Systematics, Genetics and Conservation of Golden Trout

BY

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This work is dedicated to Stan Stephens, retiring after 32 years of service to the people and resources of the State of California and 30 years devoted to golden trout conservation.

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Abstract

The evolutionary genetic relationships among rainbow trout (*Oncorhynchus mykiss*) subspecies and conservation genetic status of two native subspecies were examined using nuclear and mitochondrial DNA variability. In Chapter 1, Amplified Fragment Length Polymorphism (AFLP) markers were used to evaluate subspecies relationships among native rainbow trout groups. A deep genetic division was found between golden and redband groups. Within the golden trout, strong support exists for California golden trout (*O. m. aguabonita*), Little Kern golden trout (*O. m. whitei*), and Kern River rainbow trout (*O. m. gilberti*) as distinct subspecies. A highly divergent McCloud River redband in Sheepheaven Creek also warrants subspecific status. The Columbia redband (*O. m. gairdneri*) also formed a distinct group that included the Catlow Basin populations. Remaining redband trout groups reflect the history of hydrogeographic fluctuation and connectivity in this region.

The conservation of threatened and endangered species relies upon an accurate understanding of species composition in the native range. In Chapter 2, Single Nucleotide (SNP) markers were validated and applied to California golden trout populations to detect and quantify rainbow trout introgression. SNP data supported results from previous microsatellite and minisatellite DNA genetic studies, detecting low, localized levels of hybridization with rainbow trout in the Golden Trout Creek drainage and a hybridization cline in the South Fork Kern River. SNP markers detected rainbow trout introgression in all sampled localities within the South Fork Kern, ranging in magnitude from 2% in Upper Mulkey Creek (a headwater tributary) to 29% in Upper Trout Creek.

In Chapter 3, the comparative SNP-microsatellite approach was extended to the federally threatened Little Kern golden trout, a subspecies that has been reintroduced into large portions of its native range after chemical treatment of its native streams to eradicate rainbow trout hybrids. Both microsatellite and SNP datasets detected similar levels and patterns of rainbow trout hybridization, with hybridized individuals found in a few key localities within the native range and also in Little Kern golden trout broodstock previously used in restoration. Microsatellite analyses showed extant population genetic structure and low diversity that is likely a signature of restocking restoration efforts.

Chapter 1: Subspecies relationships among native rainbow, golden, and redband trout (*Oncorhynchus mykiss spp.*) in western North America

Abstract

Genetic relationships among 8 purported subspecies of native rainbow trout and hatchery rainbow strains (*Oncorhynchus mykiss spp.*) from western North America were evaluated using Amplified Fragment Length Polymorphism (AFLP) markers. Based on 149 polymorphic AFLP loci, multiple analysis approaches including ordination, phylogenetic tree construction and hierarchical Bayesian analysis generally corroborated one another in their support for several major groups of *O. mykiss*, including the subspecies designation of several previously established groups: California golden trout (*O. m. aguabonita*), Little Kern golden trout (*O .m. whitei*), Kern River rainbow trout (*O. m. gilberti*) and the McCloud redband of Sheepheaven Creek (*O. m. stonei* or *O. m. spp*.). Sheepheaven redband trout were the most distinct group of all rainbow trout subspecies, with a large degree of genetic distinction between this and all other rainbow trout populations evaluated. Golden and redband groups were deeply split, supporting the hypothesis of a more distant common ancestor. Several redband trout populations examined do not show significant resolution from one another based on AFLP data; however, there is limited evidence for distinction between Davis Creek (Sacramento redband) and Northern Great Basin redbands, and shared similarities between the Catlow Valley (Northern Great Basin) redband populations and redbands of the Columbia Basin. Lack of genetic differences among certain redband groups for the loci we examined requires reliance on non-molecular data in support of their taxonomic distinction.

Introduction

Various attempts have been made to elucidate the systematic relationships among native inland rainbow trout (*Oncorhynchus mykiss*) groups through a variety of techniques, from morphological to cytological to molecular genetic (Bagley and Gall 1998; Behnke 1992; Gold 1976; Gold and Gall 1975; Shreck and Behnke 1971). The shallow differentiation of these taxa (reviewed by Behnke 2002) follows from their relatively recent post-Pleistocene radiation and has made subspecies delimitation somewhat complex. After the rainbow trout lineage diverged from the cutthroat trout lineage approximately two million years ago, most native rainbow trout subspecies evolved in allopatry; however, Pleistocene hydrographic fluctuation also afforded bouts of temporary habitat connectivity and opportunities for gene flow between rainbow trout groups, particularly among the redband trout in the Great Basin (see Oakey et al. 2004 and references therein). Similarly, trout groups in the Kern River basin likely retained some connectivity with coastal rainbow trout in the absence of geographic isolating barriers.

Anthropogenic influence over the past 150 years has superimposed a signature of introgression between native and introduced rainbow trout, further complicating relationships within and among groups. Of particular conservation concern are the golden and redband trout groups, many of which have been reduced to small portions of their native ranges by habitat alteration and introgression with introduced rainbow trout strains (Behnke 1992; Cordes et al. 2006). Apparent lack of reproductive isolating mechanisms between native and non-native groups has resulted in introgression and subsequent decline in native subspecies. Establishing clear systematic relationships of

remaining non-hybridized populations of these native trout is critical to their conservation both for focusing conservation efforts at the appropriate levels and for determining appropriate reference populations for studies of introgression among groups.

The subspecific classification of native *O. mykiss* has been characterized as a tension between taxonomic "lumpers" and "splitters" (Behnke 1992; Behnke 2002), and designation of various populations and groups as species or subspecies has changed numerous times since their original identification (reviewed in Bagley 1998). Evidence from the most recent comprehensive morphological analysis of all taxa (Behnke 1992) supports to varying extents the existence of several major groups, including: coastal rainbow trout (*O. m. irideus*), interior (also referred to as "inland" or "Columbia") redband trout (*O. m. gairdneri*), McCloud River and Sacramento redband trout (*O. m. stonei*[1]), Northern Great Basin redband trout (*O. m. newberii*[1]), and California (aka "Volcano Creek") golden trout (*O. m. aguabonita*). The distinctiveness of the McCloud River redband, in particular the Sheepheaven Creek population, probably warrant subspecies designation. While evidence for morphological distinction of Little Kern golden trout (*O. m. whitei*) from the Kern River rainbow trout (*O. m. gilberti*) was found lacking by Behnke (1992), he supported the separation of the former for management purposes; Smith and Gall (1981) attribute this lack of distinction to the inclusion of hybridized Little Kern populations in the original (Schreck and Behnke 1971) morphometric analysis of specimens. Likewise, Eagle Lake rainbow trout (*O. m. aquilarum*) lacked morphometric support, although striking ecological differences exist for this population (and see molecular evidence below).

