Genetic Management Plan for the Paiute cutthroat trout

(*Oncorhynchus clarkii seleniris*)

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Section 1. Introduction

Section 1.1 Document objectives/executive summary

This genetic management plan (GMP) serves as a plan both for the restoration of the Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*, hereafter PCT) to its native range in Middle Silver King Creek and for the long-term management of the species. It utilizes new genetic analyses in the context of previous genetic work.

The GMP covers risks and benefits for different management actions designed to meet recovery criteria from the PCT Recovery Plan (USFWS 2004). A broader perspective on the species history, population trends and health, and habitat quality should inform any choice among the management actions. And though our recommendations are based on the most current and best available science, future work may provide additional insight and change genetic recommendations. Managers must be willing to adapt and change based on new information.

Section 1 of this GMP provides relevant natural history and genetic background for PCT. *Section 2* summarizes current genetics research on PCT. *Section 3* addresses achieving recovery criteria, *Section 4* discusses genetic monitoring, while *Section 5* describes adaptive management needs. *Section 6* closes with recommendations for achieving recovery criteria based on the currently available genetic data. Note that this plan is adapted from the Genetic Management Plan written for the California golden trout (*O. mykiss aguabonita*), another imperiled inland trout species that faces challenges similar to the PCT (Stephens et al. 2013).

Section 1.2 PCT *background*

Distribution

The threatened PCT (USFWS, 1975) has the most restricted range of any native North American cutthroat trout. Its native habitat is limited to the middle reaches of Silver King Creek and associated tributaries (hereafter Middle SKC). Silver King Creek (SKC) is a headwater tributary to the East Fork Carson River located in a high alpine meadow 2500 meters above sea level in the Carson–Iceberg Wilderness in Alpine County, California. The middle reaches of SKC are bounded by Llewellyn Falls, and the downstream portion is bounded by Silver King Gorge. These boundaries are fish barriers and are thought to have isolated PCT from Lahontan cutthroat trout (*O. c. henshawi*, LCT), approximately 10,000 years ago (Behnke 1992). PCT were moved upstream of Llewellyn Falls in 1912, and by the time PCT was described in 1933, the PCT had already disappeared from Middle SKC due to the introduction of other salmonid species that likely both outcompeted the PCT and drove PCT to extinction through hybridization (see Ryan and Nicola 1976, Busack and Gall 1981, Cordes et al. 2004, Finger et al. 2011).

After a complex series of introductions and translocations over the last 100 years (see Cordes et al. 2004), nine refuge populations persist above fish barriers. There are five within Silver King Basin (Upper Silver King Creek above Llewellyn Falls, Coyote Valley Creek, Corral Valley Creek, Four Mile Canyon Creek, and Fly Valley Creek) and four out-of-basin locations (Cabin Creek and North Fork Cottonwood Creek in Mono County; Stairway Creek in Madera County; Sharktooth Creek in Fresno County) (See Appendix 1, Table 1 for list of refuge populations, and Figure 1 for map of locations from Cordes et al. 2004). Each of these

populations has been determined to be non-introgressed (pure) based on microsatellite data (Cordes et al. 2004) and single nucleotide polymorphism (SNP) data (Stephens et al. 2011).

Species status and Taxonomic overview

The PCT was originally listed as endangered on March 11, 1967 (USFWS, 1967), under the federal Endangered Species Preservation Act of 1966. In 1975, the PCT was downlisted to threatened (USFWS, 1975) to allow more flexible habitat management and species restoration. Other ranking systems list the PCT as highly imperiled, such as the American Fisheries Society Endangered Species Committee, which ranks it "endangered" (Jelks et al. 2008). Moyle et al. (2008) classified it as having a "high certainty" of extinction in 50-100 years.

The PCT was originally described as *Salmo seleniris* (Snyder 1933, 1934), but it was reclassified as a subspecies of the cutthroat trout by Vestal (1947). Subsequently, all western North American trout have been reclassified from the genus *Salmo* to the genus *Oncorhynchus*, as summarized by Smith and Stearly (1989) and adopted by the American Fisheries Society's Committee on Names of Fishes, the accepted authority on North American fish taxonomy (Robins *et al*. 1991). More recently, the species name for all cutthroat trout changed from *clarki* to *clarkii* to reflect the original spelling (Nelson *et al*. 2004), changing the taxonomic status of the PCT to *Oncorhynchus clarkii seleniris*. Morphologically, the PCT is distinguished from the LCT, its closest relative, by having nine or fewer spots and a purplish hue, while LCT is more heavily spotted (Behnke 1965). Finger et al. (2009) found no differences between LCT and PCT using seven diagnostic SNPS, however these SNPs were not designed to differentiate between PCT and LCT. Pritchard et al. (2013) found that PCT only contain a subset of LCT

genetic diversity. However, recent genomic analysis found up to 91 fixed differences between PCT and LCT (author's unpublished data). These results are further discussed in Appendix 1.

Life history

The PCT's restricted range and threatened status have limited the number of studies on PCT life history, and most biological information on PCT comes from the introduced population in the North Fork Cottonwood Creek (Wong 1975, Wong 1991, Titus and Calder 2009). PCT populations consist mostly of fish from one to three years old, though a few may survive past their third year up to five or six years (Wong 1991, Titus and Calder 2009). The growth of individuals of this subspecies is nearly isometric, where size increases at a rapid rate among juveniles and slows considerably among adults (Wong 1991), though growth rates are higher in larger habitats (Titus and Calder 2009). Sexual maturity occurs at the age of two years, and peak-spawning season occurs in the months of June and July (Wong 1975).

Stream pool habitat with overhanging banks and riparian vegetation appears to be important for PCT growth and survival, perhaps because it provides areas for winter refuge and rearing juveniles (Raleigh et al. 1984, Berg 1994). Although run and riffle habitats may still be utilized by PCT, the larger fish typically occupy pools (USFWS 2004). PCT also exhibit limited dispersal pattern based on mark-recapture studies (Diana and Lane 1978). Transplanted PCT do not move far, tending to remain in their new area rather than occupying other vacant spaces within their stream (Diana and Lane 1978).

Historical and current biotic and abiotic threats

Although a more complete review of various threats to PCT can be found in the revised PCT Recovery Plan (USFWS 2004) and a 5-year Review (USFWS 2013), the two major factors

leading to the listing of the PCT as threatened are habitat degradation due to grazing and the introduction of nonnative salmonids such as California golden trout and rainbow trout which hybridize with and outcompete the PCT (USFWS 2004).

The presence of livestock grazing may reduce stream pool habitat with undercut banks and abundant riparian vegetation through streambank erosion (USFWS 2004). This negative effect of grazing has been demonstrated and quantified in studies related to a related species, the California golden trout (Knapp and Matthews 1996, Knapp et al. 1998). Although impacts of habitat degradation may linger, there has been no grazing in the Silver King Basin since the summer of 1994. The Paiute cutthroat trout Revised Recovery Plan recommended the closure of the Silver King Allotment (USFWS 2004) and, in 2012, the Humboldt-Toiyabe National Forest officially closed the Allotment to all livestock grazing for the protection of PCT (USFS 2012). Of the out-of-basin locations, only Cabin Creek has occasional active grazing allotments, resulting in some bank failure and increased sediment input (USFWS 2004).

Perhaps the biggest past threat to the PCT was competition and hybridization with introduced salmonids. When LCT or rainbow trout are present, PCT tend to lose their distinctiveness through introgressive hybridization or are displaced by competition (Schroeter 1998, USFWS 2004). Thousands of non-native plantings within Middle SKC are thought to be the major driver to their extirpation within their native habitat (USFWS 2004). As a result, a complex series of rotenone treatments to remove non-native fish and habitat restoration projects were conducted to create and maintain viable refuge PCT populations, which are isolated from downstream populations of non-native salmonids. To date, studies of introgression using both SNP and microsatellite markers have not found any introgression with

non-native rainbow trout in any of the PCT refuge populations (Cordes et al. 2004; Stephens et al. 2011). Furthermore, preliminary data also suggests that refuge populations are not introgressed with LCT (Appendix 1).

Currently, the main threats to PCT existence are a small total number of populations, demographic stochasticity associated with small population census sizes, and possible unauthorized introduction of nonnative salmonids. None of the refuge populations are within the native range of the PCT, and some may have undergone founder effect (where small numbers of individuals are used to establish a population and therefore do not represent the full genetic diversity of the source population) upon their creation (See Appendix 1, Table 1 for refuge populations). Furthermore, the native range in the middle section of SKC provided greater flows and habitat stability than the present locations above fish barriers or in out-of-basin locations. Given the smaller habitat extent and the increased variability of the refuge habitat, individual population extirpation due to a catastrophic event is more likely, though total risk of extinction for the species is lower. In addition, reduced population size and stability in addition to inbreeding and loss of genetic diversity over time may become mutually reinforcing and create an insidious cycle known as an extinction vortex (Gilpin and Soulé 1986).

While fish barriers currently prevent other salmonids from invading refuge PCT populations, there is a continuing risk of human introductions, particularly in areas like the Middle SKC where non-PCT trout occur in close proximity to refuge populations. Once non-PCT fish are removed from Middle SKC and PCT are reintroduced, the chance of unauthorized trout introduction will be greatly reduced (USFWS 2004).

History of species management

The first documented transfer of PCT into new habitat occurred in 1912, when sheepherders transplanted PCT from Middle SKC below Llewellyn Falls into formerly fishless Upper SKC (Ryan and Nicola 1976); the transplants established a self-sustaining population by 1924. By then, PCT below Llewellyn Falls had become admixed with LCT, California golden trout, and rainbow trout due to unauthorized transplantations (Ryan and Nicola 1976, Cordes et al. 2004). It is possible that no pure PCT populations existed in their native range when PCT were first described in 1933 (Snyder 1933, Cordes et al. 2004).

PCT was again successfully transplanted out of its native range, into North Fork Cottonwood Creek in 1946 (Ryan and Nicola 1976). What followed was a succession of non-native trout introductions, introgression with PCT populations, chemical treatments, and further transplantation of PCT into nine different locations (see Cordes et al. 2004 for overview). Ironically, transplanting PCT into fishless waters enabled maintenance of pure populations of PCT, as introductions of rainbow trout above Llewellyn Falls took place as early as 1949 (Ryan and Nicola 1976, Cordes et al. 2004). Efforts to restore pure populations of PCT by chemical treatments and/or electrofishing began in the 1960s. Though multiple attempts were required for certain locations, removal of hybridized trout was eventually successful in Upper Silver Creek, North Fork Cottonwood Creek, Corral Valley, and Coyote Valley Creeks (USFWS 2004). Results from genetic analyses further confirm that chemical treatments were successful in eradicating hybrids from these tributaries (Cordes et al. 2004, Stephens et al. 2011).

Section 2. PCT population genetic structure, diversity and differentiation

A brief overview of the genetic characteristics of the nine PCT refuge populations is given below. Appendix 1 offers more details concerning the most recent genetic analysis of the refuge populations.

Section 2.1 Genetic diversity of PCT refuge populations

Genetic diversity is important for managers to consider because it is the raw material for evolution and is essential for populations to adapt to changing environments (Hedrick 2000, Frankham et al. 2002). Lowered genetic diversity has been correlated with reduced fitness and population viability and increased risk of extinction (Reed and Frankham 2005, Frankham 2005). Though Lande (1998) argued that demographic factors may lead to extinction more rapidly than genetic factors, managers should be mindful of ameliorating the loss of genetic diversity, and work where possible to avoid its decline.

In this study, genetic diversity was measured using 11 microsatellites, with the standard metrics of allelic richness (R_S, number of alleles per microsatellite locus corrected for sample size using rarefaction), expected and observed heterozygosity (H_E and H_O, measures of genetic variability), and effective population size (N_E, a measure correlated with the rate of loss of genetic diversity over time due to genetic drift).

Every PCT refuge population is a transplant population with varying numbers of founders and habitat size, and the values of H_E, H_O, R_S, and N_E vary accordingly, but all are relatively low. There does not appear to be a correlation between these genetic diversity levels and status as an in or out-of-basin population, though there is genetic diversity in out-of-basin populations not represented in the Silver King Creek watershed. Some in-basin populations, such as Four Mile Canyon, have reduced heterozygosity and low R_S, while the out-of-basin

Cabin Creek has surprisingly high heterozygosity and the greatest R_S value (Appendix 1 Table 2). The genetic diversity results over all populations are not unexpected, given that transplanted populations are often created with small numbers of individuals relative to the source population and are subject to founder effect. For example, Sharktooth Creek was founded with only 29 individuals (Ryan and Nicola 1976), and Corral Valley Creek only had 20 founders (Bacon 1978). In addition, all of the refuge populations are generally small and subject to stochastic demographic events, and therefore subject to genetic drift, which causes genetic differentiation and loss of genetic diversity over time.

