The Evolutionarily Significant Unit Concept and the Role of Translocated Populations in Preserving the Genetic Legacy of Lahontan Cutthroat Trout

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Abstract.—The Lahontan cutthroat trout Oncorhynchus clarkii henshawi, a listed subspecies of cutthroat trout O. clarkii, has been extirpated from over 90% of its historic waters. The 1995 U.S. Fish and Wildlife Service Recovery Plan identified three evolutionarily significant units, or distinct population segments (DPSs), of Lahontan cutthroat trout based upon morphological, genetic, and ecological data. Teams composed of federal and state agency and university biologists were organized to formulate recovery strategies that were specific to each DPS. In this context, fish native to each DPS are considered exclusively for recovery activities. Lahontan cutthroat trout populations in the Willow–Whitehorse drainage of the Coyote Lake basin, Oregon, were initially identified as part of the Northwestern DPS, but recent genetic analyses suggest that these populations may warrant a fourth DPS designation. The Willow–Whitehorse Lahontan cutthroat trout are found in seven within-basin streams and have been translocated into multiple out-of-basin streams. Genetic analysis of seven populations translocated into out-of-basin streams of the Steens Mountain ( Alvord Lake basin), Oregon, in the 1970s and 1980s support inclusion of these streams in any DPS-based recovery strategy for the Willow–Whitehorse Lahontan cutthroat trout. Overall gene diversity per locus was similar among translocated and source populations ( Steens Mountain, 0.096–0.908; Willow–Whitehorse, 0.039–0.903). However, 135 of 300 alleles identified at 20 microsatellite loci were found in the Steens Mountain populations but not in the contemporary Willow–Whitehorse populations. The Steens Mountain populations are found in small habitats subject to the effects of genetic drift. Lahontan cutthroat trout from these out-of-basin streams should be included in conservation plans.

Translocation of individuals from populations of threatened and endangered species into habitats that have experienced local extirpations, were previously unoccupied by these species, or where translocation was meant to augment in situ populations has been widely used as a conservation strategy ( Stockwell et al. 1996; Harig and Fausch 2002; Stockwell and Leberg 2002; Minckley et al. 2003; Bouzat et al. 2009). Dating back to the debate over the role of zoos in conservation ( Ralls and Ballou 1986; Frankham and Loebel 1992; Stockwell and Leberg 2002; Minckley et al. 2003; Bouzat et al. 2009), moving individuals from degraded or shrinking habitat into “refugia” has been viewed as a risk-spreading strategy for ameliorating the chances of species extinction. The Devil’s Hole pupfish Cyprinodon diabolis ( Wilcox and Martin 2006), California condor Gymnogyps californianus ( Pimm 2000; Ralls et al. 2000), and black-footed ferret Mustela nigripes ( Miller et al. 1988; May 1989) are well-known examples of species for which this strategy has been used. However, captive and translocated populations in the wild are typically founded with small numbers of individuals that may not be representative of the source population. In addition, small refugia populations can quickly lose genetic variation through drift and inbreeding, leading to fitness declines and loss of evolutionary potential ( Hedrick 1994; Saccheri et al. 1998; Frankham 2005; Pimm et al. 2006). Wilcox and Martin ( 2006) have also

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pointed out that subtle differences in habitat may lead to rapid adaptive divergence. Therefore, the conservation value of such a risk-spreading strategy may be of limited utility for ensuring maintenance of genetic variation and long-term population and species persistence unless large effective population sizes are maintained (Fiumera et al. 2000; Harig and Fausch 2002).

During the late 19th through mid-20th centuries, trout species were translocated into waters all over the globe to create angling opportunities. This is especially true for rainbow trout Oncorhynchus mykiss, brook trout Salvelinus fontinalis, and brown trout Salmo trutta. In addition to the more widespread planting of nonnative salmonids, many of the native subspecies of cutthroat trout O. clarkii were also translocated into occupied and fishless waters within their native range as well as into out-of-basin habitats. Here, we address the conservation value of out-of-basin translocated populations of Lahontan cutthroat trout O. clarkii henshawi, a federally listed subspecies of cutthroat trout (U.S. Endangered Species Act), as potential donor populations for recovery activities under the evolutionarily significant unit or distinct population segment (DPS) approach (Federal Register notice [61FR4722, February 7, 1996], final listing determination for Klamath River–Columbia River DPS of bull trout Salvelinus confluentus). This approach uses morphological, genetic, and ecological data to define groups of evolutionarily distinct populations that are then treated as separate conservation units (Moritz 1994; Walensky 1995).

