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[Article]

Homogenization of Fall-Run Chinook Salmon Gene Pools in the Central Valley of California, USA

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Abstract.—We assessed the population genetic structure and temporal stability of genetic diversity from 1999 to 2001 in collections of fall-run Chinook salmon *Oncorhynchus tshawytscha* in California's Central Valley. Tests for genotypic differentiation at seven microsatellite loci revealed few significant pairwise comparisons between samples from five hatchery populations and eight naturally spawning populations throughout the Central Valley that were separated by 50–350 km. All collections were genetically homogeneous and failed to cluster with their nearest geographic neighbors. Likewise, evaluation of temporal change in genetic diversity revealed few changes over the 3 years of the study. Our results suggest that fall-run Chinook salmon throughout the Central Valley comprise a genetically homogeneous population that has lower among-population genetic diversity than fall-run Chinook salmon populations examined elsewhere over similar geographic scales. The lack of genetic distinction and the lack of temporal differences in allele frequencies between hatchery and naturally spawning fish indicate that considerable gene flow occurs between fall-run Chinook salmon throughout the Central Valley. Due to the prevalence of off-site release of hatchery-reared juveniles and the history of interbasin hatchery transfers and stocking within the Central Valley, homogenization of Central Valley fall-run populations is most likely the result of hatchery practices for the past 140 years.

Multiple factors have contributed to the decline in the number of fall-run Chinook salmon *Oncorhynchus tshawytscha* returning to spawn in California's Central Valley. Accordingly, concerns remain regarding the long-term sustainability of fall-run Chinook salmon populations, despite the fact that short-term trends in population size for many streams are stable or increasing. Habitat loss in California has had a major impact on all Chinook salmon life history phases. Dams have cut off migrating adults from over 72% of their original spawning habitat and have degraded habitat downstream by altering water flow regimes and eliminating gravel replenishment from upstream sources (Yoshiyama et al. 1998, 2000). Large-scale levee construction has eliminated the flood plain and estuarine rearing habitats for juveniles and has exacerbated the entrapment of juveniles in unscreened water diversions as recently as 1989 (USFWS 1995; Yoshiyama et al. 1998).

The loss of spawning and rearing habitat has led to a precipitous decrease in natural production of Chinook salmon in California rivers. To mitigate for the losses incurred by the ocean fishery, hatcheries were used to supplement the remaining natural production. However, the production of high numbers of hatchery-reared juvenile fish and the timing of their release into rivers may negatively affect multiple life history stages of Chinook salmon and the genetic diversity of naturally spawning fall-run populations. Juvenile hatchery fish may be larger and have a competitive advantage for food or territory over their smaller wild counterparts. Straying of hatchery-origin adults may result in redd imposition within limited spawning habitat. Management practices involving the transport of fish to mitigate for population declines may have contributed to even greater losses of genetic diversity of fall-run fish. Smolt transport and release into the San Francisco Estuary and San Francisco Bay in lieu of release at hatcheries have led to major increases in rates of straying (CDFG–NMFS Joint Hatchery Review Committee 2001). Greater straying rates, movement of fish among hatcheries, and out-of-basin transplants (Hvidsten et al. 1994; Pascual and Quinn 1994; Pascual et al. 1995; Machordom et al. 1999) may have contributed to genetic homogenization of Central Valley fall-run Chinook salmon. In addition to decreasing the genetic diversity between hatchery and naturally spawning populations, stray hatchery fish can negatively influence the reproductive fitness of naturally spawning populations through

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hybridization and may exacerbate the loss of genetic diversity experienced by small, fluctuating populations. Several studies of Pacific salmonids suggest that ''domestication'' selection by captive rearing results in genetic-based decreases in fitness and survival of hatchery fish in the natural environment (Swain and Riddell 1990; McGinnity et al. 1997).

A decline in the size of naturally spawning populations and an increase in hatchery output may have created a scenario in which efforts to mitigate declining population numbers result in the erosion of the genetic diversity of fall-run fish. According to Fry (1961), at least six return years during the 1950s involved fewer than 500 spawners returning to the Merced River. In addition, spawning escapement in the Merced River averaged 240 fish through the 1960s (Yoshiyama et al. 2000). During the early 1990s, there were only a few hundred spawners in the Mokelumne and Stanislaus rivers and less than 100 spawners in the Merced and Tuolumne rivers (USFWS 1995). Reduced genetic variability through random loss of alleles by intergenerational sampling can be significant when population sizes are restricted over several generations. The ecological stressor of competition at multiple life history stages and the genetic stressor of introgression with large numbers of hatchery-reared fish diminish the capacity of naturally spawning populations to contribute to the genetic diversity of future generations.

Previous studies of the population genetic structure of Chinook salmon in the Central Valley have either not focused on or have been unable to resolve intertributary differences within the fall run. Analysis of allozymes (Bartley and Gall 1990; Bartley et al. 1992; Meyers et al. 1998) and mitochondrial DNA (Nielsen et al. 1994a, 1994b; Kim et al. 1999) indicated that major genetic units were consistent with the four temporal spawning runs (fall, late-fall, winter, and spring) of Chinook salmon within the Sacramento River and San Joaquin River basins. However, these studies did not identify population structure at either an inter- or intrabasin scale within the Central Valley for individual populations of fall-run Chinook salmon. Furthermore, Utter et al. (1989) examined four putative populations of fall-run Chinook salmon from the Central Valley. Sample collections from Coleman National Fish Hatchery and Nimbus Hatchery were pooled, as were the collections from the Feather and Mokelumne rivers, since the pooled sample sets lacked significant allele frequency differences at any of the 14 allozyme loci used in their study.

Microsatellite markers offer comparatively greater resolution of population genetic diversity and structure than that offered by allozymes or mtDNA. Banks et al. (1996, 2000a) not only showed how microsatellite loci could be used to differentiate between the endangered winter-run Chinook salmon and their less-threatened relatives in the Central Valley, but also presented evidence that substantial genetic diversity and structure (the genetic differentiation index $F_{ST} = 0.082$ for 5 loci and 0.078 for 10 loci) exist among the seasonal runs. However, the fall run, which included 13 samples collected from 1993 to 1996 from hatchery and naturally spawning populations throughout the Central Valley, appeared to form a homogeneous population. Furthermore, temporal variation between cohorts was absent, similar to data from assessments of other seasonal runs. It is possible that the microsatellite loci used in the studies by Banks et al. (1996, 2000a) had not accumulated differences because populations had only recently diverged. In this case, the loci used would lack the power to resolve population substructure within the fall run at either the inter- or intrabasin scale. While the earlier studies by Banks et al. (1996, 2000a) were successful at providing information on discriminating separate seasonal runs of Chinook salmon, they were unable to discriminate between fall-run populations, which would have facilitated management decisions focusing on the fall-run stock.