Section 2.2 Population genetic differentiation and structure

An analysis of genetic differentiation among pairs of populations of PCT showed significant pairwise F_{ST} values for most pairwise comparisons (Appendix 1, Table 3). F_{ST} is a measure of population differentiation or reduced gene flow, and significant values indicate population structure. Most pairwise F_{ST} values were significant, and most fell within the moderate to large range (See Appendix 1, Table 6 for within and between-year pairwise F_{ST} values for PCT refuge populations). Detection of private alleles is also a measure of population distinction. A private allele is an allele at a locus that is unique to a particular population. Several refuge populations had private alleles (Appendix 1, Table 2), indicating that these refuges may have genetic diversity not represented in other refuge populations.

We cannot predict at this time if genetic differentiation has a negative effect on individual PCT refuge populations, but if surveys show a decline in population numbers and other factors have been ruled out, it may be attributable to genetic factors such as inbreeding. In this case, managers may consider population supplementation or translocations when all

other factors of decline have been ruled out. Finally, it is of note that the original PCT population, as it was isolated from LCT, may have been subject to a founder effect and possibly bottlenecks throughout the course of its history. PCT may be adapted to survival in small populations and with low genetic diversity. Managers should therefore continue to monitor trends in genetic diversity to determine if intervention is warranted for the purpose of retaining diversity and preventing anthropogenically induced genetic drift.

Section 3. Achieving recovery criteria for delisting

The recovery criteria addressed here, as stated in the Recovery Plan are:

1. The refuge populations in Corral and Coyote Creeks, Silver King Creek, and tributaries above Llewellyn Falls as well as out-of-basin populations are maintained as refuges and are secured from the introduction of other salmonid species
2. A viable population occupies all historic habitat in Silver King Creek and its tributaries downstream of Llewellyn Falls to fish barriers in Silver King Canyon

To meet these criteria, managers have choices regarding best management actions based on refuge population status and location, genetic diversity, and how best to restore and monitor Middle SKC following nonnative fish removal and PCT reintroduction. We do not prioritize populations in terms of importance for conservation of the species, because in the case of the PCT, there are so few populations that they are all high priority. Conservation actions should then be based on population demographic and genetic status.

As stated previously, this genetic management plan only addresses genetic concerns associated with achieving recovery criteria. Managers must regularly monitor non-genetic

factors such as habitat and population census sizes and integrate this information with estimates of genetic diversity, differentiation, and N_E before making management decisions.

The following sections are organized to present and analyze potential management actions for meeting each recovery criteria.

Section 3.1 The refuge populations in Corral and Coyote Creeks, Silver King Creek, and tributaries above Llewellyn Falls as well as out-of-basin populations are maintained as refuges and are secured from the introduction of other salmonid species

According the recovery plan (USFWS 2004) "a viable population will be achieved when the population is secure and comprises three or more age classes for five years, and consists of a minimum of 2,500 fish greater than 75 millimeters (3 inches) (Hilderbrand and Kershner 2000). Several different conservation strategies that are not mutually exclusive may be employed to secure and maintain viable populations. **Option 1** is a status quo approach, where managers continue to manage populations as has been done in the past, but do no additional management such as translocating individuals, **Option 2** is to conduct one of several translocation scenarios. We discuss these options in more detail in the following subsections.

Before selecting any of the above options, we suggest that managers follow George et al. (2009) and use the guiding principle of "do no harm". Managers must weigh the risks and benefits of doing nothing with the risks and benefits of active management actions such as translocations.

<u>Section 3.1.a Status quo</u>

Option 1 is to continue to manage the PCT refuge populations as has been done in the past, that is, maintain the status quo. This generally involves taking census sizes in test sections

of each of the in-basin refuge populations every year, but has also included habitat management in the past (such as in Four Mile Canyon Creek, where stream structure was added), founding new populations, and rotenone treatments (USFWS 2004, Cordes et al. 2004). The benefits of option 1 are that managers do not risk implementing a management action that could harm a refuge population. However, doing nothing also carries risks, which are generally the risks associated with small isolated populations: inbreeding depression, low N_E, reduced genetic variation, potentially decreased fitness and lowered long-term viability, and an increased risk of extirpation due to catastrophic events, which in the case of the PCT could represent valuable and irreplaceable genetic diversity (e.g. Frankham et al. 2002, Hedrick and Kalinowski 2000, Frankham and Ralls 1998). For more in-depth discussion of the genetic risks associated with small populations, see risks associated with small populations in *Section 1.2 Historical and current abiotic and biotic threats*. Overall, by not mixing or founding new populations, managers avoid the risk of outbreeding depression, but may increase the risk of inbreeding depression and loss of genetic diversity or even entire populations.

Section 3.1.b

One option for preserving genetic diversity and preventing further differentiation is translocations, or the intentional movement of individuals from one population to another (IUCN 1987), in order to preserve genetic diversity. We recommend this option be explored only in collaboration with geneticists, and in the context of understanding the refuge population histories, ecology, habitat, genetic status, and other biotic and abiotic factors. For the fragmented PCT refuge populations, where removal of fish barriers is not viable, managers may implement up to three major translocation options: **option a**: No translocations except in

the case of extirpation; **option b**: Active maintenance of gene flow through regular translocations of individuals between some or all populations; and **option c**: Conditional supplementation into declining populations or for the purpose of preserving unique genetic diversity. For all options, regular genetic monitoring will ensure that sufficient genetic diversity is maintained, or reveal if continued translocations are necessary (see *Section 6. Genetic Monitoring* for recommendations). We outline the pros and cons of each option.

Option a*: No translocations except in the case of extirpation*

Option a is essentially the status quo strategy described in *Section 3.1.a* and carries the same risks. Risks associated with re-founding new populations that are extirpated are addressed below in *Section 3.1.b*.

Option b: *Active maintenance of gene flow*

Before undertaking any active translocation, managers, working with geneticists, must identify the purpose of translocation, weigh the risks, and determine measures of success (e.g. target N_E or census size) before acting. For single species conservation, goals can range from maintaining overall population resilience and genetic diversity to genetic rescue (e.g. Hedrick 1995; Westemeier et al. 1998; Tallmon et al. 2004; Weeks et al. 2011). However caution is required before conducting translocations to avoid outbreeding depression (Huff et al. 2011), which has been shown to reduced fitness (e.g. Goldberg et al. 2005, Huff et al. 2011), though it may be less of a concern in PCT populations given how recently the populations were separated. In general, managers should avoid translocating individuals from dissimilar populations or environments (Edmands 2007). In addition, the translocation of individuals from

one population into another may introduce non-native pathogens, which can negatively affect the recipient population and/or other aquatic species in that environment.

Due to the above risks, active translocations should only be considered an option if multiple PCT refuge populations have declining census sizes and/or a trend of declining genetic diversity, and if conditional supplementation has been tried first and properly evaluated. In that case, there are some benefits to translocations. First, given that PCT populations are differentiated and some of them have private alleles, active translocations on an appropriate scale could create replicates of genetic resources that are only found in one or a few populations. For example, by translocating fish from Stairway Creek to another population, any unique genetic diversity from Stairway Creek might be preserved in the recipient population, preventing loss of this diversity in the event of decline or extirpation of Stairway Creek. In some cases, recipient populations end up with more genetic diversity than their donor population if the donor population has a smaller N_E or goes through a genetic bottleneck after being a donor. For example, Chen (2013) found that transplanted populations of Mojave tui chub contained more genetic diversity and private alleles than their donor population. Translocations may have the added benefit of increasing the N_E of each population, fostering long-term maintenance of genetic variation.

In order to maximize translocation success and minimize outbreeding depression, mangers should examine demographic, ecological, and genetic data when choosing founder fish. In the case of the PCT, all of the populations were once a single population and have differentiated over time (Appendix 1). This may be the result of natural selection to biotic or abiotic factors specific to each refuge population, but we believe it is most likely due to genetic

drift through inbreeding and small N_E's. For the PCT, outbreeding depression may be a higher risk when transferring individuals between basins, rather than within a particular basin. For example, there is differentiation between populations within the SKC basin, but they are geographically proximate and relatively ecologically similar, potentially reducing the risk of outbreeding depression and making viability of translocated fish in the new environment more likely. However if fish from Sharktooth Creek are translocated into one of the refuge populations in the Silver King basin, not only are these populations genetically dissimilar, their habitats are ecologically different. This combination could reduce the success of the translocations (Young 1999, but see Zeisset et al. 2012). In a meta-analysis, McClelland and Naish (2007) found that the effects of outbreeding depression cannot be predicted in fishes, so if an active translocation scenario is chosen, managers must approach it with an "experimental spirit" (Young 1999) so that managers may learn from translocation events and share knowledge with other fisheries biologists.

Once populations have been selected in terms of ecological and geographic suitability, managers must balance potential harm to the donor population through fish removal with the benefits and risks incurred on the recipient population. If too few fish are translocated, there may be no effect, defeating the purpose. On the other hand, if too many fish are added to the recipient population, the addition of donors could "swamp out" the unique genetic diversity in the recipient population. This is a risk with the PCT refuge populations given that several contain unique alleles that should be preserved. In addition, managers should not take too many fish from the donor population, which could disrupt the functional ecology of the donor stream and potentially lower the N_E of the donor population through removal of genetic

diversity. One solution to this problem is to translocate fewer individuals over a longer period of time, such as throughout a spawning season (Young 1999) or in an ongoing basis (Drauch and Rhodes 2007) rather than in a single translocation event. In addition, fish from various age classes, rather than a single age class, should be selected for translocation in order to capture as much genetic diversity from the donor population as possible (e.g. Caudron et al. 2012, Stockwell et al. 1996, Minckley 1995). Finally, selecting fish from throughout the chosen donor reach rather than just a single location will also contribute to greater genetic diversity.

Option c: *Supplement declining populations*

If managers deem the risk of outbreeding depression to outweigh the risk of inbreeding depression and choose not to start a full-scale program of regular translocations, a more cautious approach is available: if one or more refuge populations is in decline or has become highly differentiated and non-genetic factors such as poor habitat quality or pathogens have been ruled out, managers may want to supplement with individuals from other populations. The risks and benefits of one-time supplementation or supplementing only declining or highly differentiated populations are similar to those for active translocation. These risks are ameliorated because there would only be translocation in certain populations and on a smaller scale. However if supplementation is chosen for a declining population, managers should make sure that any non-genetic reason for decline is addressed so that healthy fish from a good "source" population are not deposited in a "sink" population where they will fail to thrive.

Section 3.2 Paiute cutthroat trout must be successfully reintroduced into Silver King Creek from Llewellyn Falls downstream to Silver King Canyon.

As part of the recovery criteria, the nonnative fish in Middle SKC, from Llewellyn Falls downstream to Silver King Canyon, must be removed. Following successful removal, pure PCT will be reintroduced to found a viable population, creating the largest extant population of SKC and restoring the species to its native range. This plan will address the re-founding process and future monitoring of the new population in Middle SKC.

Additional populations act as an "insurance policy" against the loss of unique genetic, ecological, or behavioral characteristics of PCT against catastrophic events. Creation of Middle SKC as an additional healthy population has the added benefit of creating another source for genetic rescue of the extant refuge populations in the event of their decline (Hedrick and Fredrickson 2010). When establishing the new population in Middle SKC, managers must use enough founders so that the risks associated with small populations are avoided. Middle SKC cannot be considered properly restored until the population is healthy and viable, and as such also useful as a robust donor population in the future. Therefore, appropriate planning and management are critical for the successful founding of Middle SKC.

When choosing donor populations from which to select founders for reintroduction into Middle SKC, there are three main considerations: 1) location, 2) demographic health, and 3) genetic health of the founder stocks and the new population.

Choosing fish from in-basin or out-of-basin populations has pros and cons as discussed in *Section 3.1.b*. Generally, donor fish should be chosen from genetically and ecologically similar populations, which in the case of Middle SKC are populations within the basin. The second consideration is which populations can withstand removal of founder fish and maintain viability. Population census sizes should be considered both in the planning phase and at the

time of capturing, moving, and planting fish into Middle SKC. If a population cannot withstand the loss of 10% of its census size, then founders should not be taken from that population. In order to found the most genetically viable population in Middle SKC, managers should aim to represent as much genetic diversity as possible from selected donor populations. Young (1999) found that translocating between salmon between genetically and ecologically similar populations will be more successful than between dissimilar populations, though Zeisset et al. (2012) found that transplanted common toads were more successful when they were from larger populations with higher diversity rather than populations with greater genetic and ecological similarity to the recipient population.

Section 4. Genetic monitoring

Genetic monitoring is critical for assessing the success of any genetic management plan. Genetic monitoring must have a temporal aspect, in that it occurs more than one time in order to monitor population trends over time (Schwartz et al. 2007). For this plan, we follow the definition of Schwartz et al. (2007), where genetic monitoring is "quantifying temporal changes in population genetic metrics or other population data generated using molecular markers." Without adequate monitoring, managers cannot detect changes and trends in genetic structure and diversity of the PCT refuge populations. Monitoring can reveal genetic decline or potential introgression in a population that is not apparent with population surveys and habitat monitoring. It can also identify bottlenecks and lowered N_E's that can lead to a loss of genetic diversity and reduced population viability, all of which can undermine genetic management goals (e.g. Laikre et al. 2008).