[1] Subspecies nomenclature after Behnke (1992), not formally recognized

Molecular approaches shed additional light on subspecies relationships within *O. mykiss*. Previous studies of introgressive hybridization have compared different populations within subspecies groups to one another or to rainbow trout in studies of introgressive hybridization. Few studies, however, have simultaneously addressed the distinctness of all native California trout subspecies relative to one other, with some notable exceptions. Bagley and Gall (1998) used mitochondrial DNA and single copy nuclear DNA (scnDNA) sequence variability to assess genetic structure and relatedness among 10 groups of rainbow trout. They found strong genetic structuring among the rainbow trout subspecies groups overall, support for the morphometric groups described by Behnke (1992), and support for the distinction of *O. m. whitei* and *gilberti* as separate subspecies based on the presence of highly divergent mtDNA. Phylogenetic patterns between marker types, however, were discordant and lack of monophyly for Goose Lake (Northern Great Basin) redband, Sacramento River coastal rainbows, Kern River rainbows and coastal steelhead even by mtDNA confounded clear distinctions among groups. Furthermore, levels of polymorphism at several single-copy nuclear DNA loci examined were insufficient to confirm the more prominent mitochondrial DNA patterns observed. A second study (Nielsen et al. 1999) focusing on relationships among McCloud and other redband and golden groups found strong evidence for the distinctiveness of Sheepheaven Creek and other McCloud fish, but limited resolution of deeper relationships among redband and golden trout groups.

To resolve these systematic issues, we performed a comprehensive nuclear genetic analysis of all native California trout subspecies relative to one another using Amplified Fragment Length Polymorphisms (AFLP). Although commonly applied to

plant taxa, AFLPs have been recognized as an underutilized technique for animals (Bensch and Akesson 2005). The AFLP technique is a relatively inexpensive, simple, reproducible method that generates large numbers of polymorphic markers presumed to have a random distribution throughout the genome (Bagley et al. 2001; Vos et al. 1995). Consequently, these markers have been useful for examining genetic variation both at and below the species level (reviewed in Mueller and Wolfenbarger 1999). Additionally, AFLP techniques have proven valuable in individual assignment analyses at levels comparable to microsatellites (Campbell et al. 2003), and are frequently used for species diagnostic markers (Tranah et al. 2003; Young et al. 2001). The dominant nature of AFLPs and associated need to estimate allelic states effectively reduces the individual locus information content they provide, complicating demographic parameter estimation (Holsinger et al. 2002; Wong et al. 2001), Palacios et al. 1999). For the purposes of understanding overall relationships, the wide sampling of the genome and the large number of loci provided by AFLPs likely compensate for this deficiency. In addition, recent advances in analytical methodology have improved the estimation of parameters from AFLP data, particularly in the extension of Bayesian analyses to dominant data (Falush et al. 2007; Holsinger et al. 2002).

The objective of this study was to improve our understanding of molecular relationships among designated subspecies of the *O. mykiss* species group to determine 1) molecular genetic support for established and proposed subspecific designations, 2) overall relationships of subspecies to one another in a phylogenetic context, and 3) whether known (or cryptic) hybridization events are apparent in the analyzed populations. Nine of the ten native rainbow trout groups evaluated by Bagley and Gall (1998) were

included in this study (we did not evaluate interior redband steelhead trout, *O. m. gairdneri*), and several additional hatchery rainbow trout strains were also examined. The genome-wide survey that the AFLP technique affords allows us to better examine the overall differentiation among these major groups using a highly polymorphic, neutral, nuclear marker.

Materials and methods

Samples

A total of 199 samples were obtained, representing the major *O. mykiss* groups and one cutthroat trout outgroup (localities and scientific names given in Table 1.1, Figure 1.1). Because the study was concerned with the overall evolutionary relationships among native trout, a relatively small number of representative samples were assayed for a large number of loci (common for systematics studies and in contrast to a typical population genetics study, which employs fewer markers but much larger sample sizes). Representatives of native groups included were selected as the best available remaining "pure" members of their group, that is, non-introgressed based on morphology or genetic information, when available. California golden trout samples were selected based on previous microsatellite analyses demonstrating little to no detectible levels of introgression with rainbow trout (Cordes et al. 2006; Cordes et al. *in press*). California golden trout samples from Upper Trout Creek, a population characterized by microsatellite analysis as highly introgressed with rainbow trout (Cordes et al. *in press*), served as a known hybridized reference sample. Little Kern golden trout were selected mainly from non-hybridized headwater populations above barriers or restored populations derived from non-hybridized populations. Kern River rainbow trout

populations were selected based on previous allozyme analyses supporting their distinction from other golden trout (Gall and May 1997). Redband trout represent a more variable group, with subspecific designations being somewhat vague. East Fork Nelson Creek and Davis Creek populations were selected to represent Sacramento redband from the Pit River drainage and the morphometrically distinct Sheepheaven Creek fish represented the Sacramento redband of the McCloud Basin group. Northern Great basin and interior redband samples were provided by Doug Markle, Oregon State University, based on allozyme analyses by Currens (1997) that evaluated redband group relationships. Two populations of Surprise Valley redbands from Mill and Cedar creek, outside the purported historic range, were included as likely transfers of unknown origin.

Wild rainbow trout strains from the Navarro, Feather, and American River drainages were obtained to be inclusive of shared ancestral polymorphisms between rainbow and other native coastal California *O. mykiss*. The hatchery strains examined were included as the strains most likely to have hybridized with native redband and golden trout populations. Lastly, Paiute cutthroat trout outgroup samples were obtained from North Fork Cottonwood creek, a transplanted population shown by microsatellite analysis to be genetically non-introgressed (Cordes et al. 2004). Genomic DNA was extracted from either dried, DMSO- or 95% ethanol-preserved fin clip tissues using Promega Wizard™ 96 well extraction kits.

AFLP data collection

Each sample was assayed for variation with the AFLP technique (Vos et al. 1995) as modified by Agresti et al. (Agresti et al. 2000), using a combined digestion-ligation step, followed by a pre-amplification step with Eco + A and Mse + C primers (6uM) to

reduce the number of potential bands to amplify and create unlimited sample for subsequent selective amplification. Selective amplification with nine primer combinations of 5' fluorescein-labeled Eco-ANN primers and an unlabeled Mse-CNN primers yielded products that were run on 5.0% denaturing (7.5M urea) polyacrylamide gels and visualized with a Fluorimager 595 (Molecular Dynamics™). Positive controls were included on each gel to ensure consistency of both amplification and visualization of fragments. Multiple samples from each subspecies were included in triplicate as positive controls for a second run of new populations to confirm that both the digestion and selective amplification of products were consistent between sample runs. Bands of equal size were treated as a putative locus and all polymorphic loci in the 100-400 base pair size range were scored visually for presence or absence of fragments.

