76	0.18	0.64	0.19
Salmon River	Spring	SRS	94	1997, 2006	0.1	10.0	13.3	0.73	0.75	0.17	0.22	0.61
Salmon River	Fall	SRF	52	2002, 2006	0.3	10.9	16.7	0.75	0.77	0.24	0.28	0.48
Trinity River Hatchery	Spring	TRHS	133	1992, 2004	0.2	9.5	15.3	0.72	0.74	0.06	0.06	0.88
Trinity River Hatchery	Fall	TRHF	124	1992, 2004	0.1	9.6	15.0	0.74	0.74	0.06	0.07	0.87
South Fork Trinity River	Spring	SFTS	7	1993						0.15	0.08	0.77
South Fork Trinity River	Fall	SFTF	19	1993	0.3	9.7	9.7	0.69	0.74	0.22	0.20	0.58
Horse Linto Creek	Fall	HLC	38	1997	0.4	10.5	12.4	0.75	0.77	0.81	0.08	0.11
Blue Creek	Fall	BC	69	2008	0.7	12.1	17.5	0.79	0.80	0.90	0.05	0.05
Terwer Creek	Fall	TC	23	2008	0.7	10.2	10.7	0.76	0.76	0.93	0.03	0.04

each collection using ML-RELATE (Kalinowski et al. 2006). Inclusion of hybrids between spring- and fall-run salmon returning to Trinity River Hatchery (Kinziger et al. 2008) was minimized by selecting samples from the early portion of the spring-run spawning period and the end of the fall-run spawning period.

Molecular methods.—The DNA was extracted using the Promega Wizard SV96 Genomic DNA Purification System, Qiagen DNeasy spin columns using a Qiagen 3000 BioRobot, or with a Chelex Resin protocol. A total of 28 microsatellite loci were assayed (Supplementary Table 4). Sixteen loci were genotyped using a Beckman Coulter CEQ 8000 Genetic Analysis System and 12 loci, which have been standardized for use with Chinook Salmon (Seeb et al. 2007), were genotyped with an Applied Biosystems 3730 genetic analyzer. Polymerase chain reaction volumes and thermocycling conditions varied between loci and that information is available from the authors upon request. Relative allele sizes were determined automatically from electropherograms and then visually inspected to avoid errors caused by automated calling.

Tests of assumptions and genetic diversity.—Loci were tested for null alleles, large allele dropout, and stutter peaks with MICROCHECKER version 2.2.3 (van Oosterhout et al. 2004). An estimate of the microsatellite-scoring error rate was generated by repeating 540 PCRs across 12 loci in 45 IGH individuals, and the copy error rate per allele was calculated as the ratio between the observed number of allelic differences and total number of allelic comparisons (Bonin et al. 2004). Tests for linkage disequilibrium between all locus pairs in each population and for conformance to Hardy–Weinberg proportions for each locus in each population, conducted using the Markov chain Monte Carlo approximation of Fisher's exact test, were implemented in GENEPOP version 4.0.10 (Rousset 2008). Corrections for multiple tests used the Bonferroni method (Rice 1989). Allelic richness (A), observed (H_o), and expected heterozygosity (H_e) were all determined with ARLEQUIN version 3.1 (Excoffier et al. 2005). Standardized private allelic richness (A_p) and standardized allelic richness (A_R), equalized using rarefaction to a sample size of 38 genes, were calculated with HP-RARE version 1.0 (Kalinowski 2005).

Genetic structure.—A standardized measure of genetic differentiation, G'_{ST}, (Hedrick 2005) was calculated between each population pair using FSTAT (Goudet 1995) in combination with RECODEDATA version 0.1 (Meirmans 2006). The G'_{ST} accounts for differences in allelic diversity within populations, which can bias traditional F_{ST} estimators. Significance of genetic differentiation between population pairs, based on F_{ST}, was estimated using permutation tests implemented in FSTAT (Goudet 1995). Corrections for multiple tests used the False Discovery Rate method (Benjamini and Yekutieli 2001), which provides increased power for detecting population divergence compared with the Bonferroni method (Narum 2006). Significance of isolation by distance, a correlation between genetic (G'_{ST}) and geographic distances (river distances in kilometers), for all populations above the confluence of the Klamath and Trinity rivers (see Results) was evaluated using a Mantel test in FSTAT (Goudet 1995; Hutchison and Templeton 1999) with 10,000 permutations of the data. The geographic location for the spring-run population at the Trinity River Hatchery was estimated as 50 km upstream from the barrier dam, which is intended to reflect predam spawning activity (Moffett and Smith 1950; Smith 1976).