A characterization of population genetic structure in the existing fall-run Chinook salmon stocks in the Central Valley will form the basis from which subsequent management practices may be measured. Ecosystem-level changes driven by global climate change or remedial efforts to restore ecosystem-level processes may influence the selective pressures upon the San Joaquin River fall-run stock, eventually resulting in genetic and ecological character changes. The genetic baseline would benefit conservation and management decisions about population, habitat, and harvest management and hatchery operations by specifically providing a census of fall-run Chinook salmon population structure. An assessment of the degree of geographic population structure may indicate restrictions in gene flow (Small et al. 1998), and the degree of temporal heterogeneity in genetic diversity may reflect variation in reproductive success (demographic stochasticity) (Tessier and Bernatchez 1999). Tetranucleotide repeat microsatellite loci (Williamson et al. 2002) (with higher levels of variability than the dinucleotide repeat mi-

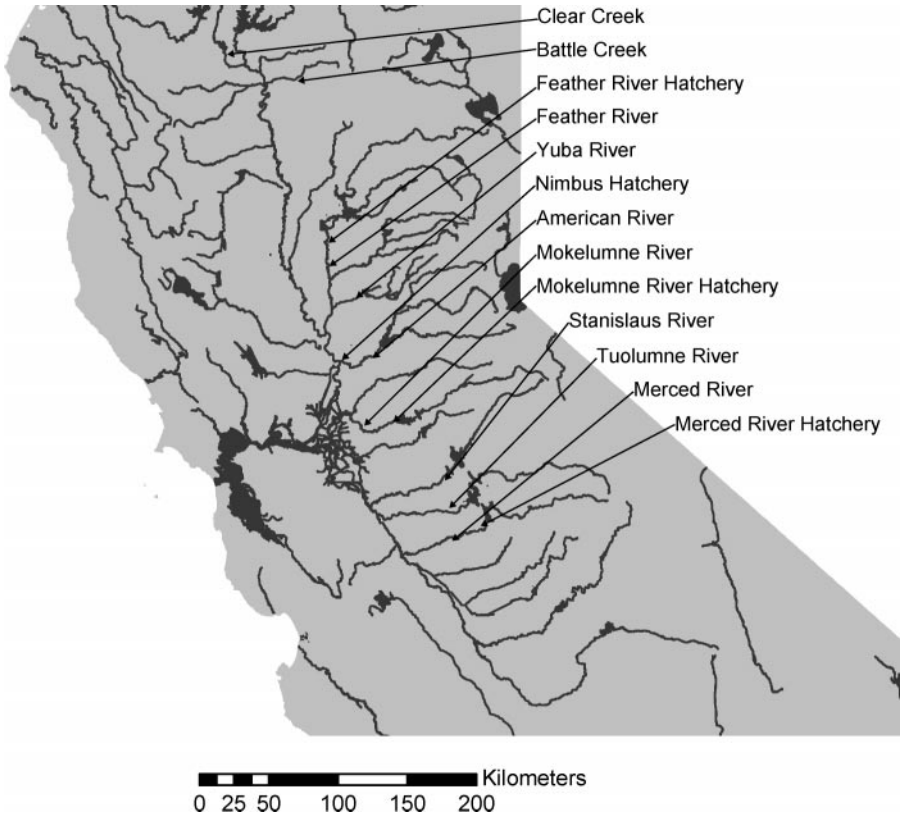

FIGURE 1.—Map showing the sampling locations for fall-run Chinook salmon collected between 1999 and 2001 within the Sacramento River and San Joaquin River basins in California.

crosatellites that comprised most of the markers used in the Banks et al. [1996, 2000a] studies) and more temporally robust sampling were used to explore intertributary-scale genetic structure and temporal stability of genetic diversity within fall-run Chinook salmon from the San Joaquin River basin from 1999 to 2001. We evaluated both spatial and temporal population genetic structure in fall-run Chinook salmon populations from tributaries of the San Joaquin River. The census of genetic structure was then used to ascertain whether San Joaquin River basin fall-run Chinook salmon populations could be discriminated from other fall-run populations in the Sacramento River basin.

Methods

Sampling sites.—California Department of Fish and Game (CDFG) personnel obtained fin clips from adult fall-run Chinook salmon during the 1999–2001 salmon carcass surveys conducted within the Sacramento River and San Joaquin River drainages. During 1999, carcass surveys were performed at seven locations in the Sacramento River basin (American River, Battle Creek, Clear Creek, Feather River, Feather River Hatchery, Nimbus Hatchery, and Yuba River) and six locations within the San Joaquin River basin (Merced River, Merced River Hatchery, Mokelumne River, Mokelumne River Hatchery, Stanislaus River, and Tuolumne River) (Figure 1). During 2000, carcass surveys were performed at two locations in the Sacramento River basin (Nimbus Hatchery and Yuba River) and four locations within the San Joaquin River basin (Merced River, Merced River Hatchery, Stanislaus River, and Tuolumne River). During 2001, CDFG carcass surveys for fall-run fish were performed only at four locations within the San Joaquin River basin (Merced River, Merced River Hatchery, Stanislaus River, and Tuolumne River). For sample sizes at each location, refer to Table A.1 in the appendix. Carcasses were sampled during the entire spawning period and on multiple occasions at several different points on each tributary. Spring-run Chinook salmon were also collected during the 1999 carcass survey of Deer Creek. Fin clips were cut from carcasses of

fish and were stored individually on ice in 10 mM of tris (pH 8) buffer. Razor blades were mechanically cleaned and rinsed between each use for sampling fish.

Microsatellite genotyping.—Genomic DNA was extracted from fin clips by means of a QIAGEN DNA tissue extraction kit, eluted into a 96-well sample plate, and quantified with a Molecular Dynamics 595 fluorimager (Sunnyvale, California). Individuals were genotyped at 10 previously developed tetranucleotide repeat microsatellite loci: *OtsG13, OtsG68, OtsG78b, OtsG83b, OtsG249, OtsG311, OtsG409, OtsG422, OtsG432,* and *OtsG474* (Williamson et al. 2002). Microsatellite alleles were amplified via polymerase chain reaction (PCR) by use of 2–20 ng of genomic DNA, 2.0 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.2 μM of each PCR primer, 0.25 units of *Taq* DNA polymerase (Promega Biosciences, Inc., San Luis Obispo, California), 20 mM of tris (pH 8.5), and 50 mM of KCl in 10-μL volumes. The forward primer of each PCR primer pair was labeled with a fluorescent phosphoramidite (HEX, FAM, or TET). PTC100 thermal cyclers (MJ Research, San Francisco, California) were programmed with the following conditions to amplify all microsatellite loci examined: one denaturation cycle at 95°C for 90 s; 35 amplification cycles of 95°C for 60 s, 52°C for 30 s, and 72°C for 60 s; and a final extension cycle of 60°C for 45 min. The lengthy final extension cycle was used to fill in the +A nucleotide additions that *Taq* DNA polymerase creates at the 3′ end of each synthesized DNA strand, thereby permitting more consistent and accurate scoring of PCR products. The PCR products and in-lane size standards (GeneScan 400HD and 500, mixed 1:1) were electrophoresed on a 5.5% denaturing polyacrylamide gel at 2,600 V for 75 min on a MJ Research BaseStation Sequencer and Genotyper. Individual genotypes were scored by use of Cartographer software (MJ Research 2003), and allele sizes were manually verified for every genotyped individual. Replicate samples of individuals were placed on separate DNA sample plates to alleviate potential variability in allele size scores between different gel runs.

Characterization of microsatellite loci.—The 10 microsatellite loci used in this study were characterized by calculating the average and total number of alleles per locus and the allelic size range (nucleotide base pairs observed over all collections) in the program GENETIX, version 4.04 (Belkhir et al. 2003). We used this information in combination with population genetic statistics to decide whether the loci were suitable as markers for the analysis of population genetic structure.