Data analysis

ARLEQUIN (ver. 3.11Excoffier and Schneider 2005) was used to calculate number and percent of polymorphic loci for each population. Nei's (1978 unbiased) measures of genetic distance and identity for pairs of sampled populations were calculated in POPGENE (Yeh 1997) TFPGA (Table 3). A dendrogram was constructed using UPGMA (Unweighted Paired Group Method with Arithmetic mean) as implemented in POPGENE, and bootstrap values obtained by 1000 permutations using TFPGA (Miller 1997). Jaccard's similarity indices were calculated using the SimQual module of NTSYSpc (Rohlf 2002) and Principal Coordinate Analysis (PCA) was used to identify clustering of groups based on Jaccard's similarity measures using the Dcenter and Deigen procedures in NTSYSpc (Rohlf 2002). Iterative PCAs of individuals based on their genetic affinities was performed to determine whether additional structure

existed among the observed clustering. Though more descriptive and visual in nature, multivariate procedures are quite useful, particularly for situations of in which admixture is known or suspected (Moazami-Goudarzi and Laloe 2002)

We used STRUCTURE version 2.2 (Falush et al. 2007; Pritchard et al. 2000a) identify population structure at and above the subspecies level. STRUCTURE applies a Markov chain Monte Carlo algorithm to cluster individuals based on multilocus genotypes and estimate the proportion of an individual's genome attributable to each cluster. The newest version 2.2, however, accounts more appropriately for the ambiguity of recessive (absent) alleles in dominant marker data sets and has been used successfully for AFLP data sets (Albert et al. 2006; Falush et al. 2007). We conducted a hierarchical analysis of individuals (after Rosenberg et al. 2001), whereby an initial evaluation of all rainbow trout individuals using five independent runs each of K=1-10 were performed using 100,000 burn-in period, 300,000 MCMC iterations, no prior probabilities, and assuming correlated allele frequencies for both the admixture and no-admixture models. Groups were selected by examining plots of negative log likelihood values visually as well as calculating the value of delta K (Evanno et al. 2005) to determine the most likely number of clusters in the data set. Each identified respective cluster was subsequently examined for additional subcluster structuring using both admixture and no admixture models, 30,000 burn-in and 100,000 MCMC iterations and individuals grouped according to the cluster accounting for the greatest proportion of their genome. Where applicable, clusters and subclusters were then associated with one or more *O. mykiss* subspecies groups. The outputs of STRUCTURE runs were visualized in the program DISTRUCT (Rosenberg 2004).

Results

AFLP statistics

We obtained 149 polymorphic loci that could be scored unambiguously in all individuals (Table 1.2). *O. mykiss* and *O. clarki* exhibited dramatic differences in AFLP genotypes and represented the only level at which fixed differences between groups were exhibited. The number of polymorphic loci scored ranged from 8 to 23 for each primer combination. Nei's pairwise genetic distances and identity for sampled populations are shown in Appendix 1.1 for each population.

UPGMA analysis

The UPGMA cluster analysis based on Nei's genetic distances (Table 1.3) between all taxa examined generated a dendrogram with a major division separating Sheepheaven Creek from all other populations, and two subsequent clades containing golden trout in one and redband and rainbow trout in the other, though these latter clades generally lacked bootstrap support for deep nodes (Figure 1.2a). Columbia redband trout and Catlow Valley Northern Great Basin redband populations formed a clade. Wild and hatchery rainbow, Mount Shasta, Coleman, and Pit hatchery trout, and Eagle Lake populations formed an unsupported monophyletic clade sister to the redband populations, while the Mount Whitney Hatchery population grouped outside the redband/rainbow clade. Non-hybridized California golden trout from both Golden Trout Creek and South Fork Kern populations form a well supported monophyletic group. The heavily rainbow-introgressed Upper Trout Creek sample did not cluster with California golden trout, but instead in a separate clade with Little Kern golden trout (Figure 1.2a). Little Kern golden trout formed a paraphyletic group, with Grey Meadow and Upper Fish Creek populations

grouping as sister to the entire golden clade. Kern River rainbow trout from Chagoopa Falls and Kern-Kaweah were nested within the Little Kern golden trout, indicative of the close relationship of Kern River rainbows to Little Kern golden trout.

The presence of a known hybridized Upper Trout Creek population and the suspected admixed Upper Coyote Creek population (see PCA section below) likely accounts for some of the unexpected topology observed in Little Kern golden trout populations. Likewise, the inclusion of hatchery populations known to have mixed ancestry (Busack and Gall 1980) may also affect tree topology. Therefore, a second UPGMA dendrogram excluding these populations, as well as the questionable East Fork Nelson Creek population (see PCA section below), resulted in a tree with slightly more consistent relationships but with some striking differences, namely the clustering of wild rainbow trout with members of the golden trout clade (Figure 1.2b). Little Kern golden trout still formed a paraphyletic group, with Upper Fish and Grey Meadow Creek populations clustering separately from other Little Kern golden trout. Relationships among redband groups remained essentially the same, except for the grouping of Davis Creek redband individuals with the Fall Creek redband population. Eagle Lake rainbow trout grouped with Northern Great Basin redband populations (Figure 1.2b), where previously they had clustered with Pit and Coleman hatchery rainbow trout strains (Figure 1.2a).

Principal coordinate analysis

A series of PCA plots shows comparisons of rainbow and cutthroat trout and comparisons between different *O. mykiss* groups based on their genetic clustering. The PCA plot of all groups revealed as expected the distinctiveness of the cutthroat trout

outgroup (Figure 1.3). With this outgroup excluded (Figure 1.4), relationships among the various *O. mykiss* groups can be examined further; the most divergent group, the Sheepheaven redband, is distinct from all other groups, including all other redbands. Golden trout subspecies form a second distinct cluster and the Upper Coyote Creek population notably shows an affinity for California golden trout, not Little Kern golden trout, its purported subspecies group. A distinct third cluster contained hatchery and wild rainbow and all remaining redband trout.

The PCA plot comparing all golden trout groups to rainbow trout reveals the relative distinctiveness of all examined golden groups from rainbow trout (Fig 1.5). The California golden trout subspecies forms a single, cohesive group, non-overlapping with rainbow trout and other golden subspecies; individuals of the Little Kern golden and Kern River rainbow trout subspecies formed a cluster, distinct from both California golden and hatchery and wild rainbow trout, but not from one another. The PCA plot comparing all three golden trout subspecies (without rainbow trout) shows no overlap between Little Kern golden and Kern River rainbow trout (Figure 1.6a). Likewise, comparison of Little Kern golden trout and Kern River rainbow trout groups alone yielded two non-overlapping clusters, concordant with subspecies identity (Figure 1.6b). The Upper Coyote Creek population grouped consistently with California golden trout (Figure 1.5, 1.6a). In plots of California golden trout and hatchery and wild rainbow trout groups together, California golden trout individuals form a single, cohesive group, non-overlapping with rainbow trout populations, with the introgressed Upper Trout Creek sample positioned intermediately between the two groups (Figure 1.7).