Before commencing any conservation action involving translocation or the founding of a new population, a genetic baseline must be created for each of the nine refuge populations. After the initial genetic baseline is established, genetic sampling and analyses should be conducted at regular intervals through time. Appendix 1 may serve as an initial microsatellite genetic baseline for the refuge populations (except for Cabin Creek), but we recommend additional markers, such as SNPs, for potentially more powerful and repeatable analysis (Morin et al. 2004). Continued regular, standardized monitoring will establish the populations' genetic status and structure and reveal trends over time that may require changes in management.

Regular long-term genetic monitoring must include standardized protocols for data collection. Different markers such as microsatellites and AFLP have been used with success in genetic monitoring, but recent advances in next-generation sequencing technologies have facilitated large-scale SNP genotyping, which has the advantage of being easily coordinated among different labs, and can be inexpensive and less time-consuming if SNP assays are already available. Regardless of the marker chosen, long-term genetic monitoring should include estimating genetic variation and structure, bottleneck detection, and estimation of N_E (Schwartz et al. 2007).

Section 5. Adaptive management

In combination with long-term genetic monitoring, regular visual surveys and evaluation of management practices are critical in order for managers to see the effects of actions, and to evaluate those actions in the context of complex and somewhat unpredictable systems affected by ecological, stochastic, and environmental interactions. Therefore, it is imperative that PCT managers make informed decisions using an adaptive management approach, which increases

the effectiveness and flexibility of a management program through incorporation of past experience with new circumstances and information. Overall, adaptive management process relies on careful planning to identify goals and active monitoring during management. This process enables managers to integrate and build upon the positive aspects of a management program and to abandon or adapt less successful management actions. Another important aspect of adaptive management is to document and share the management process, including both successes and failures so that other projects may benefit from collective experiences. A good source of information on adaptive management can be found in *Open Standards for the Practice of Conservation*, from the Conservation Measures Partnerships and available at www. ConservationMeasures.org. Another resource is the software program MIRADI (available at www.miradi.org), which is available to guide managers through the Open Standards process. Finally, an adaptive management program for the PCT should operate under the "do no harm" perspective (George et al. 2009) and an understanding that management actions are likely to differ by population and over time.

Section 6. Recommendations for achieving recovery criteria

Here we provide specific recommendations for genetic management of PCT in order to achieve specific recovery criteria. Our recommendations are based on the latest genetic analysis and our consideration of the risks and benefits for different genetic management strategies. Recommendations below are organized by recovery criteria.

Section 6.1. The refuge populations in Corral and Coyote Creeks, Silver King Creek, and tributaries above Llewellyn Falls as well as out-of-basin populations are maintained as refuges and are secured from the introduction of other salmonid species

Below we will provide recommendations specific to the two major strategies for achieving this recovery criteria: 1) Status quo and 2) Various translocation scenarios.

6.1.a Status quo

We do not recommend staying the course in managing the PCT. None of the out of basin populations are surveyed the minimum of every three years, and samples were not collected form Cabin Creek in 2010-2012 for genetic analysis. Cabin Creek had substantial genetic diversity in 2000 but was not sampled in 2010-2012 so its current genetic status is unknown. PCT populations need additional active monitoring to protect from further loss of genetic diversity. In addition, some of the populations are highly differentiated from the other refuge populations, such as Stairway and Sharktooth Creeks, or are in decline, such as Four Mile Canyon Creek. These populations may need additional management in order to prevent extirpation and/or further differentiation.

6.1.b Translocation scenarios

Option a: *No translocations except in the case of extirpation*

Given that there are so few populations of PCT, each of the nine populations is valuable and necessary for the recovery of the species. A catastrophic event could wipe out a population, an unauthorized introduction of nonnative salmonids could lead to introgression, or there could simply be a general decline of a population leading to extirpations due to habitat quality or genetic reasons. In this case, population(s) will need to be re-founded. If this option is chosen, we make the same recommendation for re-founding any refuge population as we do for restocking Middle SKC after a chemical treatment. See *Section 6.2*.

Option b: *Active maintenance of gene flow in selected populations*

Active, regular translocations to maintain gene flow between populations may be an attractive option given that the subpopulations were somewhat recently a single population (dating back to the initial transfer out of Middle SKC into Upper SKC in 1912, and possibly back to the hypothesized original founder event separating PCT from LCT), making outbreeding depression less of a concern. Translocations may have the added benefit of increasing the N_E of each population, and replicating genetic diversity in highly differentiated populations, fostering long-term maintenance of genetic variation in the species. However, as mentioned above, large-scale integration of populations could swamp out any existing local adaptation, and potentially lower global N_E (the N_E over all refuge populations). The risk of translocating healthy fish to an unhealthy environment or introducing pathogens could be high so we do not recommend active supplementation at this time, especially without screening for pathogens. However if this option is selected, continued supplementation of 5 to 10 individuals from varying age classes per generation (Mills and Allendorf 1996) between selected populations (one or more populations that can withstand the loss of founders and have high genetic diversity, such as Upper Silver King, Fly Valley, Coyote Valley, Corral Valley, Cabin Creek, North Fork Cottonwood Creek) is recommended. If this option is selected, each donor fish should and the recipient population should be both genetically analyzed and screened for pathogens. Subsequent to the translocation event, the recipient population should be genetically analyzed every three to five years, in order to create a baseline for the new population and monitor trends. We do not recommend this option without trying and evaluating conditional supplementation first.

Option c: *Supplement declining populations*

With one time supplementation of declining or highly differentiated or genetically depauperate populations, managers can add 20-30 fish from varying age classes from one or more of the most genetically diverse refuge populations that can withstand the loss of fish (Upper Silver King, Fly Valley, Coyote Valley, Corral Valley, Cabin Creek, North Fork Cottonwood Creek), with the aim of minimizing loss of polymorphism and heterozygosity while maintaining divergence in allele frequencies in recipient populations. Specifically, managers might consider a one-time supplementation for populations such as Sharktooth, Stairway, and Four Mile Canyon Creeks due to high differentiation and/or loss of genetic diversity. The caveat to this scenario is that there might be initial failure to thrive of fish introduced into Sharktooth or Stairway Creeks due to a lack of adaptation to the donor environment, or future reduced viability due to outbreeding depression. Transplanted fish may also unsuccessfully integrate into Four Mile Canyon Creek if the factors leading to this population's decline have not been addressed. Essentially, managers should not take fish from a healthy population and introduce them into one with poor habitat quality or other factors causing the initial decline of the refuge population.

Another reason for conditional one-time supplementation is to replicate unique genetic diversity found in some of the highly differentiated populations, such as Sharktooth Creek. To address this, 20-30 fish of varying age classes from Sharktooth Creek or another selected population can be reciprocally translocated into another small, differentiated population such as Stairway Creek, which could also benefit from an infusion of new genetic diversity (with the caveat that there could be outbreeding depression). This would also serve to increase the N_E of both populations. If conditional translocations are conducted, as with regular translocations,

the donor fish and the recipient population should be genetically analyzed and screened for pathogens, and recipient populations should be analyzed every two years until census sizes and levels of genetic diversity show a stable trend.

Section 6.2 Paiute cutthroat trout must be successfully reintroduced into Silver King Creek from Llewellyn Falls downstream to Silver King Canyon.

To accomplish the re-stocking of Middle Silver King Creek, our recommendations are based on results from the simulation model done by Hilderbrand (2002) that suggest that a number of mature fish within 5-10% of the habitat's carrying capacity should be sufficient for a successful population reintroduction, defined as having a lower than 10% probability of extinction in 100 years. In contrast, a reintroduction plan including immature fish (age-0 or subadults) requires far more founders (greater than 50% carrying capacity of the habitat). Due to the relatively close taxonomic relationship between PCT and the non-native trout found in Middle SKC, the number of removed non-native trout from Middle SKC may be able to serve as an approximate estimate for the carrying capacity of the area. Fish population test section data collected in 2000 suggests that there may be approximately 4,000 adult and 7,000 total PCT supported in restored habitat (W. Somer personal communication). Alternatively, model-derived estimates developed by Young et al. (2005) may also be used. Persistence of populations within these simulations (Hilderbrand 2002) varies in the optimal number of fish to be stocked depending on the carrying capacity of the stream. To reduce the risk of population extinction, more supplemental individuals are needed for a small population (e.g. streams supporting 200 fish) than that of a large population (e.g. streams supporting 2500 fish) (Hilderbrand 2002).

When reestablishing Middle SKC and other new refuge populations, we recommend using roughly equal proportions of at least 30-50 individuals (in the absence of knowledge of the carrying capacity of Middle SKC) from Upper SKC, Coyote Valley, Corral Valley and Fly Valley Creeks, limited to no more than 10 percent of the smallest donor population, to minimize genetic or demographic impacts to the donor populations. Frankel and Soulé (1981) calculated that 30 or more fish could capture more than 98% of the genetic diversity of a donor population if all founders contribute equally to the next generation (Weeks et al. 2011). If 30 fish from all refuge populations except Four Mile Canyon were reintroduced into Middle SKC, this would effectively represent most of the genetic and ecological diversity in the SKC basin in the newly established population. We do not recommend using out-of-basin fish into the new population at this time, due to the possible increased risk of outbreeding depression, introduction of non-native pathogens, and higher stress levels in transporting donor fish. Each of the donor fish should be fin clipped and genetically analyzed, and the population must be genetically monitored every three to five years for a minimum of 10 years, or until genetic and demographic trends have stabilized and the population is deemed viable.

Section 6.3 Genetic monitoring recommendations

The recommended frequency of sampling will depend on the management actions chosen. For refuge populations that are not selected to be recipients of translocations, monitoring should be conducted at three to five year intervals, as this is approximately the generation time for PCT. If supplementation is a chosen management action, genetic monitoring (both donor and recipient populations) should be conducted at the time of the first translocation event and every three to five years until populations have reached a target goal,

such as a specific or stable trend in census size or N_E over a certain time period, at which time genetic monitoring can be conducted every three years. Newly established populations (either reestablished extirpated populations or the establishment of Middle SKC) should be genetically monitored every three to five years until such targets have been reached, and every three to five years thereafter. These monitoring recommendations should be integrated into the adaptive management framework so that translocations are only occurring when needed, and are stopped when goals have been achieved.

Section 6.4 Adaptive management recommendations

As described in *Section 5*, adaptive management is the integration of planning, management actions, monitoring and reevaluation of the success of management actions, and is crucial for the success of any management plan. Adaptive management will serve as a way to evaluate individual management actions and provide a record of successes and failures. In addition, this framework encourages managers to seek and remain open to new information and data that may be revealed during the restoration and monitoring processes. Within the adaptive management framework, as technology advances and new types of information are made available, the management process will be strengthened and goals are more likely to be met.

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APPENDIX 1

GENETICS REPORT: MICROSATELLITE AND RAD-SEQ ANALYSIS ON THE PAIUTE CUTTHROAT TROUT

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INTRODUCTION

There are a total of nine populations of the federally threatened Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*, hereafter PCT; Table 1). These populations are all refuge populations for the species, located in non-native habitat and containing valuable replicates of individual fish and genetic variation. To date, genetic studies have largely focused on examining levels of introgression with nonnative salmonids in these refuge populations (Cordes et al. 2004, Stephens et al. 2011). Cordes et al. (2004) found no introgression with rainbow trout (*O. mykiss*, RT) or Lahontan cutthroat trout (*O. c. henshawi*, LCT) based on seven microsatellite markers and one scDNA (single copy DNA) marker. An additional study looking for introgression was conducted using more powerful diagnostic (fixed for alternate alleles) SNP (single nucleotide polymorphism) loci (Stephens et al. 2011). This second study supported the results of Cordes et al. (2004), and also ruled out introgression with California golden trout (*O. m. aguabonita*, CAGT).

This report complements and expands on the genetic studies of Cordes et al. (2004) and Stephens et al. (2011) by examining genetic diversity within and among refuge populations using additional microsatellite markers and preliminary next-generation sequencing data. The results from this study can provide information to managers on how the genetic diversity of the

refuge populations has changed over time, give insight into how to minimize loss of genetic diversity, and elucidate how past management practices may have affected current genetic diversity. Finally, the next-generation sequencing data will provide preliminary insight into whether or not the Paiute cutthroat trout have alleles unique to the subspecies, or simply contain a subset of the broader genetic diversity of their sister species, the Lahontan cutthroat trout. We will conclude by placing our research in the context of previous genetic work.

METHODS

Sample collection

Between 16 and 80 individual PCT fin clip samples were collected for genetic analyses by California Department of Fish and Wildlife (CDFW) personnel and volunteers from five refuge populations in the Silver King Creek watershed (Upper Silver King Creek (SKC), Fly Valley Creek (FVC), Four Mile Canyon Creek (FMC), Coyote Valley Creek (COY), and Corral Valley Creek (COR)) and four out-of-basin refuge populations (Stairway Creek (STW), Sharktooth Creek (SHK), North Fork Cottonwood Creek (NFC) and Cabin Creek (CAB)). All populations were sampled in 2000 and all but CAB were sampled again in 2010, 2011, or 2012 (see Figure 1 for map taken from Cordes et al. 2004, and Table 2 for sample codes, number of individuals sampled per population, and years collected). We used the Qiagen DNeasy DNA extraction kit to extract DNA from samples collected between 2010 and 2012.