Population genetic analyses.—Data from each sample site were analyzed for deviations from Hardy–Weinberg equilibrium (HWE) and possible sample admixture by use of the program GENEPOP, version 3.1c (Raymond and Rousset 1995). Pairwise linkage disequilibrium (LD) tests were performed on each sample collection alone to detect admixture and on all possible pairwise combinations of sample collections. The artificial mixing of sample collections by combining multilocus genotype datasets from different sample sites and then testing the combined data for LD was performed to identify which samples were genetically distinct from one another and which samples, if any, from within either the San Joaquin River basin or the Sacramento River basin could be pooled for subsequent analyses. The more genetically distinct two populations are from one another, the greater the degree of LD; significantly high inbreeding coefficients (F_{IS}) on a per-locus basis should also be detected in the combined data from those populations. The LD between all pairs of loci in each collection or combination of sample collections was determined by the Markov chain method of estimating exact *P*-values (Guo and Thompson 1992) as implemented in GENEPOP (1,000 dememorization steps; 50 batches; 5,000 iterations per batch). To decrease the chance of erroneously rejecting null hypotheses, we applied sequential Bonferroni adjustments to the significance level α of simultaneous tests when appropriate (Rice 1989).

Population genetic statistics and allelic frequencies were calculated from the data from each sample site. Expected heterozygosity (H_e) under HWE, observed heterozygosity (H_o), and multilocus F_{IS} values were calculated in the program GENETIX (Belkhir et al. 2003). Significance of F_{IS} values was determined by performing 500 random permutations in GENETIX. Allelic frequencies were calculated in GENEPOP.

Population subdivision was analyzed via pairwise comparisons of Wright's standardized measure of allele frequency variance (F_{ST}) calculated in GENETIX. Significance of F_{ST} values was determined by performing 500 random permutations in GENETIX. If the number of permuted F_{ST} values equal to or larger than the observed F_{ST} value exceeded 5% ($\alpha = 0.05$), then the observed F_{ST} value for a given pair of sample collections was judged to be statistically nonsignificant.

A Bayesian clustering approach to detect the presence of cryptic population structure was also

performed. Revealing the presence of undetected population structure can prevent erroneous associations from invalidating the assumptions of standard analyses (Ewens and Spielman 1995). Another advantage of a Bayesian clustering approach, in the case of assigning individuals to their appropriate populations, is that it makes assignment more robust to the presence of misclassified individuals and should be more accurate if only preclassified individuals are used to estimate allele frequencies (Smouse et al. 1990). The software program Structure, version 2.0 (Pritchard et al. 2000), evaluates population structure by assigning individuals to populations based on their multilocus genotypes while simultaneously estimating population allele frequencies. Under the assumptions of HWE and linkage equilibrium between loci within populations, each allele at each locus in each genotype is considered to be an independent draw from the appropriate frequency distribution, and this completely specifies the probability distribution of K clusters of sampled individuals given their genotypes. A burn-in period of 30,000 iterations was used, and data were collected for 250,000 iterations under each model that evaluated the likelihood of K clusters.

The degree of differentiation among geographic and temporal samples from the same sampling site was quantified by a hierarchical analysis of molecular variance (AMOVA; Michalakis and Excoffier 1996) available in the program Arlequin, version 2.0 (Schneider et al. 2000). Variance among collection sites grouped by river basin (Sacramento or San Joaquin; geographic component), variance between temporal samples from the same site (temporal component), and variance among individuals within samples were assessed to determine whether there were any significant associations with different possible levels of genetic structure.

The phylogenetic analysis software PHYLIP, version 3.6 (α3) (Felsenstein 2002), was used to construct neighbor-joining (NJ) phenograms (Saitou and Nei 1987) derived from raw allele frequencies. The SEQBOOT subroutine in PHYLIP was used to bootstrap 10,000 iterations of each raw data set. Bootstrapped sets of Cavalli-Sforza and Edwards' (1967) chord distance (D_{CE}) were calculated for each bootstrapped raw data set by means of the GENDIST subroutine in PHYLIP. This measure of genetic distance was chosen because it assumes that genetic divergence between populations is due solely to genetic drift and it does not assume a particular mechanism of mutation. In addition, according to Takezaki and Nei (1996), D_{CE} appears to be more efficient than other measures of genetic distance for obtaining correct tree topology from allele frequency data. A rooted NJ phenogram was calculated for each bootstrapped data set by use of the NEIGHBOR subroutine. The consensus phenogram and the significance of each node were evaluated by the CONSENSE subroutine. Finally, the consensus NJ phenogram was visualized in TREEVIEW (Page 1996).

Results

Characteristics of Examined Loci

Ten microsatellite loci were genotyped for each sample collection obtained from 1999 to 2001. Due to the degraded nature of carcasses, some samples were thrown out since they did not produce usable template DNA for analysis. Other samples could be amplified at only a subset of the loci examined, regardless of the number of attempts made. The latter samples were retained for analysis since they still provided usable data for loci that could be amplified in those individuals. As a result, the total number of individuals genotyped per locus ranged from 948 (*OtsG422*) to 1,301 (*OtsG432*) (Table A.1). The total number of alleles observed per locus (A) ranged from 17 (*OtsG474*) to 75 (*OtsG13*; not shown), and the A averaged across collections ranged from 11 (*OtsG474*) to 31 (*OtsG13*; not shown) (Table A.1). Tests for conformity to HWE (initial $\alpha = 0.005$, across 10 loci; data not shown) revealed significant heterozygote deficiencies for three microsatellite loci in the sample collections evaluated from 1999 to 2001. Significant heterozygote deficiencies for loci *OtsG13* ($P < 0.003$), *OtsG68* ($P < 0.004$), and *OtsG78b* ($P < 0.0001$) were observed in 21, 9, and 23 out of 23 total collections, respectively. This indicates the presence of one or more nonamplified alleles (null alleles) at relatively high frequencies due to a mutation in the region that is complementary to one of the PCR primers (Callen et al. 1993; Paetkau and Strobeck 1995; Pemberton et al. 1995). These three loci were excluded from further analyses. Out of the seven remaining loci, heterozygote deficiencies were found in 19 out of 161 locus comparisons with Hardy–Weinberg expectations (initial $\alpha = 0.007$, across seven loci; data not shown). The heterozygote deficiencies spanned 14 out of 23 sample collections; no more than five such deficiencies occurred at a single locus (*OtsG474*), and no more than three occurred within a single collection (Battle Creek in 1999). The H_o, H_e, A, and F_{IS} values (Weir and Cockerham

TABLE 1.—Matrix of Wright's genetic differentiation index (F_{ST}) values (Weir and Cockerham 1984) for 13 fall-run Chinook salmon populations in the Sacramento River and San Joaquin River basins, California, and a single spring-run population (Deer Creek) collected during 1999. Pairwise F_{ST} and *P*-values are presented above and below the diagonal, respectively. Asterisks denote F_{ST} values that were statistically significant at $\alpha = 0.05$.