The Lahontan cutthroat trout is endemic to the hydrographic Lahontan Basin of northeastern California, southeastern Oregon, and northern Nevada (Figure 1) and is one of approximately 14 allopatrically distributed subspecies of cutthroat trout (Behnke 1992). This subspecies evolved in pluvial Lake Lahontan and associated satellite basins in the north-central Great Basin province of western North America (Behnke 1992). The high stand of Lake Lahontan occurred about 14,000 years ago, when the lake itself covered approximately 22,100 km² in a drainage basin of about 117,000 km² (La Rivers 1962; Thompson et al. 1986). After its high stand, Lake Lahontan rapidly desiccated to near present day levels about 8,000 years ago (Benson and Thompson 1987). Pyramid and Walker lakes in western Nevada are remnants of the larger pluvial lake (see Figure 1). Alpine Lake Tahoe in the western Sierra Nevada mountain range and the large river systems in eastern Nevada (the Humboldt River and its tributaries) were never inundated by the pluvial lake. However, the Quinn River in northwestern Nevada was inundated. Before European settlement of California, Nevada, and Oregon, Lahontan cutthroat trout had access to multiple-order stream systems and large lake habitats within the Lahontan Basin.

Currently, this subspecies is found in less than 10% of its historic range and predominantly in headwater reaches of isolated streams (Coffin and Cowan 1995; Dunham et al. 1997). Genetic analyses for populations found in the few remaining interconnected stream systems suggest Lahontan cutthroat trout were affected by both landscape and metapopulation processes at multiple spatial scales, leading to complex population genetic structure within watersheds (Neville et al. 2006a, 2006b).

In the 1995 U.S. Fish and Wildlife Service Recovery Plan for Lahontan cutthroat trout (Coffin and Cowan 1995), multiple DPSs were identified: the Northwestern DPS (Quinn River drainage, Coyote Lake and Summit Lake basins), Eastern DPS (Humboldt and Reese River drainages), and Western DPS (Truckee, Carson, and Walker River drainages). Only Lahontan cutthroat trout native to each DPS are considered for recovery activities in that segment. Results of recent genetic analyses suggest that the Coyote Lake basin (Willow–Whitehorse [WWH] watershed) in southern Oregon should be considered as a separate DPS (Peacock and Kirchoff 2007). Lahontan cutthroat trout occur in seven streams in the Coyote Lake basin: Willow, Whitehorse, Little Whitehorse, Doolittle, Cottonwood, Sheep Canyon, and Fifteenmile creeks (Figure 2). Jones et al. (1998) estimated that Lahontan cutthroat trout are currently limited to 56 km of the 110 km of stream habitat in the Willow–Whitehorse streams. Barriers to movement (i.e., physical, thermal, and dry creek bed) have isolated stream reaches that were formerly interconnected within both Willow and Whitehorse creeks (Jones et al. 1998).

Specifically, we assessed (1) the degree of genetic similarity among translocated Lahontan cutthroat trout populations and their source populations, (2) levels of genetic variation within translocated populations compared with their source populations, and (3) the potential for interconnectedness among streams containing translocated populations. The third aim is of particular importance as many translocated Lahontan cutthroat trout populations are found in small, isolated streams with only a few kilometers of suitable habitat (Coffin and Cowan 1995; Dunham et al. 1997; Elliott et al. 1997; Sevon et al. 1999; Peacock and Kirchoff 2004). Because it is well appreciated that small, isolated populations are at risk of losing genetic variation through genetic drift and are vulnerable to local extirpation via demographic and environmental stochasticity (Lande 1993), such populations may be of
limited value as repositories of evolutionarily significant genetic variation. However, for translocated populations found in larger and more diverse habitats there is a greater probability of maintaining significant levels of genetic variation and gene flow among spatially interconnected subpopulations. For DPSs with few extant populations, out-of-basin populations found in interconnected stream systems could represent an important aspect of the recovery of salmonid species, both in situ and as donor populations.

FIGURE 1.—Map of the Lahontan hydrographic basin, the historical distribution of Alvord cutthroat trout (Alvord Lake subbasin only) and Lahontan cutthroat trout. Major river drainages and lakes that comprise historical habitat for Lahontan cutthroat trout are labeled. Trout Creek in the Alvord Lake subbasin contains an Alvord cutthroat trout–rainbow trout hybrid population. (Map prepared by Robert E. Elston Jr., Department of Biology, University of Nevada, Reno.)
Methods

Study populations.—Seven populations created with fish translocated from the Willow–Whitehorse watersheds into out-of-basin habitat in the Alvord Lake basin (Steens Mountain) in eastern Oregon were analyzed in this study (Figure 2). There are no remaining pure populations of the subspecies native to this basin, the Alvord cutthroat trout _O. clarkii alvordensis_ (Behnke 1992). The Alvord cutthroat trout was declared extinct in 1984 (Behnke 1992). Two drainages, Trout Creek (see Figure 2) and Virgin Creek (not shown), however, have populations of rainbow trout–Alvord cutthroat trout hybrids.