For next-generation sequencing analysis, in addition to previously extracted PCT samples, we used samples from Lahontan cutthroat trout shared by the Peacock Lab at the University of Nevada, Reno, and samples from Bonneville (*O. c. utah*, BCT), Yellowstone (*O. c.*

bouvieri, YCT), and Westslope (*O. c. lewisi*, WCT) cutthroat trout shared by Idaho Department of Fish and Game (see Table 3 for number of individuals, population code, and date collected).

Microsatellite analysis

Population genetic diversity and differentiation

We characterized genetic diversity and differentiation by genotyping the collected samples at fourteen microsatellite loci, amplified in five multiplex reactions. Each reaction had a general master mix with a total volume of 10 μL with 2 ng template DNA, 2 mmol $MgCl_2$, 125 mmol each dNTP, and 0.1–0.2 mmol forward sequencing primer labeled with a fluorescent dye (either VIC, 6FAM, NED, or PET), 0.06–0.2 mmol reverse primer, and 2 U Taq polymerase. Reactions spent 4 min at 95 °C; 25 cycles of 30 s at 95 °C, 30 s at 58 °C, 45 s at 72 °C, followed by 45 min at 60 °C. See Table 4 for primer combinations and concentrations for each multiplex. We added 8.8uL highly deionized formamide (Life Technologies, Carlsbad, California) and 1.5uL 500 LIZ (Life Technologies) to 2μL PCR product and denatured at 95°C for 3 min before electrophoresis on an ABI 3730xl Genetic Analyzer (Life Technologies). Fragments were scored using GENEMAPPER 4.0 software (Life Technologies), with allele sizes checked manually. Data was checked in Microchecker (Van Oosterhout et al. 2004) for spurious allele calls, null alleles, or large allele dropout.

We calculated standard genetic indices to evaluate overall genetic diversity. Allele frequencies for each locality were calculated in CONVERT (Glaubitz 2004). Deviations from Hardy-Weinberg equilibrium were calculated with GENEPOP version 4.0 software (updated from Raymond and Rousset 1995; Rousset 2008) using the exact test with a Markov-Chain Monte Carlo (MCMC) estimator of the probability that the observed sample was taken from a

population in Hardy-Weinberg equilibrium (Guo and Thomson 1992). Significant departures from linkage equilibrium were calculated in GENEPOP, followed by a sequential Bonferroni correction to correct for multiple tests (Rice 1989). Default MCMC parameters were used for both LD and HW tests (dememorization number = 1000, number of batches = 100, number of iterations per batch = 1000). We used Genetic Data Analysis software *(*GDA, Lewis and Zaykin 2001) to calculate expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (f_{IS}), and to detect private alleles (alleles unique to a sample group or population). We calculated allelic richness (R_S) in *HP-RARE* (Kalinowski 2005), which uses rarefaction to correct for the increased likelihood of detecting rare alleles with increased sample size (Kalinowski 2004). Pairwise F_{ST} values were calculated using the method of Reynolds et al. (1983) and Slatkin (1995) in ARLEQUIN (Excoffier and Lischer 2010) using 1000 permutations. Groups were defined as the collection of individual samples from a refuge population as sampled in a single year for a total of 17 groups so that F_{ST} values were calculated between and among samples at both time points.

We used the program STRUCTURE 2.3.3 (Pritchard et al. 2000) to investigate potential population genetic structuring by determining the most likely number of genetic clusters (*K*) and assigning individuals to specific clusters. This program uses a Bayesian model-based clustering algorithm to group individuals into populations based on allele frequency patterns. STRUCTURE analyses are useful for examining both current and historical connectedness and gene flow; populations that were once or are currently connected may have individuals that assign to multiple clusters. We ran *K* values 1-17 with three replicates for a total of 51 independent runs. We used a burn-in period of 100,000 and 750,000 Monte Carlo Markov

Chain (MCMC) repetitions using no prior information and assuming admixture and correlated allele frequencies. We used Structure Harvester (Earl and Vonholdt 2012), which provides two different methods of determining the most likely *K*. The first method is the L(*K*) method of determining *K* where the maximum value of the mean (over all independent runs) of LnP(D) for each *K* is chosen, and the second method is DeltaK (Evanno et al. 2005) where the most likely *K* has the largest second-order rate of change in negative log-likelihood.

We constructed neighbor joining (N-J) trees to visualize genetic distances among populations. We created three N-J trees: one with only samples from 2000, one with the more recent sample collections, and one with all refuge populations and both sampling times. Note that N-J trees do not necessarily portray phylogenetic relationships, just differences in frequencies of alleles that may result from evolutionary processes such as genetic drift. To construct the N-J tree, we used the *SEQBOOT* application in the software package *PHYLIP* version 3.69 (Felsenstein 1995) to simulate 1000 data sets before calculating Cavalli-Sforza and Edwards cord distances (1967; D_{CE}) for comparison between all pairs of sampling sites in *GENDIST* (Felsenstein 1995). The main assumption when calculating DCE is that differences in allele frequencies arise due to genetic drift only. We chose DCE because it does not assume that population sizes have remained constant or equal over time (Felsenstein 1995), and Takezaki and Nei (1996) found that DCE is more likely to recover true tree topology than other genetic distance estimates. We constructed N-J trees with the DCE matrices calculated in *GENDIST* using the *NEIGHBOR* application in *PHYLIP* (Felsenstein 1995). We rooted the N-J tree at the midpoint.

Population bottlenecks and N_E

N_E is a theoretical property of a population that is a function of the rate of genetic drift. Estimating N_E is important to managers since N_E, rather than census size, describes how a population responds to evolutionary forces. A smaller N_E will result in faster loss of genetic variation due to drift, possibly reducing the adaptive potential of a population. We calculated N_e values for each population with the sibship assignment method ($N_{E(SA)}$; Wang 2009), which estimates the frequencies of full and half-siblings in a cohort, and in turn uses this analysis to derive contemporary N_E. The $N_{E(SA)}$ method assumes that a random sample of individuals in the population were taken from the same cohort (no parent-offspring relationships), but does not random assume mating. $N_{E(SA)}$ can accept samples where multiple cohorts are included (such as with PCT), but power is reduced (Wang 2009). We used the software program COLONY (Jones and Wang 2009) to estimate $N_{E(SA)}$ using the options of female and male polygamy, nonrandom mating (appropriate for PCT), and the option of full likelihood long-length runs.

Directly measuring a population bottleneck is difficult without knowledge of historical population sizes. However bottlenecks can be inferred using microsatellite data with assumptions regarding microsatellite mutational models (Cornuet and Luikart 1996; Garza and Williamson 2001). We used two different tests to detect population bottlenecks: 1) the Wilcoxon signed-rank test for excess heterozygosity (H_k; Cornuet and Luikart 1996), implemented in the software *BOTTLENECK* (Piry et al. 1999), and 2) the *M*-ratio test (Garza and Williamson 2001), implemented in the software *M_P_Val* (http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298). Relative to the *M*-ratio test, the

H_k test performed in *BOTTLENECK* detects bottlenecks that are more recent, of lower severity, or where the pre-bottleneck value of θ ($\theta = 4N_E\mu$ where N_E is the effective population size, and μ is the mutation rate) was small. In contrast, the *M*-ratio test preferentially detects bottlenecks that are more severe (lasting several generations), where the pre-bottleneck θ was large, or where the population has made a demographic recovery (Williamson-Natesan 2005).

The H_K test implemented in *BOTTLENECK* operates on the theory that, during a bottleneck, rare alleles are more likely to be lost while common ones are retained, and the latter have proportionately stronger influence on heterozygosity (Cornuet and Luikart 1996). *BOTTLENECK* creates a null distribution of alleles under mutation drift-equilibrium using a chosen mutation model. A Wilcoxon signed-rank test is used to test for significant heterozygosity excess in comparison to the null distribution. We used a two phased model (TPM) with the parameters recommended in Piry et al. (1999) (variance = 12, proportion of stepwise mutations = 0.95). Each run was 5000 iterations.

The *M*-ratio (*M*), implemented in *M_P_Val,* is the ratio of the number of alleles at a locus (*k*) over the observed range of allele fragment sizes at that locus (*r*). *M* will decline after a bottleneck when alleles are randomly lost, opening "gaps" in the expected series of alleles faster than the size range declines. We calculated *M* using the conservative values of proportion of stepwise mutations (p_s) = 0.90, average size of non one-step mutations ($delta_g$) = 3.5, and θ = 10 as recommended by Garza and Williamson (2001). A bottleneck is generally inferred when *M* <0.68 (Garza and Williamson 2001).

RAD-Seq data collection and analyses

We conducted a Restriction site associated DNA sequencing (RAD-seq) study to further elucidate the relationship among Paiute and Lahontan cutthroat trout populations. To do this, two individuals from each of the nine PCT refuge populations, 14 LCT populations, two BCT populations, two WCT populations and three YCT populations were assembled into two libraries for RAD-seq analysis (Table 3). We used a modified laboratory protocol from Miller et al. (2011) that is available upon request. For our SNP discovery panel, we used a total of seven individuals (two PCT, two LCT, and one each BCT, YCT, and WCT) selected from both libraries chosen to represent the diversity of the individuals and with highest read counts (see Table 3 for populations used in discovery panel). Data was then filtered to remove loci with >20% missing data for all further analysis. To identify diagnostic or fixed loci among the pairs of subspecies, we used Python code (available upon request) which searched for alleles represented in one group but not another, investigating both the unfiltered and filtered SNP data sets.

With this SNP data set, as with the microsatellite data set, we used ARLEQUIN (Excoffier and Lischer 2010) to calculate pairwise F_{ST} values between subspecies using 1000 permutations. Groups were defined by subspecies, for a total of five groups. To visualize the genetic distances between LCT and PCT samples, we created a N-J tree using the filtered data set in the program PHYLIP version 3.69 (Felsenstein 1995), with D_{CE} in the subprograms SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE with 1000 bootstrap replicates.

We conducted STRUCTURE analyses to determine the most likely number of genetic clusters, *K*, in the PCT and LCT RAD-seq data set using no prior information and assuming

admixture and correlated allele frequencies. STRUCTURE runs for *K* values from 1 - 10, with three replicates for each *K*, were performed for a total of 30 independent runs (burn-in = 100,000, MCMC replicates = 750,000).

RESULTS

Microsatellite analysis

Population genetic diversity

Fourteen microsatellite loci (Table 4) were used to genotype 656 PCT from eight populations at two time points, and one population (00CAB) at one time point. Three loci were dropped from further analysis because they were monomorphic (OMM1108 and OCH35) or nearly monomorphic (OCH34). The remaining 11 loci had three (OMM1046 and OMM1082) to seven (OMM1088, OCH30, OCH18, OtsG85) alleles per locus (Table 4). Several of the populations were monomorphic for one locus: 2000SHK, 00NFC, 12STW, 11NFC, and 10FMCC are monomorphic for allele 203 at locus OMM1082. 00COR is monomorphic for allele 167 at locus OCH30.

Three populations showed significant departure from HWE: 10FMC ($P < 0.001$), 00COR ($P = 0.02$), and 00SKC ($P = 0.03$). For linkage disequilibrium tests, 79 out of 875 tests were significant before a Bonferonni correction, and two out of 875 tests were significant after the correction (nominal $P<0.05$), (between loci OCH18 and OMM1046 in 00NFC and between loci OMM1088 and OCH30 in 00FMC). Allelic richness (R_S) was calculated with 12 gene copies (the lowest number of gene copies in any one sample group): 00SHK had the lowest R_S (2.13) and 00CAB had the highest (2.96). H_E values ranged from 0.347 (10FMC) to 0.559 (00CAB) and observed heterozygosity from 0.284 (10FMC) to 0.570 (00CAB, Table 2). Heterozygosity levels

increased over time in some populations, such as in SKC, where H_E changed from 0.447 to 0.507, and decreased in others, like FMC, where H_E decreased from 0.419 to 0.347. Several populations had private alleles: 10SKC, 00FMC, 00FVC, 00SKC, and 00CAB each had one private allele, while 12STW and 12SHK each had two private alleles. The increase in some genetic diversity values may be due to increased sample effort in the second sampling. See Table 5 for allele frequencies at each of the 11 analyzed microsatellite loci in each sample group of PCT. Finally, f_{IS} values (the probability that two alleles are identical by descent, a measure of inbreeding) varied from -0.088 (00SHK) to 0.184 (10FMC). f_{IS} values range from -1.0 to 1.0, with negative values indicating outbreeding, and higher positive values indicating inbreeding.

Population Genetic Differentiation

F_{ST} is a measure of population differentiation or lowered levels of gene flow, with a significant value indicating population structure. F_{ST} values range from 0 to 1, with values from 0-0.05 generally being considered low genetic differentiation, 0.05-0.15 moderate, 0.15-0.25 great, and higher than 0.25 very great levels of differentiation (Hartl and Clark 1997). Within-year pairwise F_{ST} values between locations sampled in 2000 were all significant except for between 00FVC and 00COY (F_{ST} = 0.004, P=0.169; Table 4), and range from 0.004 (00FVC, 00COY) to 0.419 (00SHK, 00COR). Within-year pairwise F_{ST} for 2010-2012 range from 0.006 (between 10FVC and 10COY) to 0.376 (between 12SHK and 10FVC), and all are significant except between 10FVC and 10COY (P = 0.143, Table 4). Between-year F_{ST} comparisons for all locations ranged from <0.001 between 00FVC and 10FVC and 0.413 (between 00COR and 12SHK). Generally, between-year pairwise F_{ST} values for a refuge population were not

significant (P>0.05), except for between 00SKC and 10SKC, 00COR and 10COR, and 00FVC and 10FVC.