Sample site	American River	Battle Creek	Clear Creek	Feather River Hatchery	Feather River	Mokelumne River Hatchery	Mokelumne River
American River		0.003	0.006*	0.003*	0.001	−0.001	−0.003
Battle Creek	0.086		0.005	0.005*	0.005*	0.003	0.002
Clear Creek	0.008	0.062		0.004*	0.002	0.003	0.003
Feather River Hatchery	0.020	0.001	0.020		0.003*	0.004*	0.002*
Feather River	0.218	0.032	0.146	0.016		0.001	−0.001
Mokelumne River Hatchery	0.628	0.054	0.088	0.020	0.258		−0.002
Mokelumne River	0.980	0.206	0.108	0.108	0.644	0.870	
Merced River Hatchery	0.276	0.010	0.550	0.08	0.634	0.050	0.458
Merced River	0.276	0.682	0.046	0.324	0.274	0.124	0.676
Nimbus Hatchery	0.238	0.148	0.166	0.098	0.630	0.088	0.408
Stanislaus River	0.008	0.000	0.036	0.030	0.056	0.016	0.114
Tuolumne River	0.060	0.038	0.034	0.000	0.290	0.406	0.694
Yuba River	0.042	0.290	0.076	0.036	0.370	0.200	0.140
Deer Creek	0.000	0.000	0.000	0.000	0.000	0.000	0.000

1984) for microsatellite loci genotyped in each fall-run Chinook salmon sample collected from 1999 through 2001 are presented in Table A.1.

Linkage Disequilibrium

Significant LD was detected in 14 of 2,079 (0.67%) pairwise comparisons of collections (initial $\alpha = 0.007$ across seven loci for each pairwise combination of collections; data not shown). Significant pairwise comparisons spanned nine different locus combinations, and a maximum of three significant comparisons occurred between any given pair of loci. Five of the 14 pairwise locus combinations in LD occurred in the comparison between 1999 samples from the American and Mokelumne rivers ($P < 0.007$, 5 out of 21 pairwise locus combinations; data not shown). Significant LD within a collection ($P < 0.007$; data not shown) was only observed between *OtsG249* and *OtsG311* in the Tuolumne River samples from 1999. Physical linkage of loci, epistatic selection, and genetic hitchhiking (Maynard Smith and Haigh 1974) can produce gametic disequilibrium. To our knowledge, none of the loci used in this study has yet been mapped on salmonid chromosomes, so we cannot exclude the possibility of physical linkage. However, given the large number of tests performed for loci in LD, it is possible that a few significant tests could have occurred by chance alone.

Within-Population Genetic Variability

The H_e and H_o values were in close agreement; H_e ranged from 0.908 to 0.936 and H_o ranged from 0.836 to 0.931 across all seven loci within samples (Table A.1). However, despite the close agreement of H_e and H_o within samples, significant deviations from HWE and/or significant F_{IS} values occurred in 15 of 23 collections ($P < 0.007$; across seven loci) (Table A.1).

The mean number of alleles per locus ranged from 18.7 for the Stanislaus River collection in 1999 to 28.9 for the Merced River collection in 2001 (Table A.1). Sample size, which averaged between 26 and 80 individuals scored per locus, directly influenced the average *A.* Those sites with a significantly lower average *A* also had a significantly lower average number of individuals scored per locus (Wilcoxon's signed rank test, $P < 0.05$). Given the degraded nature of carcass samples from the Battle Creek, Merced River, and Stanislaus River collections in 1999, amplification of microsatellite loci was problematic. In these collections, the number of individuals scored per locus averaged 27 (Battle Creek), 26 (Merced River), and 34 (Stanislaus River).

Genetic Structure

All measures of genetic structure between population pairs or among population groups showed very little geographic or temporal distinction. Average pairwise F_{ST} values for 1999, 2000, and 2001 (0.0018, 0.0031, and 0.0013, respectively) were very small (Tables 1–3). We observed only three pairwise F_{ST} values that differed significantly ($\alpha = 0.05$) from zero consistently from year to year between sites. These included the Merced River and Stanislaus River comparisons from 2000 and

TABLE 1.—Extended.

Sample site	Merced River Hatchery	Merced River	Nimbus Hatchery	Stanislaus River	Tuolumne River	Yuba River	Deer Creek
American River	0.001	0.001	0.001	0.005*	0.003	0.004*	0.013*
Battle Creek	0.005*	−0.002	0.002	0.008*	0.005*	0.001	0.013*
Clear Creek	0.000	0.005*	0.002	0.005*	0.005*	0.003	0.011*
Feather River Hatchery	0.002	0.001	0.002	0.004*	0.006*	0.004*	0.013*
Feather River	0.000	0.001	−0.001	0.004	0.001	0.000	0.010*
Mokelumne River Hatchery	0.002	0.003	0.002	0.004*	0.000	0.002	0.012*
Mokelumne River	0.000	−0.001	0.000	0.003*	−0.001	0.002	0.011*
Merced River Hatchery		0.001	0.000	0.004	0.003*	0.000	0.011*
Merced River	0.252		0.000	0.003*	0.002	−0.001	0.014*
Nimbus Hatchery	0.98	0.548		0.004*	0.004*	−0.002	0.014*
Stanislaus River	0.032	0.160	0.036		0.003	0.002	0.009*
Tuolumne River	0.030	0.192	0.001	0.112		0.002	0.009*
Yuba River	0.482	0.558	0.928	0.162	0.162		0.014*
Deer Creek	0.000	0.000	0.000	0.000	0.000	0.000	

2001 (F_{ST} = 0.0029 and 0.0031, respectively; Tables 2, 3) and the comparisons between the Nimbus Hatchery and both the Stanislaus and Tuolumne rivers in 1999 and 2000 (average F_{ST} = 0.0041 and 0.0045, respectively; Tables 1, 2). Two other statistically significant differences observed in pairwise comparisons of F_{ST} values were not consistent from year to year: (1) the Yuba River in 2000 and all other 2000 collections except for the Merced River (average F_{ST} = 0.0047; Table 2) and (2) the Stanislaus River in 1999 and all other 1999 collections except for the Merced and Mokelumne rivers (average F_{ST} = 0.0046; Table 1). The latter result is probably attributable to insufficient sampling within the Stanislaus River in 1999.

The Bayesian clustering approach to detect the presence of population structure indicated that when the number of clusters (K) equaled 1, the model was the most consistent with the data for each of the 3 years (Table 4). Estimates for each value of K were consistent across independent runs, and the degree to which the model was consistent with the observed data (probability of K given the data) decreased as K increased. During the course of individual runs, α (a parameter for the degree of admixture) varied greatly (data not shown). If there is a lack of population structure, then less information about the value of α is gained as a simulation progresses. Accordingly, if real structure does not exist, then α will usually vary greatly during the course of a run (Pritchard et al. 2000). The proportion of individuals assigned to each cluster was symmetric with approximately 1/K percent of the individuals in each data set assigned to each cluster. For example, when a model of K = 3 was used, the proportion of individuals assigned to each of the three clusters was approximately 0.333. These trends were observed in all simulations for all values of K.

The pattern of a high degree of genetic similarity between sample collections was also observed in the hierarchical AMOVA of microsatellite allele

TABLE 2.—Matrix of Wright's genetic differentiation index (F_{ST}) values for fall-run Chinook salmon samples collected during 2000 from the Sacramento River and San Joaquin River basins, California. Pairwise F_{ST} and P-values are presented above and below the diagonal, respectively. Asterisks denote F_{ST} values that were statistically significant at α = 0.05.