Chord distances (Cavalli-Sforza and Edwards 1967) between population pairs were calculated from the genetic data and used to construct an unrooted neighbor-joining tree with PHYLIP version 3.68 (Felsenstein 1993). Branch support was evaluated using 1,000 bootstrap replicates with branches appearing in more than 90% of the trees considered well supported.

Population differentiation was graphically investigated by conducting a discriminant analysis of principal components (DAPC; Jombart et al. 2010) in the *adegenet* package (Jombart 2008). The DAPC performs a preliminary data transformation step using principal component analysis (PCA) to create uncorrelated variables that summarize total variability (e.g., within and between groups). These variables are used as input to discriminant analysis (DA), which aims to maximize between-group variability and achieve the best discrimination of multilocus genotypes into predefined clusters. Multivariate analyses can provide clustering power comparable with Bayesian methods but are free of assumptions about Hardy–Weinberg and linkage equilibria and take less time to calculate (Patterson et al. 2006; Jombart et al. 2010).

The Bayesian clustering algorithm employed in STRUCTURE version 2.3.3 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) was used to generate an ad hoc estimate of the most likely number of genetically distinct clusters present in the data and an estimate of the proportion of each individual's genome assigned to each cluster. Each cluster representing a genetically distinct group is assumed to be free of Hardy–Weinberg and linkage disequilibrium. Estimates of the number of genetic clusters present in the data were generated by calculating the log probability of the data [ln $\Pr(X|K)$] and by estimating ΔK (Evanno et al. 2005), assuming the data consisted of $K = 2, \ldots, 13$ genetically distinct groups. Twenty independent runs were conducted at each value of K and runs that did not converge, as indicated by divergence distances among populations and likelihoods, were discarded. STRUCTURE analyses were conducted with and without population locations as priors. To align independent STRUCTURE runs, we used the LargeKGreedy algorithm (with 10,000 random input orders) as implemented in the software CLUMPP (Jakobsson and Rosenberg 2007). Graphical depictions of CLUMPP results were generated using DISTRUCT (Rosenberg 2004).

RESULTS

Tests of Assumptions and Genetic Diversity

One locus (*OTSG311*) showed evidence of null alleles and was removed from further analyses. None of the remaining 27

loci exhibited significant problems associated with null alleles, stutter peaks, or large allele dropout. Allelic scores were identical in the 540 duplicate PCRs of fish from IGH, indicating a very low genotyping error rate. The estimated proportion of full-sibling pairs was 9% in TC, 2% in Horse Linto Creek (HLC), and 0% in all other populations. Tests of pairwise genetic differentiation (F_{ST}) between samples collected in different years but from the same location indicated no significant differences and thus were combined (Bonferroni corrected critical value = 0.01). The South Fork Trinity River Spring (SFTS) sample was excluded from all analyses, except for the Bayesian cluster and multivariate ones, due to small sample size ($n = 7$).

The loci were highly polymorphic, with an average of 24.8 alleles per locus. Of the 351 tests for departure from Hardy–Weinberg proportions (13 populations at 27 loci), seven were significant after Bonferroni correction (critical value = 0.00014). No single locus or population consistently departed from expectations, which indicated that locus- and population-specific factors were not causes for the observed departures. A total of 77 out of 4,563 tests for linkage disequilibrium were significant after Bonferroni correction (critical value = 0.000010). The majority of the significant tests (61) could be attributed to a single population, Salmon River Fall (SRF). This elevated linkage disequilibrium may be due to hybridization of this stock with Salmon River Spring (SRS), Trinity River Hatchery (TRH), IGH, or a combination of those. The remaining significant tests were not characteristic of particular populations or locus pairs. Within-population measures of genetic diversity are presented in Table 1.