The translocated populations in the Alvord Lake basin were established in fishless waters in the 1970s and 1980s with small numbers of founders from the Willow–Whitehorse drainage in Coyote Lake basin (Steens Mountain) in eastern Oregon were analyzed in this study (Figure 2). There are no remaining pure populations of the subspecies native to this basin, the Alvord cutthroat trout _O. clarkii alvordensis_ (Behnke 1992). The Alvord cutthroat trout was declared extinct in 1984 (Behnke 1992). Two drainages, Trout Creek (see Figure 2) and Virgin Creek (not shown), however, have populations of rainbow trout–Alvord cutthroat trout hybrids.

The translocated populations in the Alvord Lake basin were established in fishless waters in the 1970s and 1980s with small numbers of founders from the Willow–Whitehorse drainage in Coyote Lake basin (7–31 fish/stream; Table 1; Hanson et al. 1993). Habitat is limited to less than 3 km in most of these streams. Of these creeks, only Mosquito and Willow creeks and their tributaries are physically connected, but the waterways are only interconnected seasonally. There is no interconnection potential among the remaining five streams, which become subsurface and disappear into the alluvial fan at lower elevations. We compared the levels of genetic variation and extent of genetic differentiation among the out-of-basin populations with levels in four of the extant populations in the Willow–Whitehorse drainage (WWH-Willow, Little Whitehorse, WWH-Cottonwood, and Whitehorse creeks; hereafter, “WWH” is added to distinguish Willow and Cottonwood creeks in this drainage from the Willow and Cottonwood creeks of the Alvord Lake basin) using a suite of microsatellite genetic markers developed specifically for Lahontan cutthroat trout (Peacock et al. 2004; Robinson et al. 2008).

Study fish.—Fin clips were collected from adult fish sampled along the entire occupied reaches of streams from the Steens Mountain populations as part of an ongoing Oregon Department of Fish and Wildlife (ODFW) monitoring program using backpacking electrofishing. Fin clips were collected from 12 to 18
adult individuals per population (n = 116) and stored in coin envelopes in the field to facilitate complete drying of tissue. Jennifer Nielsen (U.S. Geological Survey, Alaska Science Center, Fisheries Research) provided dried fin clips (n = 96) collected in 1996 by ODFW biologists from populations in Little Whitehorse, Upper Whitehorse, WWH-Cottonwood, and WWH-Willow creeks in the Willow–Whitehorse drainage. Sampling locations in the Willow–Whitehorse drainage are those shown in Jones et al. (1998); samples from Whitehorse Creek included here were collected from sites above the confluence with Little Whitehorse Creek.

**Molecular markers.**—We used 20 microsatellite loci (OCH5, OCH6, OCH9, OCH13, OCH15–OCH18, OCH20–OCH22, OCH24, OCH27–OCH32, OCH34, and OCH35) that were developed from enriched genomic libraries created by Genetic Identification Services (available at: genetic-id-services.com/) for Lahontan cutthroat trout (Peacock et al. 2004, Robinson et al. 2008). These microsatellites markers have been used to characterize genetic variation in over 50 Lahontan cutthroat trout populations (Neville et al. 2006a, Peacock and Kirchoff 2007). Micro-checker (version 2.2.3, van Oosterhout et al. 2004) was used to test for null alleles and allelic dropout per locus per population.

**Extraction of DNA, polymerase chain reactions, and genotyping.**—The DNA was extracted from fin clips using Qiagen DNeasy tissue extraction kits and quantified per individual using a Labsystems Fluoroskan Ascent fluorometer. All polymerase chain reactions (PCRs) were carried out on a MBS Satellite 0.2G thermal cycler in 16-μL volumes. We used both multiplex (3–5 microsatellite primer pairs) and individual PCRs. The PCR conditions for loci OCH5–OCH17 can be found in Peacock et al. (2004), and conditions for OCH18–OCH35 are described in Robinson et al. (2008). The PCR product was diluted in deionized water to an appropriate intensity determined by dilution tests, and 1 μL was added to 19 μL of GeneScan 500 LIZ size standard with Hi-Dye Formamide (Applied Biosystems, Inc. [ABI], Perkin-Elmer Corporation). Fragment analysis was carried out on an ABI Prism 3730 DNA analyzer. Alleles were scored using ABI Prism GeneScan (version 3.5.1) and GeneMapper (version 3.0) software (ABI).