Sample site	Merced River	Merced River Hatchery	Nimbus Hatchery	Stanislaus River	Tuolumne River	Yuba River
Merced River		0.002	0.002	0.003*	0.004*	0.002
Merced River Hatchery	0.126		0.001	0.000	0.002	0.004*
Nimbus Hatchery	0.104	0.158		0.005*	0.005*	0.005*
Stanislaus River	0.024	0.412	0.000		0.003*	0.004*
Tuolumne River	0.004	0.054	0.000	0.008		0.006*
Yuba River	0.130	0.034	0.002	0.018	0.000	

TABLE 3.—Matrix of Wright's genetic differentiation index (F_{ST}) values for fall-run Chinook salmon samples collected during 2001 from the San Joaquin River basin, California. Pairwise F_{ST} and *P*-values are presented above and below the diagonal, respectively. Asterisks denote F_{ST} values that were statistically significant at $\alpha = 0.05$.

Sample site	Merced River	Merced River Hatchery	Stanislaus River	Tuolumne River
Merced River		0.001	0.003*	0.000
Merced River Hatchery	0.174		0.002*	0.001
Stanislaus River	0.000	0.034		0.001
Tuolumne River	0.572	0.246	0.070	

frequencies. The results from 1999 and 2000 indicated no significant variation in sample collections within or between river basin groups (Table 5). The 2001 data set was composed of samples from only the San Joaquin River drainage. Here, too, the degree of differentiation among samples was nonsignificant ($F_{ST} = 0.0023$, $P = 0.958$) and the largest percentage of variance occurred between individuals.

Spatial and Temporal Stability of Allele Frequencies and Genetic Diversity

A similar pattern in the partitioning of variation in microsatellite allele frequencies was observed when collections from the same site were grouped across multiple years. When the Merced River, Merced River Hatchery, Stanislaus River, and Tuolumne River collections (1999–2001) and the Nimbus Hatchery and Yuba River collections (1999–2000) were grouped across years, the largest percentage of variance (2.1%; $F_{SC} = 0.021$, $P = 1.0$) was distributed among temporal samples within groupings, whereas a much smaller proportion of the variance (0.02%; $F_{CT} = 0.0002$, $P = 0.674$) was distributed among groups.[1]

With the exception of samples from the Feather River (1999), Feather River Hatchery (1999), Mokelumne River, and Mokelumne River Hatchery, the unrooted NJ phenograms for pairwise D_{CE} among sample collections for individual years did not group collections by geographic proximity (data not shown). Consistent clustering of samples from year to year was observed only for the Merced River Hatchery and Nimbus Hatchery and the Merced and Yuba rivers between 1999 and 2000 (data not shown). Lack of replicate samples from 2000 and 2001 prevented further evaluation of tree topology. Overall bootstrap support ranged from 12% to 74% (means of 29% and 65%; data not shown), and values greater than 50% typically occurred only at the outermost nodes. The NJ phenogram of the 2001 data set (data not shown), which contained only four fall-run samples, also indicated no apparent geographic population structure. The NJ phenogram for pairwise D_{CE} among the 23 sample collections combined for all 3 years (Figure 2) indicated no temporal grouping of collections from the same site except for the Merced River Hatchery collections in 2000 and 2001. Overall bootstrap support for the phenogram was poor, and only two of the outermost

TABLE 4.—Results from the procedure for inferring the value of *K*, the number of clustered populations, in genetic baseline data sets for Central Valley fall-run Chinook salmon (1999–2001). The model assumed correlated allele frequencies and utilized a burn-in of 30,000 iterations; data were collected for 250,000 iterations. The probability of *K* clusters should be regarded as a rough guide to which models are consistent with the data sets rather than as an accurate estimate of posterior probability.

Year	*K*	Estimated Probability of data[a]	Probability of *K* given the data
1999	1	−21,785	1.00
	2	−22,494	0.27
	3	−22,991	0.15
	4	−23,835	0.11
	5	−24,898	0.08
2000	1	−13,053	1.00
	2	−14,076	0.27
	3	−15,633	0.15
	4	−16,666	0.11
	5	−19,888	0.08
2001	1	−12,343	1.00
	2	−12,988	0.27
	3	−13,806	0.15
	4	−14,393	0.11
	5	−15,419	0.08
1999–2001	1	−47,279	1.00
	2	−47,979	0.27
	3	−48,465	0.15
	4	−49,661	0.11
	5	−51,299	0.08

[a]Log_e transformed.

[1] F_{SC} is defined as the genetic variation of random genotypes within populations relative to that of random pairs of genotypes drawn from populations sampled during the same year; F_{CT} is defined as the genetic variation of random genotypes within a group of populations relative to that of random pairs of genotypes drawn from the whole data set.

TABLE 5.—Hierarchical analyses of molecular variance of microsatellite loci allele frequencies in fall-run Chinook salmon from the Sacramento River and San Joaquin River basins, California, sampled in 1999 and 2000.

Variance component	df	Variance component	% Total variance	*P*
1999 analysis				
Between basin groups	1	0.02012	0.94	1.000
Among populations within basin groupings	11	0.05033	2.34	0.937
Within basin groups	1,263	2.29221	97.79	1.000
Total	1,275	2.22088		
2000 analysis				
Between basin groups	1	0.01595	0.68	1.000
Among populations within basin groupings	4	0.04781	2.03	0.218
Within basin groups	760	2.39025	97.29	1.000
Total	765	2.33908		

nodes exhibited support greater than 50%: the Feather River Hatchery in 1999 and the Yuba River in 2000 (88%), and the Mokelumne and Tuolumne River collections in 1999 (62%). Both the rooted (based on the Deer Creek spring run) and unrooted NJ algorithms and the algorithms determined by the unweighted pair-group method with arithmetic averages produced phenograms with similar topology.

Discussion

Fall-run Chinook salmon in the Central Valley appear to belong to just one intermixing population that includes hatchery fish. Multiple analyses indicate little population genetic structure, either spatially or temporally, for Central Valley fall-run Chinook salmon. Pairwise analysis of LD provided little evidence of significant differentiation, and F_{ST} measures indicated considerable gene flow between widely geographically separate populations. Estimates of straying rates from Coleman National Fish Hatchery (70%) and the Feather River Hatchery (46%) are substantial (CDFG–NMFS Joint Hatchery Review Committee 2001). This may explain the lack of genetic distinction between hatchery and naturally spawning populations. The Bayesian modeling approach used in Structure 2.0 to detect the presence of cryptic spatial or temporal population structure indicated that a single-population model was the most consistent with each year's data set. Hierarchical AMOVA indicated that over 97% of the genetic variation was contained within populations in each case, but did not indicate significant genetic structure within or between samples grouped either by basin of origin (Table 5) or temporally from the same site (data not shown). We observed no discrete separation of populations in the NJ phenogram based on origin (hatchery versus naturally spawning fish), basin (San Joaquin River versus Sacramento River systems), or sampling year. The grouping or proximity of collections that were otherwise separated by wide geographic distances suggests that population genetic structure is not present in the Central Valley fall run. All of these lines of evidence suggest either that there is no spatial structure to genetic variation or that gene flow between rivers is great enough to dampen any potential sign of differences in temporal stability of allele frequencies between tributaries.