Genetic Structure

Most pairwise F_{ST} values differed significantly from zero (critical value = 0.00321), and the main exception was the comparisons between geographically proximate populations (Table 2). An initial test for isolation by distance indicated BC, TC, and HLC were outliers, probably because they belong to a different evolutionary lineage and ESU than the remaining populations (Gall et al. 1992). When these populations were excluded, the relationship between pairwise genetic differentiation (G'_{ST}) and river distance was strong and significant (Mantel test: $R^2 = 0.80$, $P < 0.01$; Figure 2). The intercept of the relationship was nearly zero (0.0002), which is consistent with the classic definition of an isolation-by-distance model of gene flow (Hutchison and Templeton 1999).

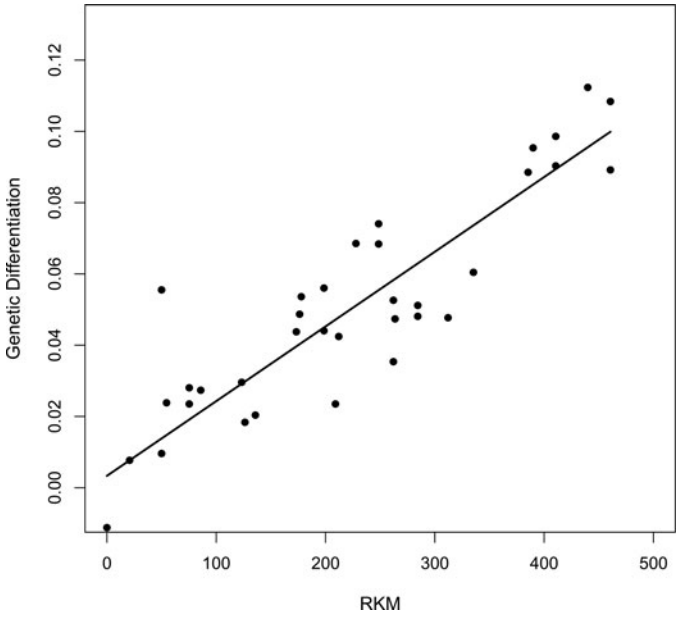

FIGURE 2. Relationship between pairwise genetic differentiation (G'_{ST}) and river distance (RKM) for Klamath River Chinook Salmon populations above the confluence of the Klamath and Trinity rivers. Analysis excludes the Horse Linto Creek population.

In the neighbor-joining tree, all nodes except two were supported by bootstrap values greater than 90% (Figure 3). A group from the lower basin (HLC, BC and TC) was resolved as distinctive from the populations in the upper basin. The hatchery stocks from the Klamath (IGH) and Trinity rivers (TRH Spring

TABLE 2. Pairwise estimates of genetic differentiation (G'_{ST}) for Klamath River Chinook Salmon populations (below diagonal) and *P*-values for significance tests of differentiation (above diagonal). Critical *P*-value adjusted for multiple tests using false discovery rate method was 0.00321. Significant tests are indicated by an asterisk. See Table 1 for definition of population abbreviations. NA = data not available.

Population	IGH	BOG	SHST	SCOT	SRS	SRF	TRHS	TRHF	SFTF	HLC	BC	TC
IGH		0.37500	0.03141	0.00064*	0.00064*	0.00064*	0.00064*	0.00064*	NA	0.00064*	0.00064*	0.00064*
BOG	−0.0112		0.76154	0.01218	0.00064*	0.00064*	0.00064*	0.00064*	NA	0.00449	0.00064*	0.00192*
SHST	0.0077	−0.0159		0.00064*	0.00064*	0.00064*	0.00064*	0.00064*	NA	0.00064*	0.00064*	0.00064*
SCOT	0.0280	0.0235	0.0238		0.00064*	0.00064*	0.00064*	0.00064*	NA	0.00064*	0.00064*	0.00064*
SRS	0.0741	0.0684	0.0685	0.0438		0.02885	0.00064*	0.00064*	NA	0.00064*	0.00064*	0.00064*
SRF	0.0560	0.0440	0.0536	0.0296	0.0096		0.00064*	0.00064*	NA	0.00064*	0.00064*	0.00064*
TRHS	0.1084	0.0892	0.1123	0.0885	0.0477	0.0354		0.00064*	NA	0.00064*	0.00064*	0.00064*
TRHF	0.0986	0.0903	0.0954	0.0604	0.0526	0.0424	0.0555		NA	0.00064*	0.00064*	0.00064*
SFTF	0.0481	0.0512	0.0474	0.0235	0.0204	0.0274	0.0487	0.0184		NA	NA	NA
HLC	0.1151	0.1267	0.1120	0.0817	0.0798	0.0743	0.1177	0.0921	0.0687		0.00064*	0.00128
BC	0.1194	0.1228	0.1213	0.0864	0.0903	0.0806	0.1207	0.1143	0.0831	0.0565		0.00064*
TC	0.2555	0.2397	0.2470	0.2225	0.1940	0.1833	0.2113	0.2413	0.2222	0.1832	0.1593	