**Statistical analyses.**—We used FSTAT (version 2.9.3.2, Goudet 2002) to (1) calculate an average gene diversity (H) per locus per watershed, (2) test for deviations from Hardy–Weinberg equilibrium (HWE) per population (inbreeding coefficient FIS), and (3) assess levels of genetic differentiation among all study populations (pairwise FST). Because Mosquito and Willow creeks are connected seasonally and therefore may exchange immigrants, we also tested for departures from HWE with fish from these streams combined into a single gene pool.

We also used a Bayesian genotype clustering approach (STRUCTURE version 2.0; Pritchard et al. 2000) to assess contemporary genetic population structure both within and across the out-of-basin and source populations. The grouping criteria in STRUCTURE include HWE and gametic phase equilibrium between loci within groups. We used an admixture model wherein individuals with novel genotypes can be identified and assigned, and we specified a range of
1–13 potential genotype clusters \((k)\). We specified a 100,000 iteration burn-in period followed by ten 100,000 Markov chain Monte Carlo (MCMC) replicates per \(k\) to approximate posterior allelic distributions against which individual genotypes were compared and assigned to a cluster (Pritchard et al. 2000). To determine the optimal \(k\), we used the \(\Delta k\) method of Evanno et al. (2005). This method calculates the largest change in the natural logarithm of the probability of the data \((\log P(D))\) between each pair of \(k\) and \(k - 1\) for all tests of \(k\). As the \(\log P(D)\) can plateau or continue to rise slightly even after the “true” \(k\) is reached (see Pritchard and Wen 2003), Evanno et al. (2005) demonstrated through simulation modeling that \(\Delta k\) (defined as the second-order rate of change of the likelihood function with respect to \(k\)) shows a clear peak at the true value of \(k\).

To verify that the study populations originated from the Willow–Whitehorse drainage, we (1) constructed a river-based phylogenetic tree and (2) conducted a Bayesian clustering analysis with population data collected from throughout the range of Lahontan cutthroat trout. For these two analyses, we used only the \(OCH5–OCH17\) loci because the data set for Lahontan cutthroat trout populations sampled throughout the range (Table 2; Peacock et al. 2004; Peacock and Kirchoff 2007) was generated before the development of the \(OCH18–OCH35\) loci. We included individuals sampled from all major watersheds with extant populations. We collapsed tributary populations into a single river designation for all major drainages with Lahontan cutthroat trout populations for which we had sufficient data (Table 2; Peacock and Kirchoff 2007). Our justification for this is based upon earlier research in which stream-based, population-level phylogenetic trees had very low bootstrap support (Peacock and Kirchoff 2007), which is probably the result of the inherent metapopulation–network dynamic that has been described for Lahontan cutthroat trout populations in intact stream networks (Neville et al. 2006a, 2006b; Umek 2007). When stream populations were combined into a watershed analysis, the bootstrap values improved dramatically and the tree topologies were consistent with geographical proximity (Peacock and Kirchoff 2007). Based upon these results, we combined the collapsed data for the Steens Mountain populations and those for the Willow–Whitehorse populations into single-watershed designations for inclusion in the phylogenetic analysis to verify origin of the translocated fish. Although the contemporary Pyramid Lake population is in the Western DPS, the native Lahontan cutthroat trout population was extirpated in the 1940s. Today, this fishery is hatchery-maintained with a broodstock founded in part with fish from the Summit Lake and Carson River populations. Therefore, we expected the Pyramid Lake, Summit Lake, and Carson River populations to be more similar to one another than to populations from other portions of the range. The phylogeny was constructed using Cavalli-Sforza genetic distance and the neighbor joining tree-building algorithm in POPULATIONS version 1.2.26 (www.cnrs-gif.fr/pge). Trees were visualized using the program TREESVIEW version 1.6 (available at: taxonomy.zoology.gla.ac.uk/rod/rod.html). All Lahontan cutthroat trout samples used in this study were genotyped on either an ABI 3700 or ABI 3730 analyzer. Control individuals were run on both sequencers to standardize allele sizes for all samples.

The Bayesian clustering analysis was conducted using the same river designations as in the phylogenetic analysis. We used an admixture model and specified a potential \(k\) range of 1–10. We specified a 100,000-iteration burn-in period followed by ten 100,000 MCMC replicates per \(k\). The \(\Delta k\) method of Evanno et al. (2005) was used to determine the optimal \(k\).