PCA plots comparing all redband and hatchery and wild rainbow trout populations show the distinctiveness of Sheepheaven Creek from all other sampled redbands, including other Sacramento redband populations (Figure 1.8a). The level of differentiation is high, such that is difficult to examine other redband-rainbow relationships without excluding the Sheepheaven population, as done in Figure 1.8b, which shows the relationships of the remaining redband populations to one another. The Davis Creek population forms a separate cluster from other redband groups, including the East Fork Nelson Creek Sacramento redband population (Figure 1.8b). Similarly, the Columbia Basin redband populations form a distinct cluster that includes Catlow Valley populations (Home Creek and Upper Home Creek). These relationships remain stable even when hatchery rainbow trout (Figure 1.9) and all rainbow trout (Figure 1.10) are removed from the analysis.

Bayesian analysis

STRUCTURE analysis established two main clusters that delimited "golden," and "redband/rainbow" individuals (Figure 1.11a). Subsequent analysis by both the admixture and no-admixture model (latter not shown) of the "golden" group individuals yielded two subclusters (K=2, -lnK = -3,945, Figure 1.11b), one containing all *aguabonita* subspecies populations and the Upper Coyote Creek population, and the other containing all other *whitei* and *gilberti* populations. The subclustering of individuals for K=3 is shown for the admixture (Figure 1.11c; –lnK = -3,755) and no-admixture models (Figure 1.11d; -lnK = 3,790, versus -3,936 for K=2) for all individuals, and results in the clustering of individuals with their respective subspecies, *aguabonita*, *whitei*, and *gilberti*. Increasing K-values greater than three under both models yielded fractional assignment of

individuals to all three clusters. Evaluation of the "redband/rainbow" cluster yielded three subclusters: 1) Sheepheaven Creek population, 2) interior and Northern Great Basin redband, Eagle Lake rainbow, and Pit Strain rainbow trout, and 3) wild rainbow trout, Mount Shasta hatchery and Mount Whitney hatchery rainbow trout strains (Figure 1.11e). The Coleman hatchery rainbow trout strain was equally divided between subclusters two and three.

Discussion

Subspecific designations and phylogenetic relationships

The multiple and varied analysis approaches used in this study generally corroborate one another in their support for several major groups as subspecies of *O. mykiss*. The nuclear AFLP data show general agreement with the California and Little Kern golden trout groups identified by Behnke (1992) and lend support for a distinct Kern River rainbow trout. In addition, the AFLP data strongly supports a separate subspecies designation for the McCloud River redband of Sheepheaven Creek., The redbands of the Columbia Basin are clearly a distinct subspecies, though the subspecies may include additional populations in the geographically proximate Catlow Basin. The AFLP data do not provide significant resolution to resolve relationships among remaining redband trout populations, beyond their distinctiveness from wild and most hatchery rainbow trout. However, limited evidence exists for a distinct group including Davis Creek (Sacramento redband) and another grouping of Columbia Basin redbands.

Overall phylogenetic relationships

The phenotypic similarities between McCloud River redband and golden trout has led other researchers (Gold 1977; Shreck and Behnke 1971) to argue for shared ancestry

of these groups. The AFLP data contradict this view, with golden and redband groups being deeply and consistently divided in PCA, UPGMA and STRUCTURE analyses. Golden trout groups clustered separately from all other groups in the initial STRUCTURE analysis of all rainbow trout individuals, form a clearly divergent cluster in ordination of individuals, and evaluations of population-level similarities in the UPGMA analysis also depict a deep split between golden and redband groups.

The relationship of golden trout and with coastal rainbow trout populations (American River coastal rainbow and Navarro River steelhead rainbow trout groups represented in this study) is somewhat ambiguous; the PCA and STRUCTURE analyses clearly argue for the similarity of coastal rainbow trout to other redband groups (Figure 1.11, Figure 1.3), as does the phylogenetic tree containing all taxa (Figure 1.8a) However, the tree based on only "native" or non-introgressed samples (Figure 1.8b) depicts a close association between all wild rainbow trout populations and the Kern River rainbow and Little Kern golden trout groups. Either scenario is consistent with a Sacramento-San Joaquin origin of these groups.

Redband group relationships

Sheepheaven Creek redband emerge from PCA plots as the most distinct from all other trout groups, including all other redbands and even other Sacramento redbands. Redband trout from Sheepheaven Creek exhibit a high degree of distinction from all other trout groups, including all other redband, as evidenced by their position within the UPGMA tree, their divergence in PCA plots, and their discrimination as a distinct subcluster in the STRUCTURE analysis. The genetic distinctiveness of Sheepheaven Creek fish from other redband trout is consistent with other meristic and genetic analyses

that suggest this population may be a relictual primitive group, likely warranting its own subspecific status. Other researchers have also found a high degree of relatedness in this sample (J.C. Garza, unpublished microsatellite data,) and although this may partially account for the genetic distinctiveness of this group, this factor alone does not explain the high level of differentiation from what should be its next closest redband relatives (i.e., Davis Creek Sacramento redband). Additional investigation of other McCloud Basin fish is needed to determine whether subspecific status should also extend to other populations in the McCloud Basin.

Redband trout as a whole were distinct from rainbow trout, though some populations showed a degree of overlap with rainbow trout groups (Figure 1.4, 1.8). It is notable that the East Fork Nelson population of Sacramento redband has an affinity for hatchery rainbow trout populations, suggesting either ancestral influence via the lower Pit River or possibly influence from hatchery rainbow trout stocks introduced in the area. The Davis Creek redband population follows next in terms of genetic distinctiveness from other redband (Figure 1.8b), though additional Sacramento redband populations need to be evaluated for more conclusive generalizations regarding whether it represents a unique Sacramento redband subspecies. The grouping of the Columbia and Catlow Basins together to form a distinct redband group is perhaps not surprising, given the likely historical hydrographic connections among basins during the Pleistocene. Goose Lake redband trout samples grouped with Warner Basin populations from the Northern Great Basin as in previous allozyme research (Currens 1997), but in contrast to hypotheses related to hydrological connectivity of Goose Lake to the Upper Pit River drainage system (Behnke 1992; Hubbs and Miller 1948).

The consistent clustering of Surprise Valley Mill and Cedar Creek populations with Willow and Honey Creek in the UPGMA analysis and strong similarities to Northern Great Basin individuals in PCA (Figure 1.8b) suggests a Northern Great Basin origin for Surprise Valley fish that is also consistent with they hydrological drainage of this region into the Warner Valley. The limited distribution of Sacramento redband populations precludes conclusive exclusion of potential Sacramento redband influence. The Eagle Lake redband sample did not yield any consistent clustering patterns or group affiliations. It was affiliated with other redband trout groups in some analyses (STRUCTURE, Figure 1.11; PCA, Figure 1.9; UPGMA tree of all taxa, Figure 1.2b), appeared somewhat distinct from other redband in one PCA plot (Figure 1.9), and grouped with Coleman and Pit strain hatchery rainbow trout in UPGMA tree of all taxa (Figure 1.2a). Although its level of genetic distinctiveness is not clear, its unique ability to withstand extreme alkalinity of Eagle Lake has clearly put this group on a distinct evolutionary trajectory. Furthermore, selective advantages may exist that are undetected by neutral molecular genetic markers.