Some populations generally had higher pairwise F_{ST} than others. For example, in 2000, within year pairwise F_{ST} values between SHK and other populations ranged from 0.172 (00CAB) to 0.419 (00COR), and within the samples collected from 2010-2012 from 0.229 (11NFC) to 0.376 (10COR). NFC also had higher F_{ST} values in comparisons between it and other samples. In 2000, pairwise F_{ST} between NFC and other populations ranged from 0.069 (00CAB) to 0.252 (00SHK), and in 2010-2012, F_{ST} ranged from 0.097 (10COY) to 0.229 (12SHK). Populations with generally lower pairwise F_{ST} in 2000 include between 00SKC and 00FVC between 00SKC and 00COY, between 00FMC and 00STW, between FVC and 00CAB, and between 00NFCC and 00CAB. In 2010-2012 there were also a number of lower pairwise F_{ST} values, such as those between 10FVC and 10SKC, 10SKC and 11NFC, 10SKC and 10COY, 10FVC and 10COR. Average pairwise F_{ST} value between populations remained similar from 2000 to 2010-2012 from 0.164 to 0.163, respectively.

STRUCTURE provided strong support for K=2 using the Ln(K) method and the Evannao et al. method, with support for additional substructure at K=5. Resulting bar plots are depicted in Figures 2a and 2b. At K=2 there is one major division within the refuge populations, with SHK, NFC, and CAB forming the first cluster, and all other refuge populations forming the second cluster (Figure 2a). At K=5, population substructure is weaker, but clusters generally consist of 1) NFC and CAB, 2) SHK, 3) FVC, COY, and SKC, 4) STW and FMC, and 5) COR (Figure 2b).

The N-J tree generally conforms to the tree from Cordes et al. (2004) (Figure 3). There are three general groupings, one consisting of FVC, COY and SKC, including COR, one consisting

of STW and FMC, and a clade comprised of NFC, CAB and SHK. Each of the oldest populations (FVC, FMC and NFC) was represented on a different branch. Two additional branches show differentiation between more recently founded populations: COR forms a branch off of the FVC clade with 87% bootstrap support, and STW forms a branch close to FMC with 82% bootstrap support. Interestingly, 00CAB formed a branch between 00NFC (49% bootstrap support) and 11NFC (51% bootstrap support). However these relationships have lower bootstrap values, so should be interpreted with caution.

Bottleneck and N_E

No bottlenecks were detected using the *M*-ratio test (*M* ranges from 0.70 in 00FMC to 0.79 in 10COY). However, bottlenecks were detected in 10SKC, 00FVC, and 00CAB using the H_K test (Table 7).

PCT have very low N_E values, ranging from six (00SHK; CI=2-20) to 31 (10SKC; CI=20-52) using the $N_{E(SA)}$ method (Table 7). N_E values from some populations increased over time, such as in SHK (00SHK = 6, 12SHK = 16), and some decreased, such as in FMC (00FMC = 15, 10FMC = 7). However, N_E must be interpreted with caution, given that the microsatellite markers had few alleles per locus, and some groups had low sample sizes (N <50).

RAD-Seq Analysis

We obtained a total of 134,929,444 reads from the two RAD-seq libraries, after removing reads due to low quality. Individual read counts (number of sequences returned per individual) ranged from 434,850 (BCT_ BL01) to 5,377,306 (YCT_HL10). The number of diagnostic loci between subspecies before filtering out loci with more than 20% missing data ranged from N=7 (between BCT and YCT) to N =5,758 (between BCT and PCT; Table 8). After

filtering, the number of diagnostic SNPs between groups ranged from N =4 (BCT and YCT) to N =4,933 (BCT and PCT). Before filtering, there were 91 diagnostic loci between PCT and LCT groups, and after filtering there were 62. Pairwise F_{ST} values on the filtered data set ranged from 0.063 (between PCT and YCT), and 0.953 (between PCT and BCT). All pairwise F_{ST} values were significant (P <0.050) except between YCT and BCT (F_{ST} =0.063, P =0.353), and between WCT and YCT (F_{ST} = 0.457, P =0.05) (see Table 8 for pairwise F_{ST} values and number of diagnostic SNPs between subspecies).

For the *STRUCTURE* analysis, the strongest supported number of clusters was K=2, with additional substructure at K =4. When K =2, PCT and LCT each comprise their own distinct clusters and fish from Independence Lake show a small amount of ancestry in the PCT cluster (Figure 4a). At K =4, PCT still form their own cluster, but the LCT groups break out into additional genetic clusters (figure 4b), with WASH and LIN grouping together, PFC, MCC and CAR grouping together, and FOR, WMR, FRZ, ABEL and TIC grouping together. SUM, INL, and SLN all show ancestry of more than one genetic cluster.

For the PHYLIP analysis, we removed individuals with more than 20% missing data (one individual from WASH and both individuals from LIN). When all remaining PCT and LCT individuals were included (N=43), individuals from each population consistently grouped more closely with each other than with individuals from any other location (Figure 5). For ease of viewing, we trimmed the tree to include only one individual from each location. The N-J tree grouped all PCT refuge populations together with strong support (100% bootstrap replicates). LCT individuals from Independence Lake grouped closely with this clade as a sister taxon (81% bootstrap support). Two additional clades of LCT were well-supported: one consisting of WMR,

FOR, FRZ, BEAV, ABEL, WASH, TIC (78% bootstrap support), and one consisting of CAR, MCC, SUM, and PFC (63% bootstrap support).

DISCUSSION

We addressed three major questions in this genetics report: 1) what is the current population genetic structure and diversity in PCT refuge populations, and has this structure and diversity changed over time, and 2) Does next-generation sequencing data shed additional light on the phylogenetic relationship between LCT and PCT, and 3) how do our analyses compare with previous genetic analyses. We will address these questions separately in the sections that follow.

1) Population genetic structure and diversity of refuge populations

The PCT refuge populations have relatively low heterozygosity levels for inland native trout (Stephens et al. 2013), though direct comparisons cannot be made due to the different microsatellite markers used. Further, the population genetics supports the findings of Cordes et al. (2004) by generally reflecting the stocking history of the species and the effect of small isolated populations differentiating over time. Bottlenecks and inbreeding were detected in some refuge populations, and N_E values are very low. Pairwise F_{ST} values indicate significant genetic differentiation between populations that were once a single population. This is most likely due to founder effect at the time of stocking, genetic drift, and/or bottleneck events, all of which can cause allele frequency differences that increase F_{ST} values.

Bottlenecks were detected by the H_K test (which detects more gradual bottlenecks that may be the result of a slow decline in population census size) in some of the populations. It is somewhat surprising that the *M*-ratio test did not detect more severe bottlenecks, but this test

may not have enough power to detect bottlenecks due to the low heterozygosity of the microsatellite markers used in this study. The low number of alleles per microsatellite locus may be due to the initial bottleneck during the PCT colonization event, meaning the PCT never had much genetic diversity to begin with, or it may be due to more recent bottlenecks and genetic drift.

FVC, FMC and NFC are the oldest refuge populations, founded in the 1940s or earlier (Table 1). They also represent the three main clades in the N-J tree, with their recipient populations forming closer branches with their founders. The additional branches represent differentiation in recipient populations, such as COR and STW, which form their own sub-branches with strong bootstrap support. FVC, FMC, and NFC also assign to a different cluster in STRUCTURE when *K*=5. This differentiation is most likely due to founder effect and genetic drift.

SHK is one of the most differentiated populations based on these analyses. SHK was stocked with 29 founders from FVC and Delaney (which was founded with fish from FMC and FVC) in 1968. 12SHK also has two private alleles, is fixed for a non-private allele at OMM1082, and has lower allelic richness. This evidence suggests substantial differentiation from the other refuge populations since the time of founding.

STW is somewhat differentiated from the other refuge populations and is most closely related to FMC based on the analyses herein. STW was founded with 70 fish derived from FMC and FVC. STW has relatively low heterozygosity levels, yet it has two private alleles. This could be due to sampling error, but there is a possibility that this allele has been lost in FVC and FMC since the establishment of STW, or they represent two new mutations (though this scenario is

less likely). This population should be monitored more frequently, as the private alleles indicate that there is genetic diversity in this population not represented elsewhere.

NFC and CAB are less differentiated, reflecting the fact that NFC was stocked with 401 fish that trace back to FVC (via COY, SKC, and COR), and CAB was stocked with 60 fish from NFC in 1968, 22 years after NFC was founded. These populations also had low pairwise F_{ST} values between each other and clustered together in the STRUCTURE analysis with K=5. CAB is also located downstream of NFC, but it is thought that there is no current gene flow between these two populations. Given the low number of founders for CAB, it is surprising that it has the highest allelic richness and heterozygosity values, and a private allele, while NFC, the donor population, has no private alleles despite robust sampling size in 2011. As such, CAB is an important refuge population and is important to resample to assess the current genetic status.

SKC underwent some genetic changes in the intervening years between sampling. This could be due, in part, to the difference in sample size between 2000 (N =38) and 2010 (N =80). For example, heterozygosity values increased substantially, as did allelic richness. SKC sampled at both time points each had a single private allele. However both SKC sample groups formed a clade in the NJ tree. Overall SKC is the most robust population demographically, occupies the largest habitat area, and has high allelic richness and heterozygosity values. This population is a good founder population candidate for future translocation efforts.

The population in FMC should be of concern to managers. Heterozygosity levels and N_E fell between 2000 and 2010, and population surveys indicated that census size had also declined. The f_{IS} level indicates inbreeding, which would be more likely to occur in the event of a bottleneck, which was detected using the H_K test. This population should continue to be

monitored closely. Habitat restoration may be needed, or conditional supplementation if the reason for decline is addressed.

2) Next-generation sequencing analysis of phylogenetic relationships

Preliminary analysis with the RAD-seq dataset found additional support for monophyly of the Paiute cutthroat trout clade. The 6,626 filtered SNPs provided new information about the differences between PCT and LCT. By using three PCT and three LCT individuals in our SNP discovery panel, we found markers that showed frequency differences between these groups. We also found 91 loci that were potentially diagnostic between the groups. In addition, there has been discussion regarding how genetically distinct PCT is from LCT. Pairwise F_{ST} values and the N-J Tree using SNP data support the PCT as a monophyletic clade branching off from the LCT, and STRUCTURE identifies PCT as a single genetic cluster. One would expect the LCT populations in the Carson River Drainage, downstream of Silver King Creek, to be most closely related to the PCT due to geographic proximity, but the closest sister taxa to the PCT clade in the N-J tree was Independence Lake (81% bootstrap support). Independence Lake is a small self-sustaining population of LCT within the Truckee river drainage. Further analysis is warranted to explore this finding. STRUCTURE results supported the close relationship between INL and PCT, with the individuals from INL assigning in part to the PCT clusters for both K =2 and K =4 (Figures 4a and 4b).

3) Comparison with past genetic analyses

Our work here more fully resolves the relationship between LCT and PCT. Most work to date supports a single colonization event that created the PCT through isolation in Silver King Creek (Behnke 1992). Nielsen and Sage (2002) used 10 microsatellite markers to examine

differentiation between 10 LCT populations, PCT (represented by 16 fish from FMC), and two populations of "Humboldt cutthroat trout" (represented by fish from West Mary's River and Frazer Creek). In their analyses, PCT had no unique alleles, but they found relatively high pairwise F_{ST} values between LCT and PCT (F_{ST} =0.667), as well as HCT and PCT, F_{ST} =0.619). They suggest that the significant genetic differentiation is largely due to founder effect from the colonization event which reduced genetic diversity overall, and that the PCT colonization event likely occurred before Lake Lahontan receded and isolated Lahontan and Humboldt cutthroat trout from each other (Benson and Thompson 1987, Behnke 1992). This conclusion was based on the lack of unique alleles in PCT, the paraphyletic relationship revealed between LCT and HCT revealed by an N-J tree and similar pairwise F_{ST} values between PCT and HCT, and PCT and LCT. We believe that it is likely that PCT arose during a single colonization event, but the data does not support the timing of the colonization event, or a paraphyletic relationship. We will address their three sources of evidence below.

The Nielsen and Sage (2002) study design only used 16 individuals from one population of PCT. Given the complicated history of PCT, it is important to use individuals from more than one, if not all refuge populations. In addition, the PCT may indeed have a subset of alleles from the Lahontan and Humboldt cutthroat trout analyzed, but if these three groups diverged from each other, they will also share a subset of alleles, so it is not surprising that PCT would have a subset of alleles from both groups.