To assess the genetic effects of small founder size and bottlenecks, we used the two-phase mutation (TPM) model (Di Rienzo et al. 1994) in the computer program BOTTLENECK (Cornuet and Luikart 1996). BOTTLENECK uses heterozygosity excess criteria to determine reductions in population size. The specified
variance for TPM and specified proportion of single step mutation model in TPM were as recommended by Di Rienzo et al. (1998).

**Results**

**Molecular Markers**

There was no evidence of null alleles or allelic dropout in any of the Willow–Whitehorse populations or in the Mosquito Creek population. Null alleles were possible for multiple loci in the remaining Steens Mountain populations, but there were no systematic patterns; no locus was out of HWE in all populations, and no population was out of HWE at all loci.

**Genetic Variation**

Two-hundred eight individuals were genotyped for this study (Table 2). Four individuals were dropped from the analysis due to degraded DNA or incomplete genotyping across all loci. Three-hundred alleles were identified exclusively in the Steens Mountain populations and 40 were found only in the Willow–Whitehorse populations (Table 3). All major river systems with Lahontan cutthroat trout populations that were included in the rangewide analysis at the OCH5–OCH17 loci had rare or unique alleles at some loci (133 alleles; number of rare and unique alleles: mean ± SD, 5.5 ± 6.5 per river system; range, 0 [Pyramid Lake] to 18 [Humboldt River]). On a rangewide basis, three alleles were unique to Willow–Whitehorse and seven were unique to the Steens Mountain populations. Sample size was a good predictor of the number of rare and unique alleles found in each river system ($r = 0.952$, $P < 0.000$). Average $H_t$ among the Steens Mountain (0.096–0.908) and Willow–Whitehorse (0.039–0.903) populations was comparable (Table 3) and similar to $H_t$ at OCH5–OCH17 for populations genotyped from across the Eastern and Western DPSs (Peacock et al. 2004; Robinson et al. 2008). Multiple Steens Mountain populations (i.e., Cottonwood, Big Alvord, Little Alvord, and Pike creeks) were out of HWE at one or two loci and Little McCoy Creek had significant $F_{IS}$ values at three loci, but no population was out of HWE at all loci and no single locus had significant $F_{IS}$ values across all populations ($P = 0.0003$, corrected for multiple comparisons). When Mosquito and Willow creeks were combined into a single gene pool, no locus deviated from HWE ($P > 0.00035$, corrected for multiple comparisons).

The Willow, Mosquito, and Cottonwood Creek populations of Steens Mountain showed evidence of recent genetic bottleneck or founder effects under the TPM model ($P ≤ 0.02$). However, when combined into a single gene pool, there was no evidence of genetic bottlenecks for Willow and Mosquito creeks under the TPM model ($P = 0.82$). The WWH-Cottonwood Creek population was the only population among the Willow–Whitehorse streams that was significantly bottlenecked under the TPM model ($P = 0.006$).

**Table 3.**—Overall gene diversity ($H_t$), total number of alleles sampled ($n$), number of alleles sampled in each basin, and unique alleles per locus for the Steens Mountain (Steens) and Willow–Whitehorse (WWH) Lahontan cutthroat trout populations.

<table>
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<tr>
<th>Locus</th>
<th>Steens</th>
<th>WWH</th>
<th>$n$ alleles</th>
<th>Steens</th>
<th>WWH</th>
<th>Unique alleles</th>
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<td>OCH33</td>
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<td>0.078</td>
<td>4</td>
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<td>Overall</td>
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<td>0.662</td>
<td>300</td>
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The Steens Mountain populations formed a clade with the Willow–Whitehorse populations in the river-based phylogenetic analysis, with 100% bootstrap support (Figure 3). Four genotype clusters were identified in the river-based Bayesian clustering analysis \( \log_2 P[D] \): mean ± SD, \(-18,086.7 ± 3.16; \Delta k = 173.65\); Figure 4a). Genotype clusters coincided with watershed classifications as predicted, with the Steens Mountain and Willow–Whitehorse populations forming a cluster distinct from the other watersheds (Figure 4b).

**Origin of Translocated Populations**

The Steens Mountain populations formed a clade with the Willow–Whitehorse populations in the river-based phylogenetic analysis, with 100% bootstrap support (Figure 3). Four genotype clusters were identified in the river-based Bayesian clustering analysis \( \log_2 P[D] \): mean ± SD, \(-18,086.7 ± 3.16; \Delta k = 173.65\); Figure 4a). Genotype clusters coincided with watershed classifications as predicted, with the Steens Mountain and Willow–Whitehorse populations forming a cluster distinct from the other watersheds (Figure 4b).