Golden group relationships

California golden trout exhibit a level of genetic distinction similar to that of Sheepheaven Creek redband. Non-hybridized California golden trout from both Golden Trout Creek and South Fork Kern River drainages form a clear, well-supported monophyletic group (Figure 1.2a). Perhaps surprisingly, this group also includes the Upper Coyote Creek sample, a tributary to the Kern River that contains what were thought to be Little Kern golden trout transplanted from Rifle Creek (tributary to Little Kern River) in 1887 (Ellis and Bryant 1920). This suggests either influence from

California golden trout coming from nearby Golden Trout Creek via the mainstem Kern River, a possible undocumented transplant of California golden trout into the Coyote Creek area, or a mistake in stream identification by the original transplanters.

The Little Kern golden trout was first described as one of three species (Evermann 1906). Some (Legendre et al. 1972; Shreck and Behnke 1971) have considered it synonymous with Kern River rainbow trout, though Smith and Gall (1981) showed these conclusions reflected samples determined to contain anthropogenically hybridized fish (Green Meadows, Little Kern River, Rifle Creek, and possibly Mountaineer Creek populations). Berg (1987) and Gall (G.A.E. Gall, unpublished reports) found substantial allozyme evidence supporting *O. m. whitei* as a distinct subspecies. Mitochondrial and nuclear evidence from Bagley (1998) and nuclear AFLP data from this study clearly show the Little Kern golden trout is distinct from other golden trout and rainbow trout groups. While it is not surprising that Grey Meadow and Upper Fish Creek populations group together (fish from Upper Fish Creek were transplanted to Grey Meadow Creek in 1995 following a successful chemical treatment of Grey Meadow Creek), it is unclear why they do not group with the remaining Little Kern populations; additional analyses with SNP and microsatellite markers (Chapter III) shows little (3% or less) evidence of rainbow trout introgression in Fish Creek populations and Grey Meadow Creek was not examined. Regardless, Bayesian and ordination analyses clearly support *whitei* as a distinct subspecies.

There is significant evidence to support *O. m. gilberti* as its own subspecies, based on its delimitation as a unique cluster in both Bayesian and ordination analyses. Evidence from the UPGMA dendrogram demonstrates minimally that Kern River

rainbow trout are a monophyletic group that shares some genetic similarity with rainbow trout and also with Little Kern golden trout. We are unable to differentiate with this AFLP data set whether the similarity is due to ancestral associations or more recent rainbow trout influence. The position of the Kern River samples is consistent with other recent analyses that suggest an intermediate position of Kern River rainbow trout (Bagley 1998), though our data set suggests a closer affinity to Little Kern golden trout, as opposed to California golden trout, evident in the UPGMA clustering of genetic distances and Bayesian clustering of individuals, and PCA.

The signature of hybridization is difficult to assess by hierarchical means such as a bifurcating dendrogram. Although the Bayesian and ordination methods have the potential to uncover such patterns, the variability in this data set did not allow us to detect introgression between less differentiated groups. A known heavily hybridized population of California golden trout from Upper Trout Creek was positioned intermediately between its parental groups in PCA as expected; however, in the case of many redband groups, clear parental groups could not be established. The affinity of the East Fork Nelson Sacramento redband population for rainbow trout (as opposed to other Sacramento redband trout) in the PCA warrants further investigation. The grouping of Mount Whitney hatchery strain as sister to the redband/rainbow group in overall UPGMA analysis of genetic distances is perhaps also indicative of its admixed origins from coastal rainbow trout, Lahontan cutthroat trout, Klamath and Eel River steelhead, Kamloops and possibly even Kern River rainbow trout (Busack and Gall 1980).

Subspecies delimitation is complex and controversial (Groves 2001; Wilson and Brown 1953; Zink 2004), yet essential to the conservation of threatened and endangered

taxa (Haig et al. 2006). The dynamic processes shaping the landscapes and species of Western North America have generated a unique group of salmonids that is as diverse as it is complex. Relationships among rainbow trout of the Kern River Basin and among redband trout of the Northern Great Basin and adjacent areas reflect a complex history of evolutionary processes including isolation and secondary contact, and in some cases, anthropogenic mixing of lineages. Such diversity is difficult to categorize into a trinomial nomenclature system, though it is certainly a desirable and necessary endeavor from a species management perspective. Additional efforts, perhaps using coalescent approaches and evaluating non-neutral genetic variation are needed to better understand the genetic variance underlying the diverse redband phenotypes observed in the Sacramento and Northern Great Basins.

Tables and Figures

Table 1.1. Sampling locality information and sample ID codes for sampled populations from different subspecies groups. Number and percent of polymorphic loci out of 149 total AFLP loci are given. Subspecies designation after Behnke, 1992, though not formally accepted taxonomy. Known hatchery rainbow trout-hybridized population is indicated with an *.

Subspecies	Basin	Code	Locality	Sample ID	N	P	P (%)
O. clarki seleniris Paiute cutthroat	Walker (Transplant)	0	North Fork Cottonwood Creek	NFCC	8	–	–
O. m. aguabonita	Golden Trout Creek	1	Volcano Creek	VC	8	30	20.1
California golden		2	Volcano Creek Left Stringer	VCLS	4	17	11.4
	S. Fork Kern River	3	Upper S. Fork Kern	UMC	4	34	22.8
		4	Upper Mulkey Creek	FCC	5	37	24.8
		5	Four Canyons Creek	USFK	5	42	28.2
	Owens River	6	Ash Meadow Creek	AMC	4	23	15.4
O. m. whitei	Little Kern River	7	Fish Creek, Upper	UFC	5	15	10.1
Little Kern golden		8	Grey meadow Creek	SC	4	22	14.8
		9	Soda Spring Creek	SSC	4	32	21.5
		10	Upper Soda Spring Creek	USSC	3	17	11.4
		11	Upper Wet Meadow Creek	UWMC	4	21	14.1
		12	Upper Willow Creek	UWC	5	23	15.4
		13	Sheep Creek	GMC	1	–	–
O. m. gilberti	Kern	14	Chagoopa Falls	CH	4	21	14.1
Kern River rainbow	Kaweah	15	Kern-Kaweah	KK	5	22	14.8
O. m. stonei	McCloud	16	Sheepheaven[1]	SHP	9	8	0.1
Sacramento redband	Lower Pit	17	East Fork, Nelson Creek	EFN	5	27	0.2
	Upper Pit	18	Davis Creek	DC	10	42	28.2
O. m. newberii	Goose Lake	19	Cottonwood Creek (Lassen Cr.)	CCL	5	22	14.8
N. Great Basin redband		20	Camp Creek	CM (93-2216)	1	–	–
		21	Cox Creek	CX (93-2135)	1	–	–
		22	Beaver Creek	BV (93-2130)	1	–	–
	Warner	23	Upper Twelve Mile Creek	UTMC	7	57	38.3
		24	Willow Creek	WIL1 (93-2398), WIL2 (93-2356)	2	14	9.4
		25	Deep Creek	HNY1 (93-2191), HNY2 (93-2181)	2	17	11.4
		26	Honey Creek	DP (93-2157)	1	–	–
	Klamath	27	Fall Creek @ Power Lines	FLL (90-301)	1	–	–
		28	Jenny Creek	JC1 (90-390), JC2 (90-228)	2	19	12.8
		29	Johnson Creek	JNS1 (90-169), JNS2 (90-267)	2	16	10.7
	Catlow Valley	30	Home Creek	HM1 (93-2107), HM2 (93-2062)	2	4	2.7
		31	Upper Home Creek	UHM (93-2071)	1	–	–