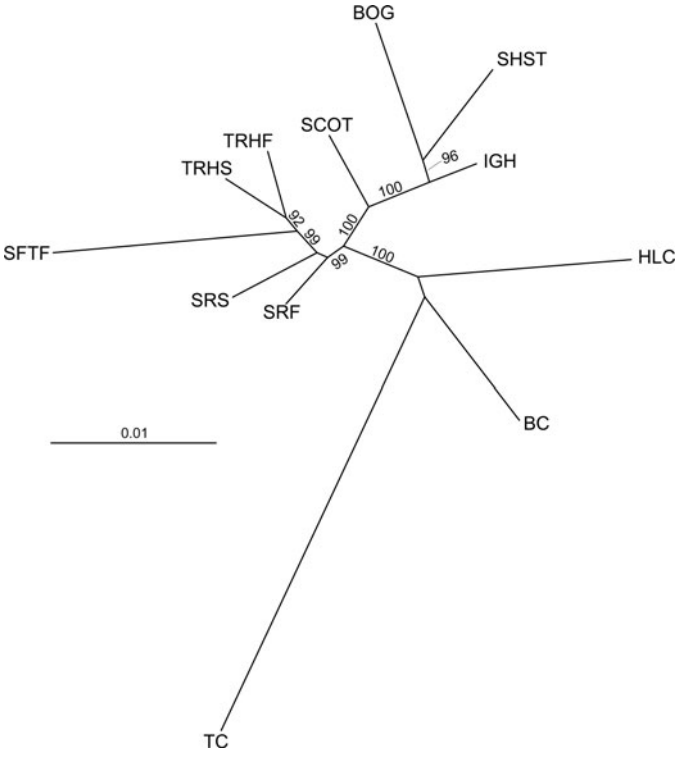

FIGURE 3. Unrooted neighbor-joining tree of Klamath River basin Chinook Salmon populations. Branch lengths are proportional to chord distances and bootstrap support (>90%) is indicated along the branches. Population abbreviations are defined in Table 1.

[TRHS] and TRH Fall [TRHF]) exhibited a large divergence from each other, and the other upper basin populations plotted in intermediate positions based upon their geographic distance from the main hatchery groups (Bogus Creek [BOG], Shasta River [SHST], Scott Creek [SCOT], South Fork Trinity River Fall [SFTF], SRS, and SRF). Long terminal branches associated with TC are probably due to inclusion of siblings (see above).

In the DAPC, 90% of the total genetic variation was captured by the first 205 principal components of PCA and these were used as input to DA. The eigenvalues resulting from DA indicated that the first two axes were sufficient to summarize the genetic structure of Klamath River Chinook Salmon (Figure 4, inset). The lower basin group (HLC, BC, and TC) diverged from the remaining populations along the first axis (Figure 4). Along the second axis, the largest divergence was between hatchery populations (IGH versus TRHS and TRHF), and the remaining tributary populations were situated along a line connecting the hatchery populations. The coordinates of these populations along this line were related to their geographic distance from the hatcheries.

In the Bayesian cluster analysis, the ad hoc statistic ΔK indicated the strongest level of structure at $K = 3$ without the use of location information (Supplementary Figure 1 available in the online version of this article). The three clusters included: (1) the lower basin (HLC, BC, and TC), (2) Klamath River (IGH, BOG, and SHST), and (3) Trinity River (TRHS, TRHF, and SFTS) (Table 1; Figure 5). Populations SFTF, SCOT, SRS, and SRF were resolved as admixtures between the three primary clusters. Inspection of individual admixture proportions at $K = 4$ indicated a unique component primarily associated with mid-basin tributaries (SCOT, SRS, and SRF), and inspection of the plot at $K = 5$ indicated a division between TRHS and TRHF, which were resolved as distinct in a previous study (Kinziger et al. 2008), supporting an assertion of at least $K = 5$ distinct clusters in our data. Even finer-scale structure was suggested by inspection of outputs at $K > 5$, but these results should be treated with caution because Bayesian clustering methods can overestimate the number of genetic clusters in data sets characterized by isolation by distance (Frantz et al. 2009; Kalinowski 2011), as is the case here (see below). Bayesian cluster analysis using location information provided similar patterns (results not shown).