**Genetic Population Structure**

**F-statistics.**—The Whitehorse Creek population was not significantly differentiated from populations found in the same drainage, Little Whitehorse and WWH-Cottonwood creeks (Table 4). The WWH-Willow Creek population was differentiated from both Whitehorse and Little Whitehorse creeks but not from the WWH-Cottonwood Creek population. This latter result, however, is consistent with ODFW translocation records showing that fish from both Whitehorse (\( n = 40 \)) and WWH-Willow (\( n = 31 \)) creeks were planted into WWH-Cottonwood Creek in 1971 and 1980, respectively.

**Figure 3.**—Watershed-level phylogenetic tree for extant Lahontan cutthroat trout populations throughout their range, showing that Willow–Whitehorse Lahontan cutthroat trout are the source of the Steens Mountain Lahontan cutthroat trout populations (100% bootstrap support; tree constructed using Cavalli-Sforza genetic distance and neighbor joining tree-building algorithm).

**Figure 4.**—(A) Mean (±SD) natural logarithm of the probability of the data \( \log_2 P[D] \) for each number of potential genotype clusters \( k = 1−7 \); upper panel), and \( \Delta k \) showing a \( k \)-value of 4 as providing the best fit to the data (lower panel) for the rangewide river-based analysis of Lahontan cutthroat trout. (B) Bayesian genotype clustering analysis, showing four distinct genotype clusters among sampled Lahontan cutthroat trout populations. Each river designation is represented in a single genotype cluster on the x-axis, with average proportional membership per genotype cluster on the y-axis (population abbreviations are listed in Table 2). The Willow–Whitehorse (WWH) and Steens Mountain populations form a single distinct genotype cluster, suggesting a fourth distinct population segment (DPS).
Among the Steens Mountain populations, Mosquito, Willow, and Cottonwood creeks were significantly differentiated from each other and most of the other populations (Table 4). All populations except that in Little McCoy Creek were significantly differentiated from Whitehorse, Little Whitehorse, and WWH-Willow Creek populations. However, Little McCoy, Mosquito, Cottonwood, and Pike Creek populations were not differentiated from the WWH-Cottonwood Creek population, which is consistent with the ODFW translocation records. The pattern of genetic differentiation among the Steens Mountain populations did not differ when Mosquito and Willow Creek populations were combined into a single gene pool ($P = 0.001$).

**Bayesian clustering analysis.**—The data for Willow–Whitehorse and Steens Mountain populations were best described by three distinct genotype clusters (log$_P[D]$: mean ± SD, $-10.933.7 ± 2.2$; $Δk = 301.4$; Figure 5a). The Willow–Whitehorse populations formed primarily two distinct genotype clusters, with some membership in a third, smaller cluster (Figure 5b). The Whitehorse and Little Whitehorse creeks are interconnected, and individuals from these populations assigned to one major genotype cluster, whereas populations in WWH-Willow and WWH-Cottonwood creeks, which are disjunct streams, assigned to the other major cluster. Again, this result is consistent with the ODFW translocation records for this drainage. Of the Steens Mountain populations, Little McCoy and Mosquito creeks had greater than 90% proportional membership in genotype clusters that were found in their source populations. The Willow Creek population had proportional membership in two clusters, one of which was the Mosquito Creek genotype cluster and the other was the primary genotype cluster found in its source population, WWH-Willow Creek. The individuals in the remaining Steens Mountain populations assigned primarily to two of the three genotype clusters, reflecting the genotype clusters found in their source populations.

**Discussion**

The phylogenetic and Bayesian genotype clustering analyses support a Willow–Whitehorse origin for the Steens Mountain Lahontan cutthroat trout populations in concordance with the translocation records. In addition, individuals within the Steens Mountain populations assigned to the primary genotype clusters found in their known source population with two exceptions. First, the Little McCoy Creek population was founded with individuals from WWH-Willow Creek, but all individuals assigned to the small, third genotype cluster that had membership in the Little Whitehorse, WWH-Willow, and WWH-Cottonwood Creek populations but in small numbers. This result suggests the founders for Little McCoy Creek were a nonrandom sample of the source population. Although there was no evidence of a genetic bottleneck, the Little McCoy Creek population did have significant $F_{IS}$ values at three loci, and ODFW sampling crews observed physical abnormalities (e.g., short opercula) in some fish sampled from this population, suggesting the possibility of a small effective population size. Second, all individuals in the Steens Mountain Willow Creek population, which was founded with fish from the WWH-Willow Creek population, have proportional membership in both of the primary genotype clusters, one found in Whitehorse Creek and the other in WWH-Willow Creek. The genotype clustering membership in Willow Creek supports ongoing gene flow between Mosquito and Willow creeks during periods of connectivity as these populations were founded a decade apart and with fish from two separate, spatially disjunct streams in the Willow–Whitehorse drainage.