Table 1.1, continued

Subspecies	Basin	Code	Locality	Sample ID	N	P	P (%)
O. m. gairdneri	Hells Canyon	32	Lonesome Creek	LNS (93-1279)	1	–	–
Columbia redband	Umatilla River	33	E. Birch Creek	EBR (93-343)	1	–	–
		34	SF Umatilla River	SFU (93-12)	1	–	–
	Owyhee River	35	W. Little Owyhee	WLO (92-276)	1	–	–
O. m. aquilarum	Eagle Lake	36	Darrah Springs Hatchery[2]	EL	5	39	26.2
Eagle Lake rainbow							
O. m. irideus	Navarro	37	Upper Indian Creek	UIC	5	42	28.2
coastal rainbow		38	John Smith Creek (Upper N. Fork Navarro R.)	UNF	5	39	26.2
	American	39	N. Fork American River	NFAR	7	53	35.6
O. m. spp							
redband spp.	Surprise Valley[3]	40	Mill Creek	MC	5	26	17.4
		41	Cedar Creek	CDC	5	35	23.5
transplanted Little Kern golden	Kern River	42	Upper Coyote Creek	UCC	4	–	–
hybrid California golden x hatchery rainbow	S. Fork Kern	43	Upper Trout Creek *	UT	4	–	–
hatchery rainbow	Mt. Shasta Strain	44	Mt. Shasta Hatchery	MSS	9	55	36.9
	Mt. Whitney Strain	45	Mt. Whitney Hatchery	WH	2	23	15.4
	Coleman Strain	46	Crystal Lake Hatchery	CS	5	30	20.1
	Pit Strain	47	Crystal Lake Hatchery	PS	5	24	16.1
Total					190		

[1] Sheepheaven redband trout currently considered members of *O. m .stonei*, however, have exhibited morphological and genetic distinctiveness that warrant separate consideration and possible unique subspecific status
[2]Surprise Valley populations not part of native range; likely transplanted from an adjacent drainage
[3] Fish collected from the wild but reared (not propagated) in the hatchery

Table 1.2. Restriction Enzyme primer combinations and levels of polymorphism based on Number of polymorphic bands for scoreable Amplified Fragment Length Polymorphism fragments.

AFLP Selective Primer Extensions		No. Polymorphic bands
Eco	*Mse*	
ACT	CGG	14
ACT	CCG	23
ACT	CTG	17
ACC	CCG	20
ACC	CGA	15
ACC	CGG	16
AAC	CCG	15
AAC	CGG	21
AAC	CGA	8

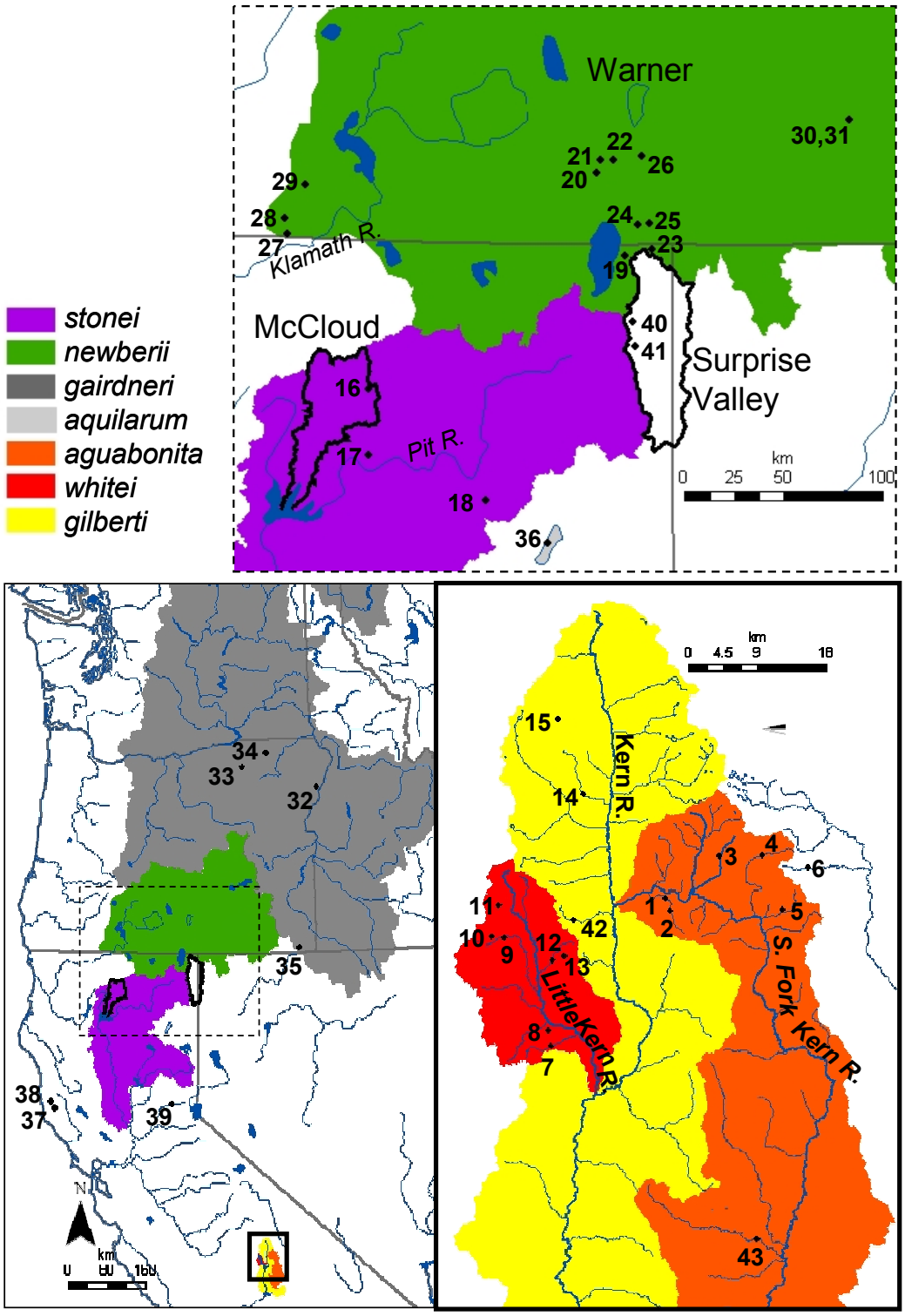


Figure 1.1. Map of approximate historic geographic range for each native *O. mykiss* subspecies (adapted after Behnke 2002) and individual sampling localities for subspecies group samples used in the current study. Numbers correspond with localities given in Table 1.1.