DISCUSSION

Genetic Structure

Chinook Salmon from the Klamath River basin exhibit a complex multilevel pattern of genetic structure defined primarily by geography. The three major genetic groups in the basin originate from separate geographic regions: the lower basin, the Klamath River, and the Trinity River. This result is supported by concordant patterns in neighbor-joining trees, multivariate plots, and Bayesian model-based clustering, all of which indicated divergence between populations from these regions. The importance of geography in describing genetic structure in Klamath River Chinook Salmon is consistent with large-scale phylogeographic assessments that indicate geography explains the majority of genetic structure within this species (Waples et al. 2004; Beacham et al. 2006; Seeb et al. 2007; Moran et al. 2013).

A distinct lower basin group of Chinook Salmon was identified, including populations in Blue, Terwer, and Horse Linto creeks. This finding is consistent with previous studies that designated Chinook Salmon populations from below the confluence of the Klamath and Trinity rivers into the Southern Oregon and Northern California Coast ESU (Figure 1; Gall et al. 1992; Myers et al. 1998). Populations below the confluence are more closely related to Chinook Salmon from other basins in northern California and southern Oregon than to stocks above the Trinity and Klamath river confluence (Gall et al. 1992; Myers et al. 1998). A similar pattern has been observed for Klamath River basin steelhead *O. mykiss* populations (Pearse et al. 2007). However, contrary to previous investigations (Gall et al. 1992), our analysis identified Horse Linto Creek as part of the lower basin group, despite its location upstream of the ESU boundary at the confluence of the Klamath and Trinity rivers (Myers et al. 1998). This result suggests a shift in ESU affiliation between 1987 and 1997, the dates of field collections for the previous and present studies. Horse Linto Creek has a relatively small population of Chinook Salmon, and it is possible that annual