However, $F$-statistics show that the Steens Mountain populations are overall differentiated from each other

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### Table 4.—Pairwise genetic differentiation index ($F_{ST}$) estimates among stream populations of Lahontan cutthroat trout (abbreviations defined in Table 1). The $F_{ST}$ values in bold are significantly differentiated ($P < 0.009$, adjusted for multiple comparisons obtained after 1,100 permutations).

<table>
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<tr>
<th>Population</th>
<th>LM</th>
<th>MC</th>
<th>WC</th>
<th>CW</th>
<th>BA</th>
<th>LA</th>
<th>PC</th>
<th>LWH</th>
<th>WH</th>
<th>WWH-Cottonwood</th>
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and from their source populations. This result is undoubtedly due to the small number of founders for these populations. However, high levels of heterozygosity and allelic diversity, similar to other extant Lahontan cutthroat trout populations observed at most of the loci genotyped for this study, suggest that founder groups were genetically diverse even if they were not fully representative of the source populations. When Mosquito and Willow creeks were treated as a single population, Cottonwood Creek was the only Steens Mountain population that showed evidence of a genetic bottleneck.

In addition, a full 45% of the alleles identified in the Willow–Whitehorse and Steens Mountain populations were found in the Steens Mountain populations exclusively. Ten of 133 alleles genotyped for the six loci used in the rangewide analysis were found only in the Oregon Lahontan cutthroat trout populations, and seven of these alleles were observed only in the Steens Mountain populations. Although the number of rare or unique alleles observed in each watershed was highly correlated with sample size, we would not expect to see big differences in allelic representation between the Steens Mountain populations and their source populations in the Willow–Whitehorse drainage unless (1) population structure was evident in the source populations and (2) founders were a nonrandom sample of the source populations. The Bayesian clustering analysis reveals three distinct genotype clusters among the source populations, with a single genotype predominating in each stream. The same three genotype clusters are represented in the translocated populations but not in the same proportions. The small, third genotype cluster is overrepresented in the Steens Mountain populations, and close examination shows that many of the rare alleles are from individuals that assign to this cluster. These results suggest that proportional membership in the third genotype cluster has changed in the source populations since fish were collected for translocation. Population structure predominates in the few remaining interconnected stream systems in the Humboldt River drainage that contain Lahontan cutthroat trout (Neville et al. 2006a; Peacock and Kirchoff 2007; Umek 2007). In such systems, the observed patterns support a complex population dynamic, including aspects of metapopulation processes and variable age-class and life history strategies (Neville et al. 2006a, 2006b; Peacock and Kirchoff 2007; Umek 2007). Although Lahontan cutthroat trout were sampled from throughout the occupied reaches in the Willow–Whitehorse streams at the time of sampling, spatial and temporal barriers had isolated some trout into smaller reaches within these creeks (Jones et al. 1998). Inherent in a metapopulation
dynamic is the probability of recolonization after a local extirpation event. If barriers preclude movement among subpopulations, recolonization potential is lost together with any rare or unique genetic variation found in local subpopulations.

The Steens Mountain populations were formed as a risk-spreading conservation action to preserve Willow–Whitehorse Lahontan cutthroat trout. Although the Willow–Whitehorse populations were not at risk of immediate extirpation, habitat restoration began in the late 1980s by the Trout Creek Mountain Working Group, which consisted of local ranchers, environmental groups, and both federal and state land and species management agencies to secure, expand, and improve habitat. The Willow–Whitehorse populations are robust in terms of genetic variation (Peacock and Kirchoff 2007), population size, and habitat quality in comparison with populations in other DPSs. However, the results presented here suggest the Steens Mountain populations contribute significantly to overall genetic diversity of the Willow–Whitehorse gene pool and maintain genetic variation that may no longer be present in the contemporary Willow–Whitehorse populations.