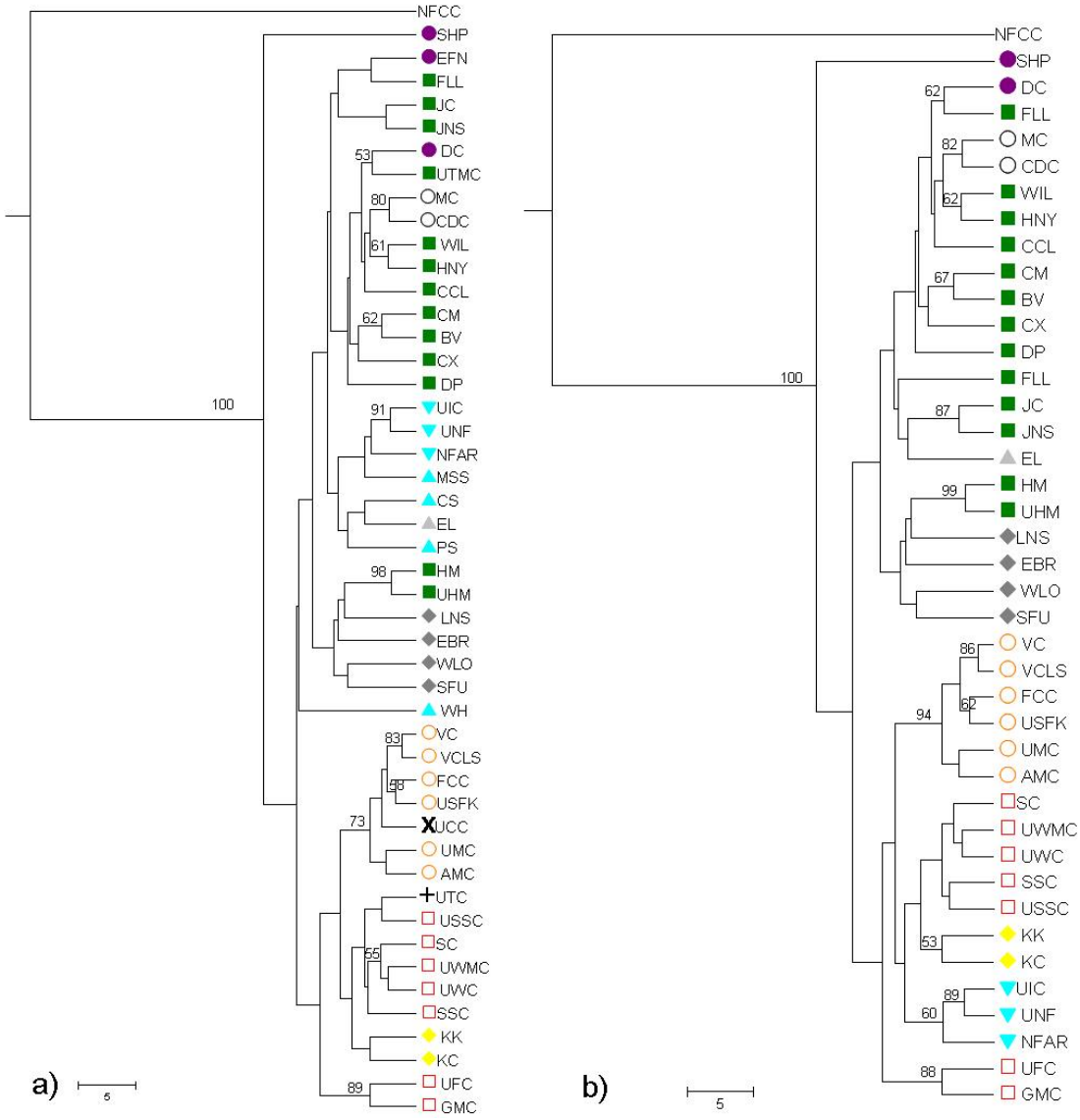


Figure 1.2. UPGMA trees for all taxa examined (a) and for a subset excluding known or suspected introgressed groups (b). Bootstrap values are shown for nodes with greater than 50% support out of 1000 pseudoreplicates. Symbols as given in Figure 3 and abbreviations as in Table 1.1.

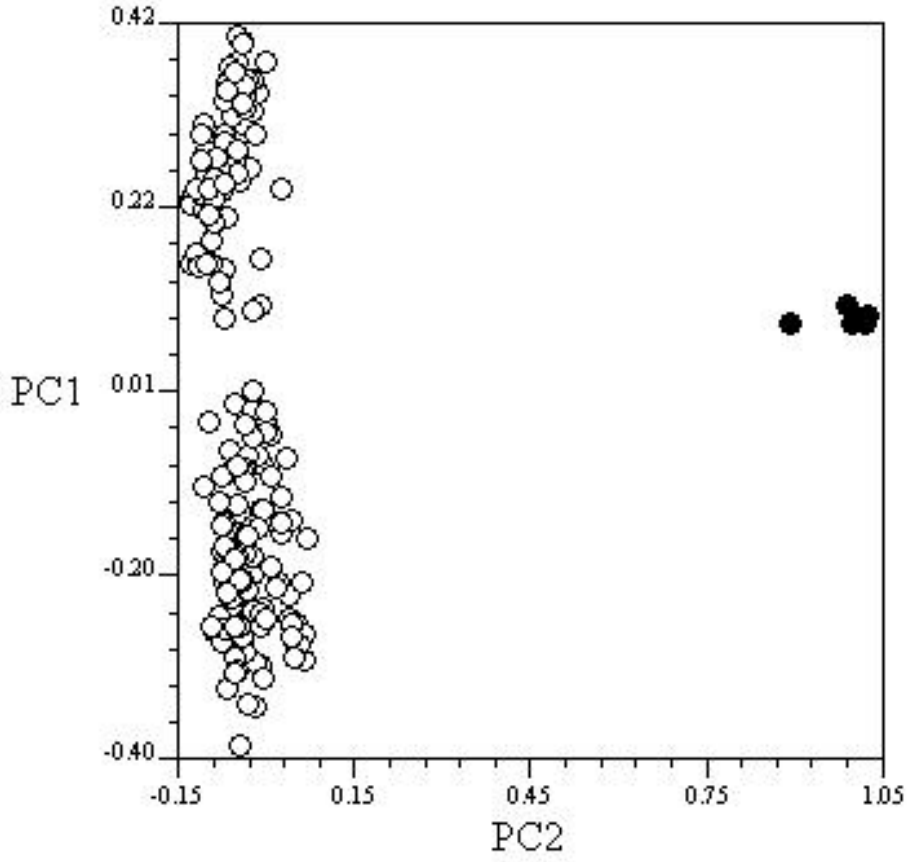


Figure 1.3. Principal Coordinate Analysis of AFLP data for Paiute cutthroat trout individuals (closed circles) and all rainbow trout individuals from all subspecies (open circles).