The Nielsen and Sage (2002) N-J tree did not resolve the relationships between the PCT and specific LCT and HCT populations with strong bootstrap support. Hillis and Bull (1993) suggest that nodes with <70% bootstrap support do not provide reliable recovery of the true

tree topology. When applying this to Nielsen and Sage (2002), when branches with <70% bootstrap support are collapsed, there is a polytomy (unresolved clade) including Edwards Creek, Macklin Creek, Pilot Peak, PCT (from FMC), Frazer Creek and West Mary's River. This lack of resolution is probably due to the low power of the microsatellite markers used and insufficient taxon sampling.

Finally, F_{ST} values cannot reliably be used to date a colonization event or phylogenetic relationships between groups. At the time of publication, they suggest that further work with samples from additional PCT populations was warranted to further elucidate these relationships, and we concur with this statement.

Peacock and Kirchoff (2007) used 10 microsatellites to examine the relationships within and among various LCT groups, for which they added 48 PCT samples (collection location not reported) to the analyses as a phylogenetic outgroup. This report was not primarily concerned with resolving phylogenetic relationships between PCT and LCT. In their analyses they created various N-J trees with different population groupings. In an N-J tree of the Carson River watershed LCT populations, the PCT formed its own clade with 42% bootstrap support (Figure 30 in Peacock and Kirchoff 2007). In a second N-J tree that included western basin populations (Figure 36 in Peacock and Kirchoff 2007), PCT formed a branch consisting of a polytomy with Independence Lake (43% bootstrap support). In a third watershed-based analysis produced a tree with a closer relationship to the Carson River populations of LCT (Pyramid Lake, Summit Lake, Carson River and O'Harrel Creek, 49% bootstrap support; Figure 37 in Peacock and Kirchoff 2007). Again, nodes with bootstrap support <70% are considered unreliable (Hillis and Bull 1993), and these relationships were probably not resolved due to insufficient taxon

sampling and/or a lack of power in the microsatellite data. Additionally, resolving phylogenetic relationships between PCT and LCT was not the aim of the report.

Pritchard et al. (2013) conducted more recent work using next-generation sequencing data agreed with Nielsen and Sage (2002) in that PCT only contain a subset of the genetic diversity found in the LCT. This is likely the result of ascertainment bias, where a small number of individuals are used in the SNP discovery process (e.g. Morin et al. 2004). This biases SNPs towards the groups included in the discovery panel. Pritchard et al. (2013) did not include PCT in their discovery panel, so it is not surprising that they did not find SNP loci that are diagnostic between LCT from PCT. By including PCT in our SNP discovery panel we have identified potentially diagnostic markers, or loci that may be unique to the PCT.

CONCLUSION

This report provides new information regarding the population genetic status of the PCT refuge populations and preliminary data resolving the phylogenetic relationship between LCT and PCT. In general, the PCT refuge populations are moderately to highly differentiated from each other, have relatively low heterozygosity levels, and low N_E's. This is likely due to genetic drift because each population has gone through a founder event upon its establishment. However, very few genetic bottlenecks were detected, though this could be due to low power of the microsatellite markers. In addition, relationships between populations reflect stocking history.

We further resolved some phylogenetic relationships between PCT and LCT, and identified 91 potentially unique SNP alleles and diagnostic loci to distinguish between PCT and LCT. In addition to loci informative between PCT and LCT, we have identified loci that may

distinguish PCT from other CT subspecies. These loci may be useful in the future for identifying unknown samples, or looking for hybridization. Our analyses are preliminary, and more LCT individuals should be genotyped to determine if the loci are truly diagnostic. However it is likely that the PCT do indeed have unique alleles that have either arisen since the time of their isolation or have been maintained in this subspecies since the colonization event.

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Table 1. Table showing information about the nine refuge populations, including name, code used in this study, number of founders and date of founding event, source of founders, and notes.

Location	Code	Number of founders	Date	Source	Notes
Upper Silver King creek	SKC	139	1994	COY	
		49	1995	FVC	
		109	1995	COY	
		134	1996	COY	
		145	1997	COY	
		30	1998	FVC	
Fly Valley Creek	FVC	54	1947	COR, COY	
Four Mile Canyon Creek	FMC	?	Unknown, before 1956	Original SKC transfer or fish movement	Thought a plant by "stockmen"
Coyote Valley Creek	COY	54	1989	FVC	Originally stocked from SKC ~1860-1890s by loggers
Corral Valley Creek	COR	20	1978	FVC	Originally stocked from SKC ~1860-1890s by loggers
North Fork Cottonwood Creek	NFC	401	1946	COY (61%), SKC (32%), COR (7%)	
Cabin Creek	CAB	60	1968	NFC	
Sharktooth Creek	SHK	29	1968	FVC (79%), DEL (21%)	Stocked in Sharktooth Lake
Stairway Creek	STW	77	1972	DEL	
Cabin Creek	CAB	60	1968	NFC	
Delany Creek	DEL	43	1966	FMC (93%), FVC (7%)	Population extirpated due to brook trout

Table 2. Number of individuals analyzed (N), expected and observed heterozygosity values (H_E and H_O), number of alleles per population (N_A), number of private alleles, f_{IS} values, and allelic richness (R_S) based on 12 gene copies. Values are based on microsatellite analysis.

Population	**N**	H_E	H_O	N_A	**Private alleles**	f_{IS}	R_S
00STW	44	0.370	0.372	32		-0.007	2.22
12STW	39	0.404	0.385	32	2	0.048	2.22
00SKC	38	0.447	0.475	32	1	-0.063	2.36
10SKC	80	0.507	0.526	36	1	-0.037	2.61
00SHK	16	0.383	0.415	26		-0.088	2.13
12SHK	40	0.422	0.417	30	2	0.012	2.28
00FVC	38	0.458	0.476	31	1	-0.039	2.35
10FVC	40	0.446	0.398	31		0.109	2.31
00NFC	33	0.432	0.389	30		0.099	2.29
11NFC	50	0.462	0.444	34		0.041	2.45
00COR	33	0.391	0.341	29		0.130	2.16
10COR	50	0.424	0.406	31		0.043	2.30
00COY	40	0.476	0.482	32		-0.012	2.43
10COY	40	0.451	0.464	35		-0.028	2.43
00FMC	37	0.419	0.435	30	1	-0.038	2.17
10FMC	42	0.347	0.284	31		0.184	2.15
00CAB	49	0.559	0.570	42	1	-0.020	2.96
Mean	41	0.433	0.427	32	0.588	0.020	2.34
Total N	656						

Table 3. Samples included in RAD-seq libraries. Information includes subspecies, creek, or lake the individual was sampled from, population code, general area or drainage, year sampled, and number (N).

Subspecies	Creek/Lake	Code	General area/Drainage	Year sampled	N
Lahontan cutthroat trout	East Fork Carson River*	CAR	Carson River	2001	2
	Foreman Creek	FOR	Humboldt River	2001	2
	Frazer Creek*	FRZ	Humboldt River	2000	2
	Tierney Creek	TIC	Reese River	2000	2
	Poison Flat Creek	PFC	Carson River	2001	2
	Murray Canyon Creek	MCC	Carson River	2001	2
	Independence Lake	INL	Truckee River	2001	2
	Slinkard Creek	SLN	Walker River	2001	2
	Line Canyon Creek	LIN	Quinn River	2001	2
	Washburn Creek*	WASH	Quinn River	2001	2
	Summit Lake	SUM	Quinn River	2001	2
	Beaver Creek (Maggie)	BEAV	Humboldt River	2001	2
	West Mary's River	WMR	Humboldt River	2001	2
	Abel Creek	ABEL	Humboldt River	2001	2
Paiute cutthroat trout	Cabin Creek	CAB	Mono County, CA	2000	2
	Corral Valley Creek	COR	Alpine County, CA	2000	2
	Coyote Valley Creek	COY	Alpine County, CA	2000	2
	Stairway Creek	STW	Madera County, CA	2000	2
	Sharktooth Creek	SHK	Fresno County, CA	2000	2
	North Fork Cottonwood Creek	NFC	Mono County, CA	2000	2
	Four Mile Canyon Creek	FMC	Alpine County, CA	2000	2
	Fly Valley Creek	FVC	Alpine County, CA	2000	2
	Upper Silver King Creek	SKC	Alpine County, CA	2000	2
Yellowstone cutthroat trout	Henrys Lake	HL	Upper Snake River/Henry's Fork, ID	1998	2
	Blackfoot River*	B	Upper Snake River/Blackfoot, ID	2002	2
	Geode Creek	GE	Yellowstone River Drainage, WY	2005	2
Westslope cutthroat trout	Cannuck Creek	C	Moyle River/Kootenai Drainage, ID	2005	2
	Garden Creek*	G	Main Fork Salmon River Drainage, ID	2002	2
Bonneville cutthroat trout	Glenwood Fish Hatchery	GW	Sevier River, UT	2004	2
	Bear Lake*	BL	Bear Lake, ID/UT	2003/2004	2
Total					**60**

* indicates used in SNP discovery

Table 4. Microsatellite markers used in this study. Information includes multiplex, primer concentration and dye used for amplifying markers. Results include number of alleles per locus (N_A), observed and expected heterozygosity values (H_O and H_E), inbreeding coefficient (f_{IS}), and size range of alleles in each marker.

Locus	Multiplex	[Primer]	Dye	N_A	H_E	H_O	f_{IS}	Size Range
OMM1058[1]	2	0.15	PET	6	0.584	0.500	0.145	214-234
OMM1108[1]	2	0.10	6FAM	1	-	-	-	152
OCH20[2]	2	0.10	VIC	5	0.527	0.457	0.133	289-317
OMM1088[1]	3	0.15	PET	7	0.676	0.585	0.135	207-231
OCH30[2]	3	0.08	6FAM	7	0.364	0.290	0.204	113-183
OCH34[2]	3	0.02	VIC	2	-	-	-	293-297
OCH18[2]	5	0.15	PET	7	0.525	0.495	0.057	245-289
OCH35[2]	5	0.15	VIC	1	-	-	-	179
OtsG85[3]	10	0.20	6FAM	7	0.541	0.462	0.146	223-227
OMM1097[1]	10	0.08	VIC	4	0.532	0.437	0.179	279-291
OMM1046[4]	10	0.06	NED	3	0.479	0.413	0.138	138-146
OMM1082[1]	11	0.08	6FAM	3	0.176	0.149	0.153	199-203
OMM1051[1]	11	0.10	VIC	6	0.592	0.453	0.235	404-424
OMM1011UW[5]	11	0.08	NED	6	0.643	0.555	0.136	172-240

[1] Rexroad et al. (2002b)

[2] Robinson et al. (2009)

[3] Williamson et al. (2002)

[4] Rexroad et al. (2002a)

[5] Spies et al. (2005)

Table 5. Allele frequencies for each population at the 11 microsatellite markers used in this study.

Locus	Allele	00 STW	00 SKC	00 SHK	00 FVC	00 NFC	00 COR	00 COY	00 FMC	00 CAB	12 STW	10 SKC	12 SHK	10 FVC	11 NFC	10 COR	10 COY	10 FMC	Overall
OMM1058	214											0.01							<0.01
	218					0.07									0.08				0.01
	222		0.16	0.36	0.13	0.14	0.42	0.16	0.06	0.49		0.33	0.24	0.24	0.18	0.17	0.11		0.19
	226	0.92	0.51	0.43	0.48	0.79	0.33	0.50	0.83	0.38	0.82	0.46	0.63	0.51	0.74	0.37	0.46	0.82	0.58
	230	0.08	0.32	0.21	0.38		0.24	0.34	0.11	0.13	0.15	0.20	0.14	0.25		0.46	0.43	0.18	0.21
	234										0.03								<0.01
OCH20	289	0.30	0.11		0.29	0.02	0.15	0.34	0.26	0.35	0.27	0.23		0.36	0.11	0.10	0.36	0.23	0.21
	293								0.10									0.04	0.01
	309		0.37	0.28	0.14			0.11	0.03		0.04	0.26	0.28	0.09		0.10	0.10	0.08	0.11
	313	0.70	0.51	0.72	0.56	0.98	0.85	0.51	0.61	0.64	0.69	0.48	0.71	0.55	0.89	0.80	0.51	0.65	0.66
	317		0.01					0.04		0.01		0.04	0.01				0.03		0.01
OMM1088	207												0.01						<0.01
	211			0.56		0.52				0.47			0.46		0.40				0.12
	215	0.10	0.27		0.08	0.02	0.02	0.04	0.12	0.08		0.09		0.03	0.03	0.08	0.09	0.01	0.07
	219	0.69	0.59		0.59	0.42	0.45	0.64	0.38	0.19	0.62	0.58		0.71	0.44	0.50	0.71	0.23	0.47
	223	0.00	0.11		0.14	0.05	0.06	0.16		0.05		0.16		0.11	0.13	0.13	0.08	0.00	0.08
	227	0.21	0.03	0.44	0.18		0.47	0.16	0.49	0.21	0.38	0.17	0.53	0.15		0.29	0.13	0.76	0.26
	231								0.01										<0.01
OCH30	113					0.03				0.01									<0.01
	163											0.01					0.01		<0.01
	167	0.67	0.95	0.25	0.90	0.68	1.00	0.88	0.54	0.69	0.59	0.83	0.26	0.91	0.61	0.99	0.88	0.83	0.75
	171													0.01	0.01	0.01			<0.01
	175										0.01								<0.01
	179	0.32	0.05	0.75	0.10	0.29		0.13	0.46	0.30	0.40	0.16	0.74	0.08	0.38		0.11	0.15	0.24
	183	0.01																0.01	<0.01
OCH18	245				0.01														<0.01
	269									0.11					0.02				0.01
	273						0.09						0.03			0.03			0.01
	277	0.06		0.31		0.06				0.08	0.22		0.50	0.01	0.12			0.01	0.07
	281	0.88	0.62	0.69	0.46	0.68	0.41	0.65	0.80	0.42	0.73	0.59	0.48	0.50	0.65	0.51	0.70	0.87	0.62
	285	0.07	0.38		0.53	0.26	0.48	0.35	0.20	0.38	0.05	0.41		0.49	0.21	0.46	0.30	0.12	0.29
	289						0.02			0.01									<0.01
OtsG85	223					0.50				0.15	0.01				0.18				0.05
	227	0.10	0.32		0.38	0.21	0.32	0.46	0.39	0.31	0.15	0.32		0.31	0.24	0.37	0.35	0.14	0.27
	231			0.67	0.02					0.02		0.01	0.47					0.01	0.04
	270								0.01					0.01			0.01		<0.01
	274	0.03									0.03	0.05					0.01	0.18	0.02
	278	0.83	0.68	0.33	0.61	0.29	0.68	0.54	0.59	0.52	0.77	0.63	0.53	0.68	0.57	0.63	0.61	0.65	0.61
	282	0.04									0.04				0.01		0.01	0.01	0.01