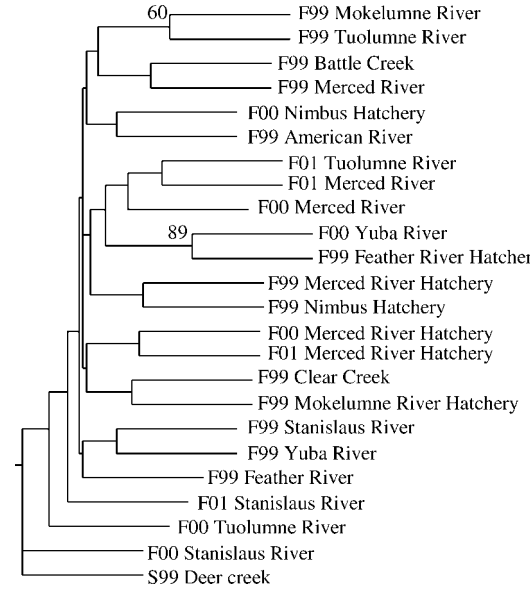

FIGURE 2.—Rooted neighbor-joining phenogram based on Cavalli-Sforza and Edwards' (1967) chord distance for fall-run Chinook salmon collections in the Central Valley, California, 1999–2001. The phenogram was constructed with data from seven microsatellite loci and was rooted with data from the Deer Creek spring run, sampled in 1999. For 10,000 bootstrap replicates, node values of 50% or greater are given.

Intrapopulation diversity within the San Joaquin River drainage and the lower portions of the Sacramento River drainage was stable within the time frame of this study. This suggests that possible demographic changes have not been large enough to cause important deviations from migration or drift equilibrium that would measurably alter intrapopulation diversity. However, these observations do not mean that disturbances caused by declining numbers of returning adults or transport of fish have had no impact on the genetic integrity of Chinook salmon populations within the Central Valley. Instead, they strengthen the position that unless demographic changes are extreme, especially given the short time frame of this study, temporal changes in intrapopulation genetic diversity are not easily depicted (Waples and Teel 1990).

Our study corroborates recent findings by Banks et al. (2000a) that spatial and temporal homogeneity of genetic diversity exists in fall-run Chinook salmon populations within the Central Valley. Out of the 20 samples of fall-run subpopulations, 17 formed a subset of samples with homogeneous allele frequencies. Temporal variation of genetic diversity within fall-run sample sites was not significant relative to the differences among seasonal subpopulations (Banks et al. 2000a). Thus, separate studies of Central Valley Chinook salmon that used the same marker type and that spanned a total time period of 6 years revealed very little spatial and temporal genetic diversity for the fall run. Our results suggest that despite their greater potential to resolve population structure, even microsatellite loci do not have the power to discriminate small genetic differences among Central Valley fall-run stocks, should such differences indeed exist.

There are several explanations as to why small genetic differences between Central Valley fall-run stocks may not have been resolved. First, historical factors, such as obliteration of spawning habitat on the Yuba, American, Feather, and Bear rivers due to logging and mining activities (Olin 1996; Yoshiyama et al. 1998), may have promoted genetic homogeneity by forcing adults to return to nonnatal tributaries. Second, the habitat into which Central Valley fall-run fish have expanded may be younger than other geographic regions, and thus genetic differences between populations have not yet had a chance to evolve. Third, the intensity of off-site releases of juvenile fall-run Chinook salmon and the admixture of spawning adults through high levels of straying (discussed below) may have increased gene flow to the point where spatial genetic structure is no longer discernible.

The lack of genetic distinctiveness even between fall-run populations separated by the maximum extent of distribution (~350 km) in the Central Valley is in stark contrast to the geographic structuring of Chinook salmon populations seen in other studies and attests to the magnitude of admixture in Central Valley fall-run Chinook salmon. Population diversity on a basin scale has been demonstrated for Chinook salmon in Alaska (Gharrett et al. 1987), the Snake River (Waples et al. 1991), and British Columbia (Teel et al. 2000), and throughout the Pacific Northwest (Utter et al. 1989). Teel et al. (2000) determined an average D_{CE} of 0.071 (based on 31 allozyme loci) for the subset of Chinook salmon populations with an ocean-type life history. In contrast, the overall average D_{CE} between populations of Central Valley fall Chinook salmon was 0.017. In a recent analysis of 11 fall Chinook salmon populations in the Klamath River basin, Banks et al. (2000b) found that no two populations were genetically homogeneous. Neither of the two hatcheries (Iron Gate and Trinity hatcheries) sampled by Banks et al. (2000b) release their fall-run Chinook salmon off site.

The contrasts between the results of this study and studies conducted in regions where off-site releases of hatchery fish do not occur lend support to the growing body of evidence that supplementation of depressed anadromous and nonanadromous salmonid stocks with hatchery fish in both North America (Utter et al. 1989; Krueger and May 1990; Hindar et al. 1991; Utter 1998; Reisenbichler and Rubin 1999) and Europe (Hindar and Balstad 1994; Cagigas et al. 1999; Machordom et al. 1999) can have a negative impact on genetic diversity and, potentially, reproductive productivity on a regional scale. There are three primary concerns regarding the genetic impact of hybridization between straying hatchery fish and naturally spawning populations (Waples 1991). First, if straying of hatchery fish results in introgressive hybridization with natural populations over a wide geographic range, genetic variation among populations may be reduced and outbreeding depression may occur. An accumulating body of evidence (Reisenbichler and Rubin 1999) indicates that individual populations of anadromous salmonids become selectively adapted to particular spawning localities and times. Habitat affinity, disease resistance, and the timing and extent of migration are unique adaptations that provide populations with a reproductive advantage in particular environments, thereby increasing the probability of persistence in those environments. Relative to na-

tive populations, transferred fish have a greatly impaired ability to persist when planted in areas to which they are not locally adapted (Withler 1982; Reisenbichler 1988; Altukhov at al. 2000). Second, genetic changes (domestication selection, drift, or stock transfers) incurred by hatchery fish may be transferred from hatchery strays to natural populations, thereby causing reduced fitness and lower productivity of natural populations. In turn, hybridized populations would be more susceptible to environmental stress (Altukhov et al. 2000). Third, indirect genetic effects mediated by altered selection regimes or population size reductions (i.e., from competition, predation, disease, or other factors) may decrease genetic variation. Hybridization between hatchery and native fish, even if it goes no further than one generation, promotes displacement of native fish through cooption of gametes and habitat (Utter 1998; Fleming and Petersson 2001).

Supplementation programs for fall- and spring-run Chinook salmon within California have included the transport of juvenile hatchery fish to sites downstream in an attempt to boost ocean catch rates despite the poorer ''fitness to survive'' (Unwin 1997) of hatchery fish than wild fish. The assumption is that if enough fish are planted, sufficient numbers will survive to enter the fishery, regardless of mortality rates. Given the importance of hatchery fish in the sport and commercial fisheries, this assumption seems justified. Our genetic evidence also supports it. It is the transport of juvenile fish that results in the straying of returning hatchery adults (Sholes and Hallock 1979; Pascual et al. 1995). A review by Quinn (1993) indicates that, in general, salmon and steelhead *O. mykiss* return as adults to the release site rather than the rearing site. It is believed that transport of salmonids during smolting interferes with their ability to imprint upon their natal stream through chemical cues (Scholz et al. 1976), thereby increasing the likelihood that they will stray to nonnatal streams when they return to spawn in freshwater as adults (Heard 1996). Tagging and genetic studies (Candy and Beacham 2000; Mackey et al. 2001) have shown that off-site releases of hatchery-reared fish promote straying rates that far exceed natural levels.