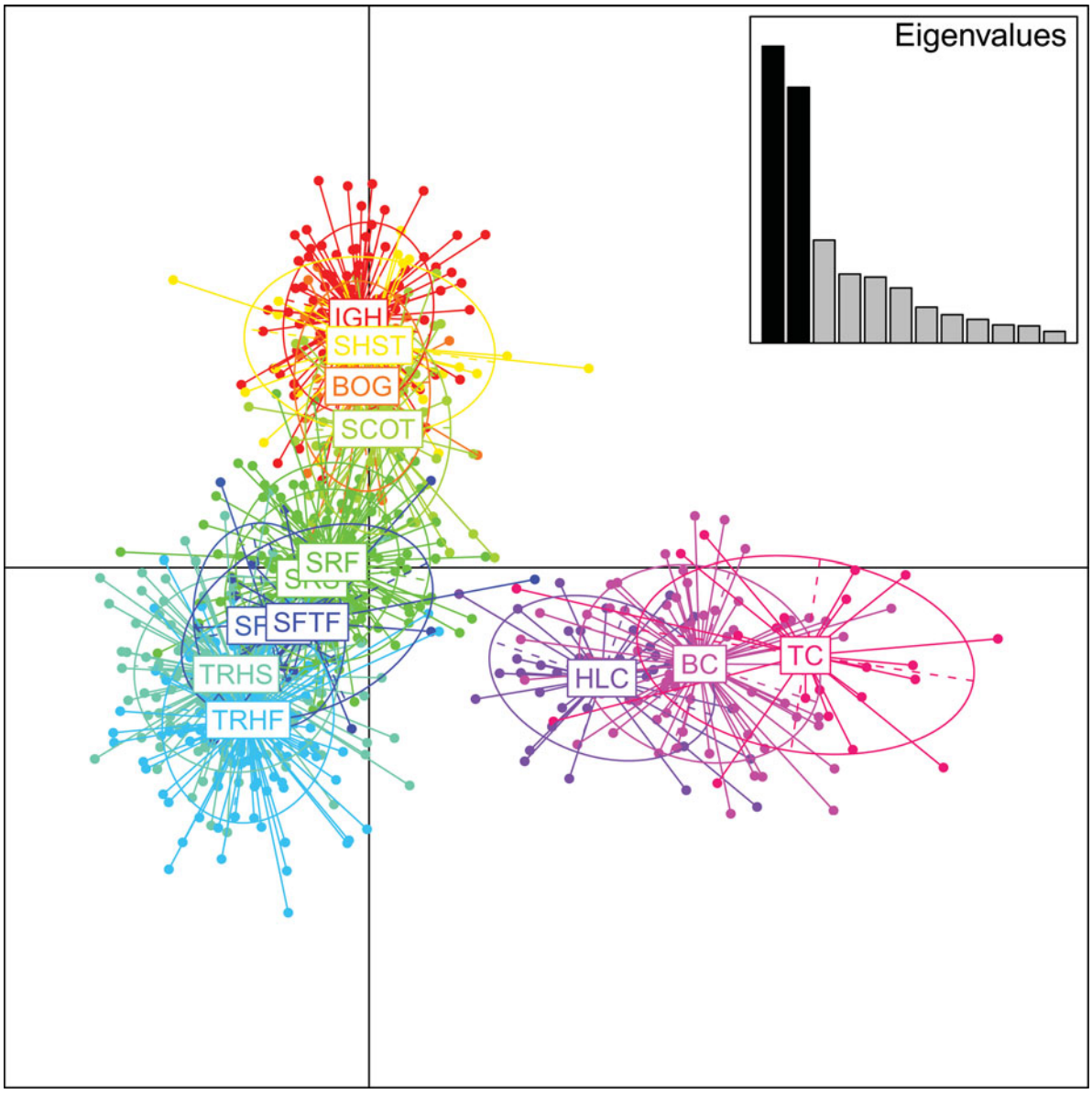

FIGURE 4. Scatterplot of the first two principal components of DAPC using population locations as prior clusters. Populations are labeled inside their 95% inertia ellipsis and dots represent individuals. The inset indicates the eigenvalues of the first 12 principal components. Population SFTF superimposes SFTS and SRF superimposes SRS. Population abbreviations are defined in Table 1. [Figure available online in color.]

influxes of migrants from larger populations cause changes in genetic composition of this population that results in temporal variability in phylogeographic affiliation. However, if the affinity of this population with the lower basin group remains stable, a reconsideration of the ESU boundary may be warranted.

The Upper Klamath and Trinity Rivers ESU was resolved as a genetically distinct lineage, but composed of two divergent subgroups, identified as the Klamath and Trinity herein. These two groups were found to be connected by processes that can be described by a one-dimensional, linear, stepping-stone model where gene exchange occurred primarily, but not exclusively, between adjacent populations (Kimura and Weiss 1964). The boundaries of the model are defined by Iron Gate Hatchery and Trinity River Hatchery, which exhibited the highest levels of genetic differentiation in pairwise tests and large separation in multivariate plots, and are geographically located at the termini of anadromy (Table 2; Figures 1, 4). Spawning populations in geographic areas between the hatcheries, such as the Shasta, Scott, Salmon, and South Fork Trinity rivers, were at intermediate positions in tree-based and multivariate analyses and their coordinates were related to geographic distance from the hatchery populations. The strong relationship between genetic and geographic distance further attests to the importance of river distance in mediating gene flow among populations (Figure 2).