OMM1097	279			0.08						0.15		0.04	0.18						0.03
	283	0.52	0.45	0.04	0.46	0.45	0.94	0.39	0.77	0.47	0.49	0.27	0.12	0.61	0.56	0.77	0.54	0.79	0.51
	287	0.48	0.55	0.88	0.54	0.55	0.06	0.61	0.23	0.31	0.51	0.69	0.70	0.39	0.43	0.23	0.46	0.21	0.45
	291									0.07					0.01				0.01
OMM1046	138		0.01																<0.01
	142	0.15	0.26	0.80	0.53	0.56	0.06	0.45	0.28	0.58	0.23	0.44	0.83	0.45	0.46	0.20	0.49	0.21	0.40
	146	0.85	0.72	0.20	0.47	0.44	0.94	0.55	0.72	0.42	0.77	0.56	0.18	0.55	0.54	0.80	0.51	0.79	0.60
OMM1082	199	0.08	0.20		0.03		0.35	0.09	0.01	0.04		0.13		0.14		0.30	0.08		0.09
	203	0.92	0.80	1.00	0.97	1.00	0.65	0.91	0.99	0.96	1.00	0.87	0.99	0.86	1.00	0.70	0.93	1.00	0.91
	207												0.01						<0.01
OMM1051	404									0.02									<0.01
	408			0.04		0.02	0.02			0.17			0.08		0.03				0.02
	412	0.01	0.07	0.75		0.32		0.05		0.18	0.01	0.06	0.77		0.15	0.01	0.04	0.05	0.12
	416	0.28	0.09	0.21	0.29	0.45	0.33	0.24	0.39	0.29	0.21	0.38	0.15	0.23	0.39	0.18	0.21	0.62	0.30
	420	0.66	0.83		0.67	0.21	0.56	0.70	0.59	0.32	0.78	0.56		0.76	0.43	0.78	0.74	0.33	0.55
	424	0.05	0.01		0.04		0.09	0.01	0.01	0.02		0.01		0.01		0.03	0.01		0.02
OMM1011UW	172	0.32	0.04	0.06	0.12	0.15	0.02	0.29	0.01	0.18	0.29	0.16	0.16	0.14	0.18	0.06	0.19	0.02	0.15
	224							0.01		0.02									<0.01
	228	0.18	0.55	0.06	0.66	0.58	0.35	0.45	0.47	0.51	0.21	0.47	0.13	0.65	0.59	0.33	0.61	0.23	0.43
	232	0.01	0.07		0.01			0.03			0.03	0.07			0.01	0.02	0.04		0.02
	236	0.48	0.34	0.81	0.21	0.27	0.64	0.23	0.51	0.23	0.45	0.30	0.63	0.21	0.22	0.59	0.16	0.75	0.39
	240	0.01		0.06						0.05	0.03		0.09						0.01

Table 6. Pairwise F_{ST} values estimated in ARLEQUIN for microsatellite data. Values in bold are not significant (P>0.05) after 1000 simulations.

	00 STW	00 SKC	00 SHK	00 FVC	00 NFC	00 COR	00 COY	00 FMC	00 CAB	12 STW	10 SKC	12 SHK	10 FVC	11 NFC	10 COR	10 COY
00SKC	0.117	-														
00SHK	0.341	0.343	-													
00FVC	0.146	0.040	0.330	-												
00NFC	0.160	0.170	0.215	0.130	-											
00COR	0.192	0.149	0.419	0.163	0.252	-										
00COY	0.082	0.033	0.291	**0.004**	0.123	0.166	-									
00FMC	0.063	0.136	0.298	0.126	0.147	0.156	0.111	-								
00CAB	0.170	0.137	0.172	0.077	0.069	0.165	0.091	0.128	-							
12STW	**0.008**	0.119	0.293	0.126	0.158	0.199	0.076	0.055	0.156	-						
10SKC	0.100	0.035	0.241	0.015	0.106	0.154	0.009	0.115	0.080	0.098	-					
12SHK	0.332	0.348	**0.002**	0.322	0.211	0.413	0.298	0.295	0.186	0.284	0.258	-				
10FVC	0.121	0.038	0.354	**0.000**	0.145	0.129	**0.014**	0.113	0.093	0.115	0.035	0.351	-			
11NFC	0.101	0.128	0.228	0.093	**0.008**	0.203	0.089	0.090	0.060	0.099	0.087	0.229	0.098	-		
10COR	0.136	0.065	0.369	0.091	0.207	0.030	0.091	0.128	0.152	0.139	0.103	0.376	0.072	0.164	-	
10COY	0.104	0.037	0.332	**0.005**	0.139	0.173	**0.001**	0.112	0.103	0.098	0.034	0.334	**0.006**	0.097	0.094	-
10FMC	0.132	0.222	0.351	0.215	0.220	0.170	0.190	0.061	0.196	0.132	0.176	0.341	0.209	0.183	0.164	0.206

Bold = Not significant (P>0.05)

Table 7. This table depicts results from the *M*-ratio and H_K bottleneck tests. For the *M*-ratio test, M is shown. A bottleneck is generally inferred when *M*<0.68 (Garza and Williamson 2001). For the H_K test, the *P*-value is reported and bottlenecks are inferred when *P*<0.05 for the one-tailed Wilcoxan sign ranked test for heterozygosity excess for the TPM model (Variance = 12, proportion of non-stepwise mutations = 0.95). Estimated $N_{E(SA)}$ values as estimated in COLONY are reported with 95% confidence intervals.

Location and year	***M***	**TPM *P*-value**	$N_{e(SA)}$	**95% CI**
00STW	0.75	0.768	17	9-34
12STW	0.77	0.385	18	10-38
00SKC	0.76	0.232	13	7-30
10SKC	0.77	**0.051**	31	20-52
00SHK	0.75	0.313	6	2-20
12SHK	0.76	0.139	16	9-34
00FVC	0.70	**0.087**	16	8-34
10FVC	0.78	0.207	20	12-38
00NFC	0.71	0.138	13	7-30
11NFC	0.76	0.065	22	13-42
00COR	0.75	0.138	16	9-35
10COR	0.78	0.232	17	9-36
00COY	0.77	0.062	15	8-31
10COY	0.79	0.551	21	12-39
00FMC	0.75	0.139	15	9-31
10FMC	0.77	0.862	7	4-21
00CAB	0.72	**0.027**	26	16-47

Table 8. Below diagonal: pairwise F_{ST} results calculated in ARLEQUIN for SNP data. Values in bold are significant after 1000 simulations (P<0.05). Above diagonal: number of diagnostic markers before filtering/after filtering out loci with more than 20% missing data.

Subspecies	BCT	LCT	WCT	PCT	YCT
BCT	-	4982/4278	1726/1554	5758/4933	7/4
LCT	**0.922**	-	2686/2363	91/62	3304/2698
WCT	**0.818**	**0.906**	-	3398/2970	136/79
PCT	**0.953**	**0.487**	**0.951**	-	4112/3339
YCT	0.063	**0.886**	0.457	**0.905**	-

Bold = significant (P<0.05)

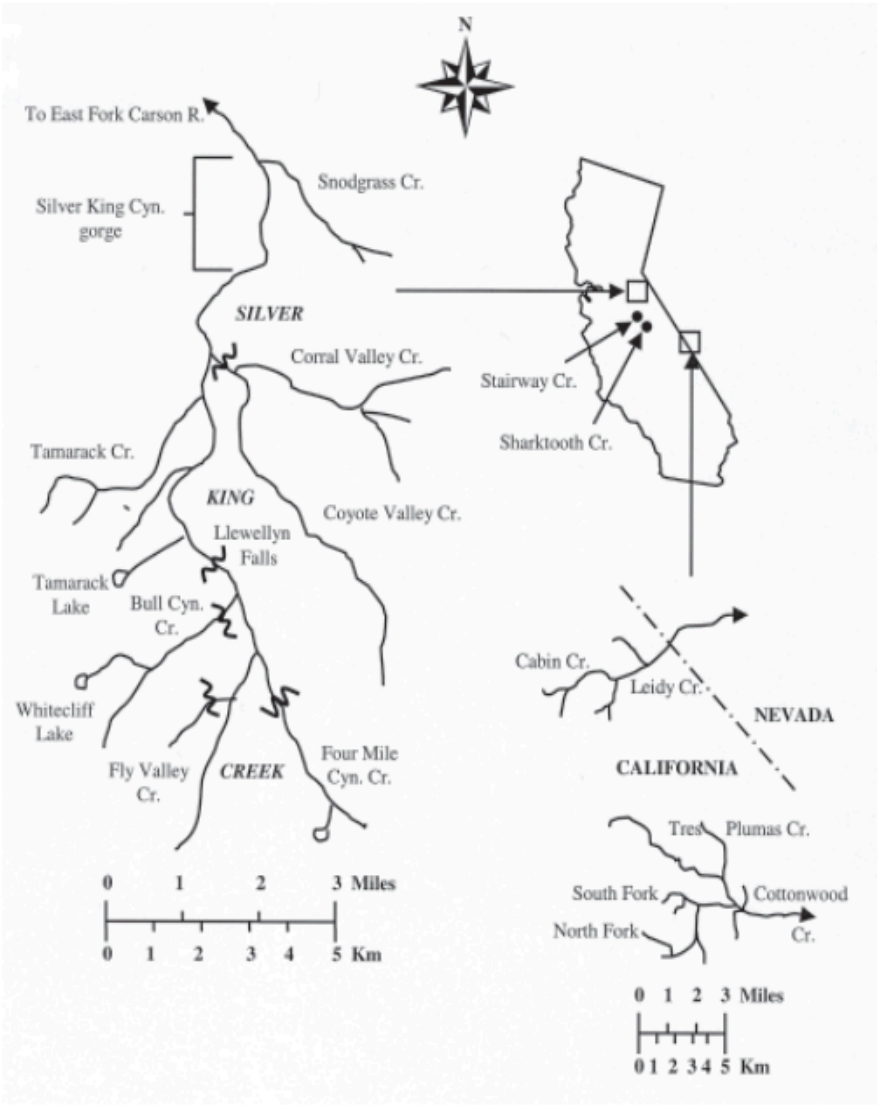

Figure 1. Map of the nine refuge populations of the PCT taken from Cordes et al. (2004).

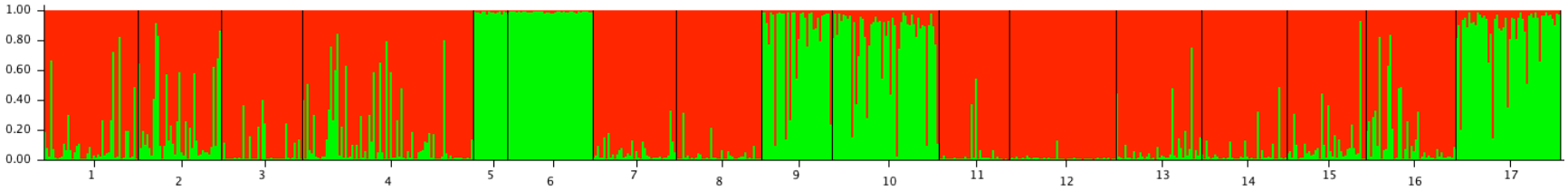

Figure 2a. Structure output with *K*=2. The bar graph is broken into 17 groups, representing as follows: 1-2 = STW, 3-4 = SKC, 5-6 = SHK, 7-8 = FVC, 9-10 = NFC, 11-12 = COR, 13-14 = COY, 15-16 = FMC, 17=CAB. Each vertical bar represents an individual fish and its proportion of assignment to each of *K*=2 groups.