Management Considerations

Modification of certain management practices will be essential to the recovery of regional genetic variation, which is in turn necessary for maintaining the evolutionary potential of fall-run Chinook salmon to persist in an ever-changing environment. First, the transport of hatchery-reared fall Chinook salmon to release sites in the San Francisco Bay, Sacramento–San Joaquin Delta, or nonnatal tributaries should be discontinued. Instead, juveniles could be released at or near the hatchery where they were produced. This would permit downstream-migrating smolts to sequentially imprint, thereby maintaining fidelity to their natal stream when returning to spawn. Without such imprinting, the straying of hatchery fish throughout the Central Valley will continue to be a significant factor contributing to the homogenization of fall Chinook salmon gene pools. Secondly, all fish released from hatcheries could be marked so that only returning adults that originated from a particular hatchery can be used in that hatchery's supplementation program. By eliminating inadvertent interbreeding of fish produced in different hatcheries, potential negative adaptive impacts caused by outbreeding depression would be prevented. Third, fewer hatchery-reared fish should be produced. If hatchery production has indeed masked declines in natural production of fall-run fish in Central Valley streams, then it would seem prudent to engage in management practices that facilitate the ability to evaluate natural production.

Conclusions

Fall-run Chinook salmon throughout California's Central Valley comprise a genetically homogeneous population that has lower among-population diversity than fall-run Chinook salmon populations examined elsewhere over similar geographic scales. The lack of spatial structure to genetic variation and the lack of temporal differences in allele frequencies in Central Valley fall-run fish are best explained by the high degree of interpopulation gene flow mediated by stray hatchery fish. Release of juvenile hatchery fish at sites other than the rearing site is the most likely cause of adult straying within and between drainages.

The lack of geographic genetic structure among Central Valley fall-run Chinook salmon does not justify stock transfers within or among basins. A lack of divergence between populations based on selectively neutral molecular markers, such as microsatellite loci (Tautz and Renz 1984), does not equal an absence of ecologically or physiologically adaptive differences between populations (Utter 1981; Utter et al. 1992; Lynch 1996). Similarly, temporal changes in intrapopulation genetic diversity are not easily detected unless demographic changes are extreme (Waples and Teel

1990). Thus, our data may not have been able to fully differentiate fall-run Chinook salmon populations from different tributaries within the San Joaquin River drainage or from the Sacramento River and San Joaquin River basins. The conservative interpretation of our findings, therefore, is that fall-run Chinook salmon in the Central Valley constitute just one population.

The continued transport of hatchery Chinook salmon to off-site release points in the Central Valley and the resultant high straying rates may degrade adaptive genetic variability within the fall-run gene pool, reduce the overall fitness of populations, and subsequently lead to greater fluctuations in population sizes. If hatchery and naturally spawning populations were managed as separate units, this would permit the continued evolution of environmentally adaptive genetic variation within the naturally spawning populations. Despite the lack of genetic distinctiveness between hatchery and naturally spawning fall-run populations, a high degree of microsatellite variability remains within the run as a whole. If variation at microsatellite loci is a surrogate for adaptive genetic diversity elsewhere in the genome, then it is likely that hybridization between hatchery and naturally spawning fish has not yet substantially diminished the variability that evolutionary processes have molded. If, however, hatchery and naturally spawning populations continue to be managed as one unit, and if the genetic homogenization of the fall-run populations (mediated by off-site releases and consequent straying and introgression) continues unchecked, then it is probable that the pool of available adaptive genetic diversity will diminish.

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Appendix: Genetic Variation

TABLE A.1.—Genetic variation at seven microsatellite loci for fall-run Chinook salmon collected at 13 sampling locations from 1999 to 2001 in the Sacramento River and San Joaquin River basins. For each location, values are given for sample size (n), number of alleles (A), expected (H_e) and observed heterozygosities (H_o), inbreeding coefficient (F_{IS}; Weir and Cockerham 1984), and exact probability for Hardy–Weinberg equilibrium (HWE). The means in the next to last column are for all locations. Both the total number of individuals genotyped and number of alleles observed per locus are shown in the last column. Asterisks denote significant values (initial $\alpha = 0.007$ across all 7 loci).

	Sacramento River drainage								
						Nimbus Hatchery		Yuba River	
Locus	American River, 1999	Battle Creek, 1999	Clear Creek, 1999	Feather River, 1999	Feather River Hatchery, 1999	1999	2000	1999	2000
OtsG83b									
n	41	25	31	40	54	44	48	33	45
A	28	25	23	26	31	30	28	31	29
H_e	0.958	0.966	0.958	0.953	0.964	0.958	0.952	0.970	0.957
H_o	0.951	0.800	1.000	0.825	0.944	0.932	0.979	0.970	0.933
F_{IS}	0.008	0.175*	−0.044	0.136*	0.019	0.028	−0.029	0.000	0.025
OtsG249									
n	40	35	31	41	55	46	58	39	51
A	30	24	25	27	30	33	30	28	31
H_e	0.957	0.953	0.957	0.960	0.960	0.967	0.959	0.963	0.958
H_o	0.900	0.943	0.903	0.902	0.927	0.935	0.914	0.974	0.941
F_{IS}	0.060	0.011	0.057	0.061	0.035	0.034	0.047	−0.012	0.017
OtsG311									
n	47	31	35	46	55	38	59	37	42
A	23	21	24	27	28	26	29	24	25
H_e	0.938	0.937	0.949	0.938	0.958	0.946	0.947	0.939	0.932
H_o	0.915	0.935	0.914	0.848	0.927	0.868	0.932	0.811	0.857
F_{IS}	0.025	0.001	0.038	0.097	0.033	0.083	0.016	0.138*	0.081
OtsG409									
n	41	33	33	47	54	44	59	33	50
A	24	19	25	25	26	27	29	24	25
H_e	0.947	0.936	0.958	0.957	0.926	0.943	0.943	0.952	0.943
H_o	0.976	0.818	0.970	0.872	0.870	0.955	0.915	0.758	0.920
F_{IS}	−0.031	0.128*	−0.012	0.089*	0.061	−0.012	0.029	0.206*	0.025
OtsG422									
n	42	14	25	36	58	42	54	25	31
A	32	19	27	31	31	38	33	22	27
H_e	0.966	0.968	0.967	0.965	0.953	0.978	0.962	0.960	0.960
H_o	0.905	0.929	0.960	0.917	0.948	0.857	0.926	0.920	0.935
F_{IS}	0.065*	0.042	0.008	0.051	0.005	0.125*	0.038	0.042	0.026
OtsG432									
n	47	38	35	49	59	48	57	39	54
A	20	17	15	21	19	20	21	20	23
H_e	0.889	0.887	0.871	0.877	0.845	0.890	0.885	0.895	0.860
H_o	0.957	0.816	0.857	0.776	0.797	0.896	0.842	0.872	0.870
F_{IS}	−0.078	0.081	0.016	0.116	0.058	−0.007	−0.049	0.026	0.012
OtsG474									
n	45	25	34	42	57	46	58	34	44
A	11	13	10	10	12	9	12	9	11
H_e	0.779	0.880	0.777	0.758	0.792	0.868	0.826	0.826	0.789
H_o	0.911	0.720	0.647	0.714	0.825	0.826	0.879	0.765	0.773
F_{IS}	−0.172	0.181*	0.170	0.058	−0.042	−0.030	−0.065	0.075	0.021
Mean									
n	43	27	32	43	56	44	56	34	45
A	24.1	19.7	21.2	23.8	25.3	26.1	26	22.6	24.4
H_e	0.919	0.932	0.92	0.915	0.914	0.936	0.925	0.929	0.914
H_o	0.931	0.852	0.893	0.836	0.891	0.896	0.913	0.836	0.89
F_{IS}	−0.013	0.088*	0.029	0.087*	0.025	0.034*	0.013	0.068*	0.027
HWE	0.040	0.010	0.011	0.001*	0.210	0.004*	0.003*	0.001*	0.081

TABLE A.1.—Extended.