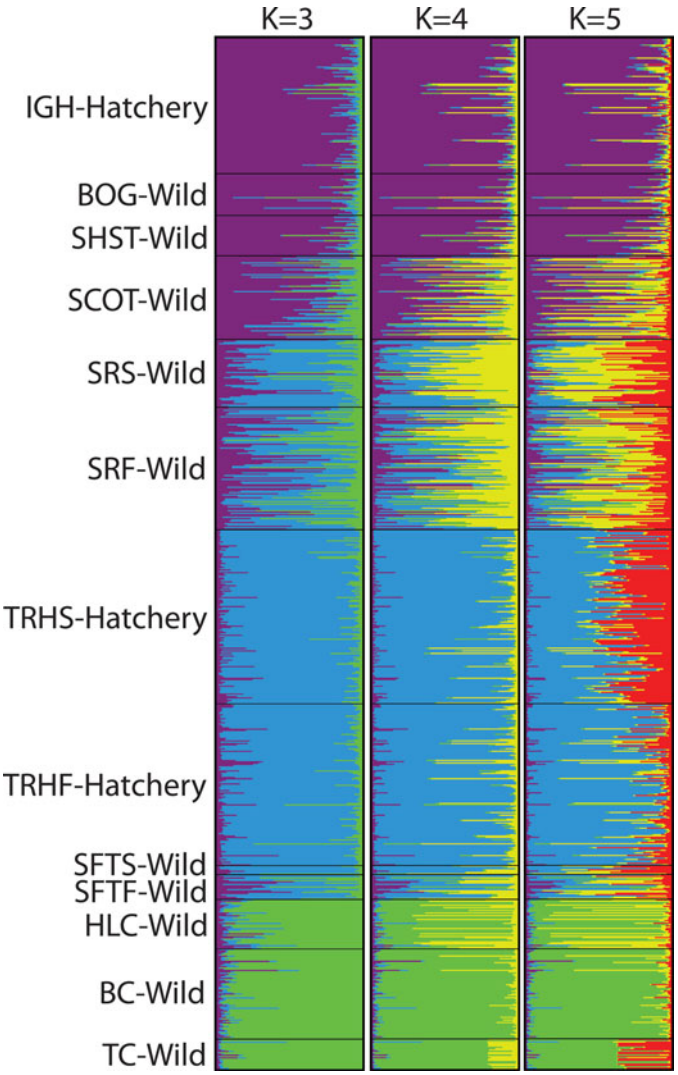

FIGURE 5. Individual membership coefficients for Klamath River Chinook Salmon for $K = 3$–5 clusters without the use of population location information from Bayesian cluster analysis. Concordance of the alignments across the independent STRUCTURE runs (H') was 0.99, 0.87, and 0.79 for $K = 3$, 4, and 5, respectively. Each individual is represented by a single horizontal bar divided in K colored segments, with lengths that are proportional to cluster membership assignments. Population abbreviations are defined in Table 1. [Figure available online in color.]

Our proposed gene flow model for the Klamath and Trinity groups is concordant with the linear arrangement of populations along the river course, strong natal homing behavior of Chinook Salmon (Quinn 1993; Dittman and Quinn 1996), and predictions about gene flow based on tag recoveries of hatchery-origin salmon. The proportions of naturally spawning fish found to be of Iron Gate Hatchery origin in tributaries situated 0 (Bogus Creek), 21 (Shasta River), and 75 river kilometers (RKM) (Scott River) downstream from the hatchery were 0.33, 0.12, and 0.00, respectively (California Department of Fish and Wildlife, unpublished data). Long-distance straying from Iron Gate Hatchery to Trinity River Hatchery (400 RKM apart) is rare and migrants between them number only a few individuals each year (California Department of Fish and Wildlife, unpublished data). While both genetic and field data have inherent problems for estimating the extent of migration among populations (Koenig et al. 1996; Whitlock and McCauley 1999), both support the contention that gene exchange decreases with river distance and occurs primarily between adjacent populations. Other populations of anadromous salmonids, including steelhead in the Klamath River, conform to isolation-by-distance models of gene flow (Hendry et al. 2004; Primmer et al. 2006; Palstra et al. 2007; Pearse et al. 2007; Narum et al. 2010), and our inferred pattern for Klamath River Chinook Salmon is consistent with patterns from these other closely related species.