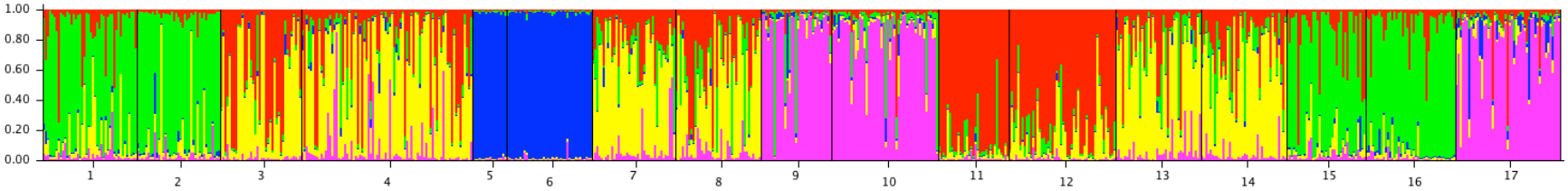

Figure 2b. STRUCTURE output with *K*=5. Populations are numbered as in figure 1a, and each vertical bar represents an individual sample and its proportion of assignment to each of *K*=5 genetic clusters.

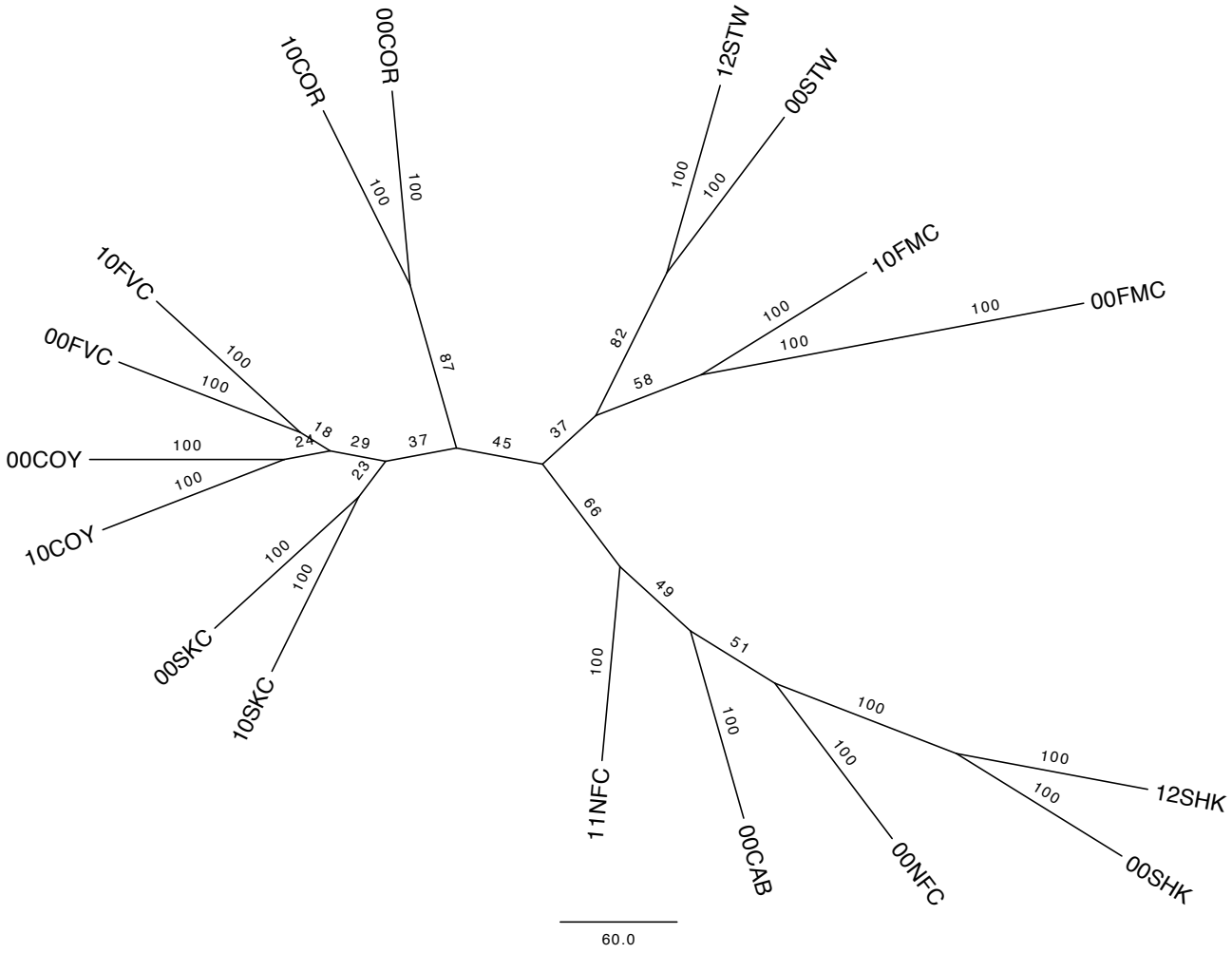

Figure 3. Neighbor-joining tree depicting genetic distances between Paiute refuge populations sampled at two time points using 11 microsatellite loci. Numbers indicate percent bootstrap support (100 replicates

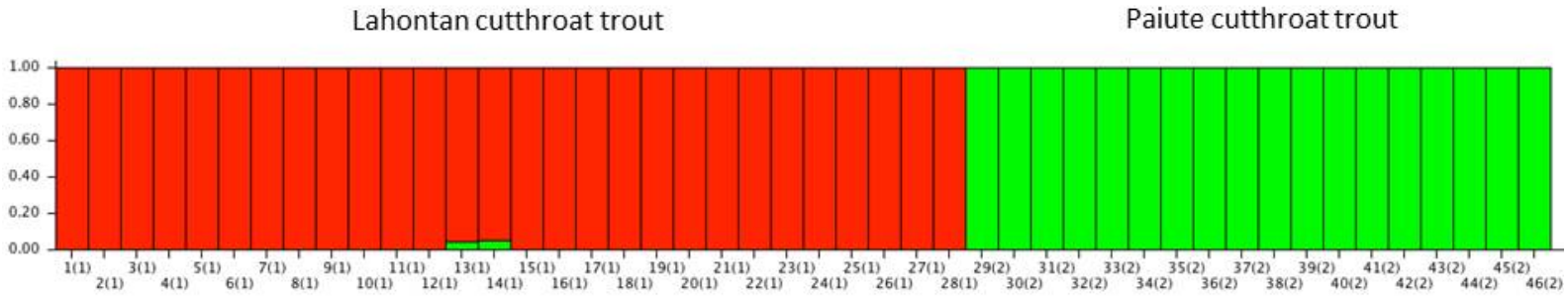

Figure 4a. Structure results with RAD-seq data where *K*=2. Each vertical bar represents and individual: 1-2 (PFC), 3-4 (SUM), 5-6 (FOR), 7-8 (CAR), 9-10 (BEAV), 11-12 (15 and 16 are from Washburn Creek, 13 and 14 are from Independence Lake, and 25 and 26 are Line Canyon Creek). Colors represent proportion assignment to *K*=2 genetic clusters.

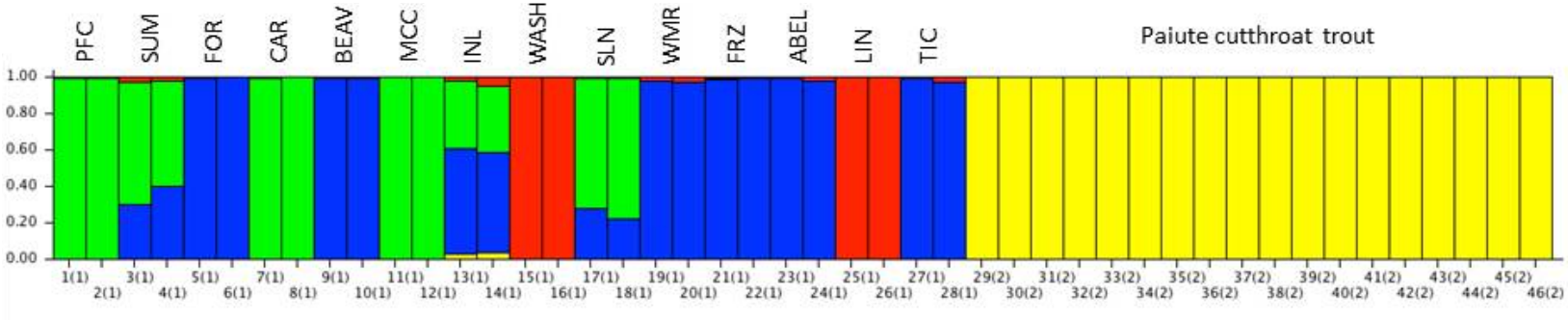

Figure 4b. STRUCTURE results with RAD-seq data where *K*=4. Individual vertical bars are as in Figure 2a. Colors represent proportion assignment to *K*=4 genetic clusters.

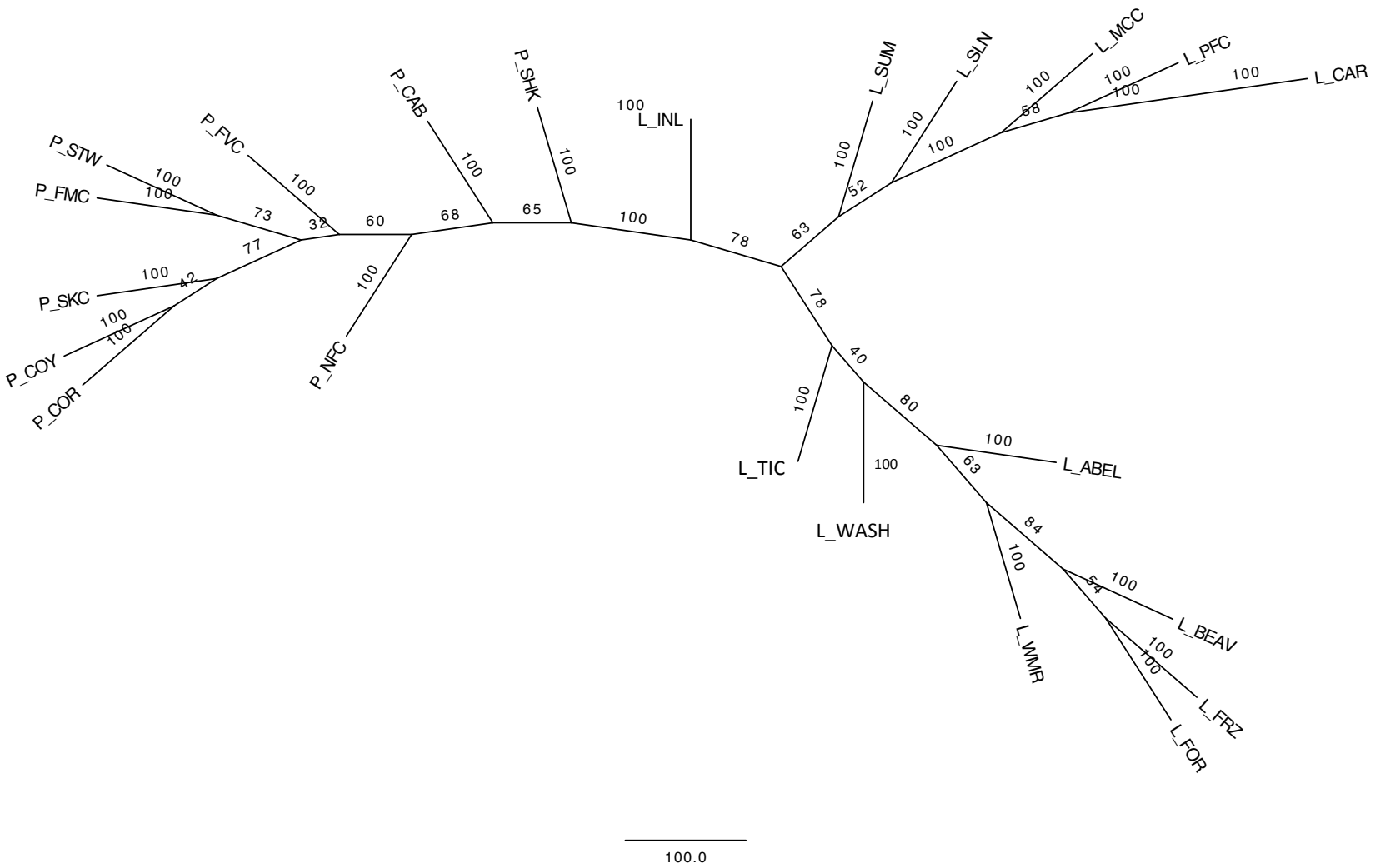

Figure 5. Neighbor-joining tree depicting Cavalli-Sforza-Edwards cord distances (DCE) between PCT and LCT using 6,626 SNP loci. Numbers at nodes indicate percent bootstrap support after 1000 replicates.