Here, we make a number of arguments regarding these out-of-basin populations in terms of overall recovery goals for this subspecies. First, these populations may represent an important part of this DPS in terms of evolutionarily potential and as such should be included in formulating a recovery strategy for the Willow–Whitehorse drainage. Second, efforts should be made to ensure stream-level connectedness where possible, such as among Willow and Mosquito creeks and their tributaries, with habitat restoration including mitigation of water-diversion structures to maximize population size and maintain extant genetic variation. Third, where it is not possible to establish migration corridors among streams, management practices should improve in situ habitat where necessary and expand the length of occupiable stream habitat where possible. However, for most of these streams the upper limits of these populations are defined by waterfalls, high stream gradients, or both, and the lower limits are defined by alluvial fans, subsurface water, and frequent shifts in channel location over the fans. Fourth, because there is no opportunity to create a large interconnected stream system among the Steens Mountain populations, we also recommend the exploration of opportunities to translocate Steens Mountain Lahontan cutthroat trout into larger interconnected stream systems or to translocate fish back into the source population. For example, the Trout Creek drainage (see Figure 2), part of the historic distribution of Alvord cutthroat trout, currently contains Alvord cutthroat trout–rainbow trout hybrids only. If genetic analyses show that the hybrids have primarily rainbow trout genomes, removal of this population may be justified and this stream system could represent potential habitat for transplants from the Steens Mountain Lahontan cutthroat trout populations. Fifth, we also recommend establishing a genetic monitoring program to assess the effectiveness of restoration, with the aim of correlating on-site restoration activities and the maintenance of genetic variation.

For critically endangered species, translocation has been repeatedly used as conservation tool. Examples include the Devil’s Hole pupfish (Baugh and Deacon 1988), Yaqui chub *Gila purpurea* (DeMarais and Minckley 1993), snail darter *Percina tanasi* (Shute et al. 2005), and four New Zealand endemic lizards (robust skink *Cyclodina alani*, marbled skink *C. oliveri*, Whitaker’s skink *C. whitakeri*, and Suter’s skink *Oligosoma suteri*; Towns and Ferreira 2001). In these cases, transplants were made from populations in peril to other locations both within and out of the historic range of these species. These refugia populations were founded to preserve gene pools and to act ultimately as donor populations for restored habitats. In extreme cases, such as the Arabian oryx *Oryx leucoryx*, black-footed ferret, and California condor, most if not all individuals in the wild were brought into captivity to avoid their extinction (Russell et al. 1994; Corp et al. 1998; Ralls et al. 2000). Captive breeding programs represent final efforts to save species and are fraught with issues of inbreeding and loss of genetic diversity (Frankham 2002).

In contrast to the previous examples, most of the movement of salmonids into nonnative habitats has been to create angling opportunities. Lahontan cutthroat trout have been translocated into stream systems in California, Oregon, Utah, Washington, and possibly other states as well as nonnative waters in southern Nevada. This subspecies was listed as endangered in 1970 (Federal Register Volume 35, page 13520) and was down-listed to threatened status in 1975 to facilitate management and allow regulated angling (Coffin and Cowan 1995). Recovery activities since that time have focused on securing isolated populations above headwater barriers and transplanting fish into fishless waters within the historic range to offset local extirpation risk (Elliott et al. 1997; Sevon et al. 1999). However, this strategy has had mixed results as such populations continue to be extirpated (Harig et al. 2000; Harig and Fausch 2002; Novinger and Rahel 2003). The Lahontan cutthroat trout is a good example of the pitfalls of such an approach as at least 30 isolated populations have been lost over the last 10–15 years.
(Dunham et al. 1997, 1999; Elliott et al. 1997) despite the Endangered Species Act listing. In both the western Lahontan Basin and Willow–Whitehorse watersheds, there are fewer than 10 extant native Lahontan cutthroat trout populations remaining. Out-of-basin populations do exist for fish native to the Carson and Truckee River watersheds in the Western DPS, but these populations tend to be genetically depauperate, with a small subset of the genetic variation that has been described for their extant source populations (Peacock and Kirchoff 2007).

This is not the case for Steens Mountain translocated populations. Although there are no opportunities to create additional interconnected stream networks among the other Steens Mountain populations, which are currently found in small stream habitats, these populations have maintained high levels of genetic variability, which may indicate high habitat quality in these small streams. However, small, isolated populations are subject to demographic and genetic stochasticity; therefore, there is a high likelihood of losses of genetic variation over time. For such situations, in which the genetic variation of a DPS is spread among within-basin and out-of-basin habitats, these populations should be considered collectively in formulating recovery strategies. An evolutionarily significant unit framework for salmonid recovery provides a platform for investigating both demographic population viability and significant genetic population structure across a species range. As evolution is an ongoing phenomenon, the DPS approach provides the best opportunity for prioritizing recovery strategies to maximize maintenance of genetic variability and therefore evolutionary potential.

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References


Fiumera, A. C., P. G. Parker, and P. A. Fuerst. 2000. Effective population size and maintenance of genetic diversity in


immigration of one individual into small partially inbred populations. Zoo Biology 11:343–351.