Genetic structure was also evident among populations from the same subbasin that exhibit temporally distinct migration timing, although divergence between these populations was less than genetic differences observed between stocks from separate geographic regions. We examined three sympatric spring- and fall-run pairs from the Trinity River Hatchery, South Fork Trinity River, and Salmon River. Pairwise genetic differentiation was low but significant between runs from the Trinity River Hatchery, nonsignificant between Salmon River runs, and sample sizes were insufficient for comparison of South Fork Trinity River runs. We selected samples from the early portion of the spring-run spawning period and the end of the fall-run spawning period for the Trinity River stock. Employing a similar sampling strategy in the Salmon and South Fork Trinity rivers may provide improved power for resolving potential genetic differences between spring- and fall-run salmon in these tributaries. Model-based clustering analysis further supported Trinity River Hatchery runs as distinct groups, but not the sympatric pairs from other tributaries (Figure 5). Previous work on Trinity River Hatchery Chinook Salmon has demonstrated hybridization and gradual transition in genetic composition between spring- and fall-run salmon throughout the spawning season (Kinziger et al. 2008). Here, we found that spring- and fall-run fish from the same tributary of the Klamath River basin were genetically most similar to one another and divergent from other groups in the basin (Figures 3, 4), which indicates that ecotypic variation appears to have evolved independently through a process of parallel evolution, which is relatively common in Chinook Salmon (Waples et al. 2004; Beacham et al. 2006; Moran et al. 2013).

Midbasin Group and Hatchery Stocking

Bayesian cluster analysis resolved an additional genetic group that primarily contained individuals from midbasin Klamath River tributaries (Scott River and Salmon River spring- and fall-run stocks). Individual assignment coefficients indicated substantial introgression of the midbasin group by the Trinity and Klamath hatchery stocks (Figure 5). The geographic intermediacy of this group between the Iron Gate and Trinity River hatcheries also makes this a logical location for admixture (Figure 1). The cluster analysis also suggested strong asymmetry in introgression between the hatchery stocks and the

midbasin group. Population admixture coefficients indicated the hatchery stocks comprised 40% of the Salmon River ancestry, whereas the midbasin group comprised only 10% of the hatchery stocks ancestry. While it is possible that these patterns may reflect historic gene flow patterns in the basin, we hypothesize that asymmetric introgression is a result of construction of impassable dams and associated mitigation hatcheries, which have consistently released millions of fish annually since the early 1960s. The dramatically larger number of fish produced in these programs, relative to any natural population, should result in higher immigration pressure from the hatchery programs into natural populations than vice versa. While hatchery-origin fish are almost never observed in the Salmon River, populations located immediately below both hatcheries in the upper reaches of both the Trinity and Klamath rivers receive enough migrants to result in complete integration with the hatchery stocks (see above). While the effects of the hatchery stocks attenuate with distance, we hypothesize that migrants from populations below the hatcheries and dams serve as stepping stones across multigeneration time scales for extending introgressive influence to downstream Chinook Salmon populations outside the direct influence of hatchery stocks.

Supplementation Impacts on Genetic Structure

Despite a history of large-scale hatchery supplementation spanning more than half a century, Klamath River basin Chinook Salmon exhibited significant genetic differentiation among populations and retained genetic structure patterns similar to that predicted by an equilibrium between migration and genetic drift, as well as that observed more broadly in the species (Hendry et al. 2004; Waples et al. 2004; Beacham et al. 2006; Moran et al. 2013). This stands in stark contrast to population structure of California Central Valley Chinook Salmon, where fall-run populations have been homogenized as a consequence of hatchery activities (Williamson and May 2005). While our results indicate introgressive pressure from the hatchery stocks into natural populations, they also suggest that the geographic scale of the Klamath River basin is large enough to have allowed retention of significant genetic structure among Klamath River Chinook Salmon populations despite such supplementation. Nevertheless, it is possible that current patterns are unstable and do not reflect historic conditions prior to initiation of large-scale hatchery programs. Comparison of historical population genetic structure to contemporary patterns, through analysis of historical tissue collections, would provide a conclusive evaluation of the extent to which hatchery operations and habitat loss from impassable dams has modified Chinook Salmon genetic population structure in the Klamath River basin.

ACKNOWLEDGMENTS

Funding for this project came from the Hoopa Valley Tribe and the National Marine Fisheries Service, Southwest Fisheries Science Center. We thank A. Antonetti, S. Borok, P. Brucker, B. Chesney, D. Hillemeier, G. Kautsky, E. Logan, B. Matilton, N. Pennington, and A. Sprowles for providing tissue samples used in this study, and A. Abadía-Cardoso, V. Apkenas, D. Faulkner, E. Gilbert-Horvath, and H. Starks for assistance with laboratory data collection. Reference to trade names does not imply endorsement by the U.S. Government.

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