	San Joaquin River drainage							
	Merced River			Merced River Hatchery			Mokelumne River	Mokelumne River Hatchery
Locus	1999	2000	2001	1999	2000	2001	1999	1999
OtsG83b								
n	22	60	81	52	47	85	28	43
A	20	34	34	28	25	33	22	28
H_e	0.955	0.960	0.952	0.958	0.949	0.958	0.951	0.956
H_o	0.955	0.950	0.889	0.904	0.894	0.929	0.964	0.977
F_{IS}	0.000	0.010	0.067*	0.057	0.059	0.030	−0.015	−0.022
OtsG249								
n	23	60	80	45	47	87	37	44
A	20	34	36	31	32	32	25	28
H_e	0.946	0.965	0.965	0.966	0.964	0.966	0.958	0.959
H_o	0.870	0.967	0.950	0.933	0.915	0.977	0.865	0.864
F_{IS}	0.082	−0.002	0.015	0.034	0.052	−0.011	0.099*	0.100*
OtsG311								
n	29	50	80	66	47	76	39	44
A	24	26	30	30	26	31	23	27
H_e	0.944	0.950	0.955	0.955	0.951	0.954	0.946	0.956
H_o	0.966	0.940	0.913	0.939	0.979	0.934	0.949	0.932
F_{IS}	−0.023	0.011	0.045	0.016	−0.029	0.020	−0.002	0.026
OtsG409								
n	27	56	83	65	47	80	40	45
A	24	27	30	29	25	26	23	26
H_e	0.941	0.931	0.957	0.955	0.958	0.953	0.951	0.955
H_o	0.852	0.875	0.904	0.923	0.894	0.925	1.000	0.978
F_{IS}	0.097	0.061	0.056	0.033	0.068	0.029	−0.053	−0.024
OtsG422								
n	9	35	71	53	46	67	25	42
A	13	28	36	34	34	36	25	32
H_e	0.961	0.964	0.964	0.967	0.968	0.960	0.958	0.965
H_o	0.889	0.857	0.944	0.962	0.913	0.955	0.960	1.000
F_{IS}	0.079	0.112*	0.021	0.005	0.057	0.005	−0.003	−0.037
OtsG432								
n	39	58	85	64	47	77	43	45
A	20	19	23	18	20	25	20	18
H_e	0.906	0.875	0.870	0.872	0.863	0.869	0.888	0.890
H_o	0.923	0.793	0.871	0.766	0.872	0.792	0.884	0.889
F_{IS}	−0.019	0.094	−0.015	0.123	−0.011	0.089*	0.005	0.001
OtsG474								
n	34	65	90	66	47	81	43	46
A	12	13	13	13	11	10	12	8
H_e	0.834	0.805	0.745	0.797	0.796	0.788	0.784	0.789
H_o	0.794	0.769	0.811	0.788	0.766	0.802	0.651	0.804
F_{IS}	0.049	0.042	−0.089	0.011	0.039	−0.018	0.171*	−0.020
Mean, all Loci								
n	26	55	77	59	47	79	36	44
A	19	25.7	28.9	26.3	24.7	27.6	21.4	23.8
H_e	0.927	0.924	0.921	0.921	0.915	0.921	0.919	0.924
H_o	0.893	0.888	0.879	0.890	0.897	0.902	0.896	0.92
F_{IS}	0.038*	0.046*	0.018	0.039*	0.034	0.021	0.026	0.004
HWE	0.002*	0.014	0.005*	0.004*	0.033	0.008	0.015	0.002*

TABLE A.1.—Extended.

	San Joaquin River drainage							
	Stanislaus River			Tuolumne River				
Locus	1999	2000	2001	1999	2000	2001	Mean	Total observed
OtsG83b								
n	21	55	69	31	63	83		1,101
A	18	27	27	23	34	30	28	53
H_e	0.941	0.943	0.942	0.951	0.959	0.953	0.955	
H_o	0.857	0.982	0.913	0.839	0.952	0.940	0.925	
F_{IS}	0.091	−0.042	0.031	0.118*	0.007	0.014		
OtsG249								
n	23	58	60	32	67	73		1,132
A	19	29	36	23	34	33	29	48
H_e	0.946	0.962	0.955	0.942	0.966	0.965	0.959	
H_o	0.870	0.914	0.900	0.938	0.985	0.973	0.924	
F_{IS}	0.082	0.050	0.058	0.005	−0.020	−0.008		
OtsG311								
n	32	42	65	49	73	79		1,161
A	20	27	27	28	31	30	27	52
H_e	0.938	0.955	0.949	0.908	0.951	0.962	0.946	
H_o	0.938	0.929	0.892	0.857	0.932	0.924	0.914	
F_{IS}	0.001	0.028	0.060	0.057	0.021	0.040		
OtsG409								
n	46	53	71	50	71	81		1,209
A	24	27	27	29	35	32	27	57
H_e	0.944	0.957	0.947	0.955	0.954	0.961	0.949	
H_o	0.891	0.925	0.930	0.920	0.775	0.951	0.904	
F_{IS}	0.057	0.032	0.019	0.037	0.188*	0.011		
OtsG422								
n	19	48	66	20	50	70		948
A	21	31	39	26	33	34	28	57
H_e	0.960	0.967	0.970	0.974	0.967	0.963	0.965	
H_o	0.895	0.979	0.894	0.950	0.920	0.929	0.928	
F_{IS}	0.070	−0.031	0.094*	0.026	0.049	0.036		
OtsG432								
n	51	64	80	52	80	90		1,301
A	17	21	18	16	21	19	19	27
H_e	0.840	0.862	0.878	0.870	0.862	0.868	0.875	
H_o	0.804	0.906	0.838	0.846	0.850	0.867	0.851	
F_{IS}	0.044	−0.051	0.046	0.028	0.015	0.001		
OtsG474								
n	46	64	71	51	79	84		1,252
A	12	12	10	11	12	11	11	17
H_e	0.784	0.787	0.754	0.753	0.802	0.782	0.795	
H_o	0.609	0.766	0.634	0.627	0.797	0.774	0.759	
F_{IS}	0.226*	0.027	0.160*	0.169*	0.006	0.011		
Mean, all Loci								
n	34	56	69	41	69	80		
A	18.7	24.8	26.3	22.3	28.4	27		
H_e	0.908	0.919	0.914	0.908	0.923	0.922		
H_o	0.838	0.914	0.857	0.854	0.887	0.908		
F_{IS}	0.078*	0.005	0.064*	0.060*	0.039*	0.015		
HWE	0.001*	0.001*	0.041	0.005*	0.001*	0.026		