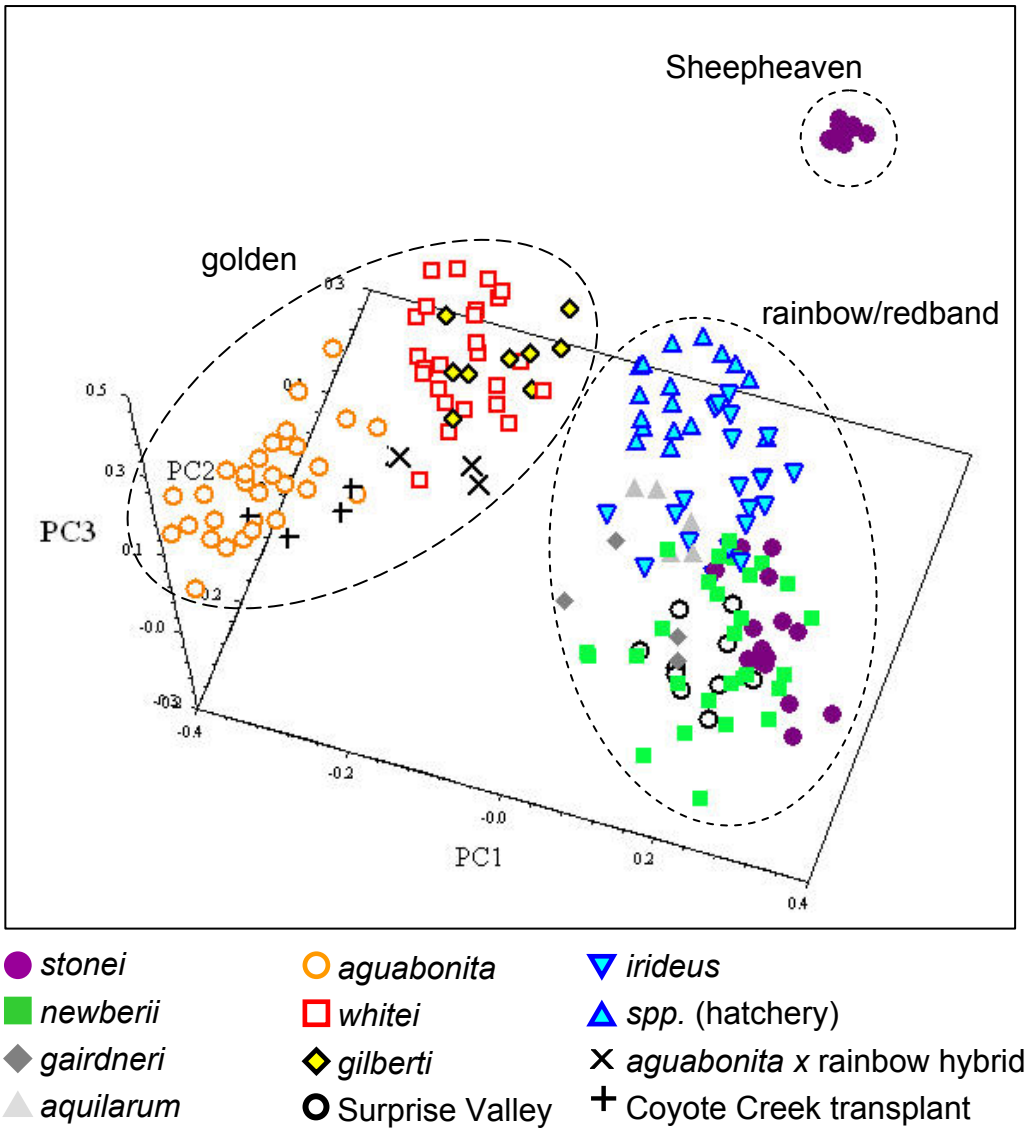


Figure 1.4. Principal Coordinate Analysis of all rainbow trout subspecies group individuals (first 3 dimensions shown), excluding cutthroat trout and known hybrids (Upper Trout Creek population). Subspecies groups as described in Table 1.1.

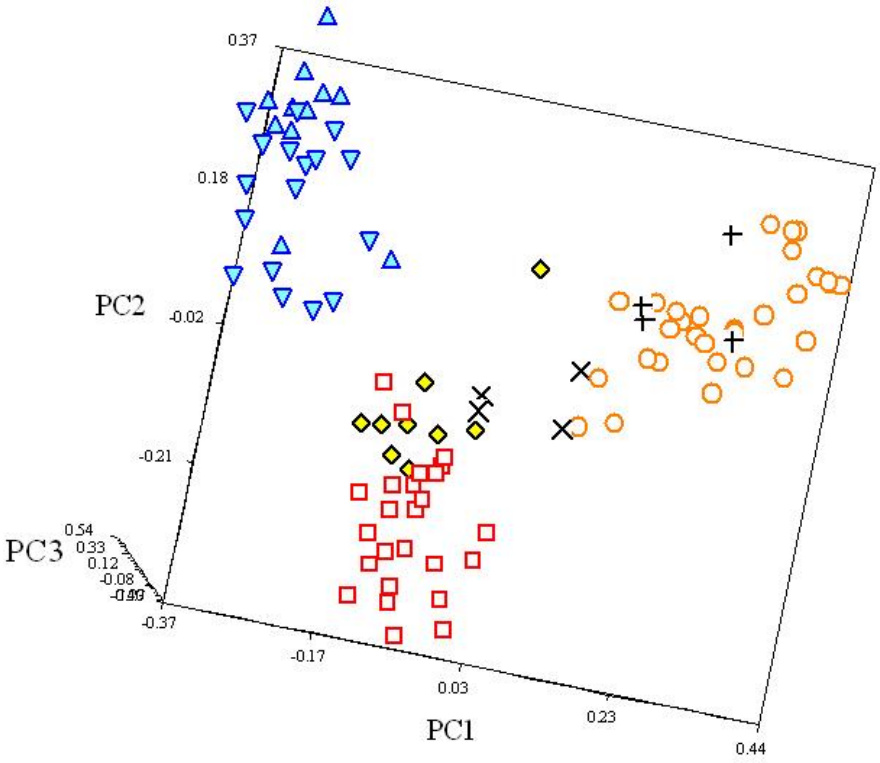
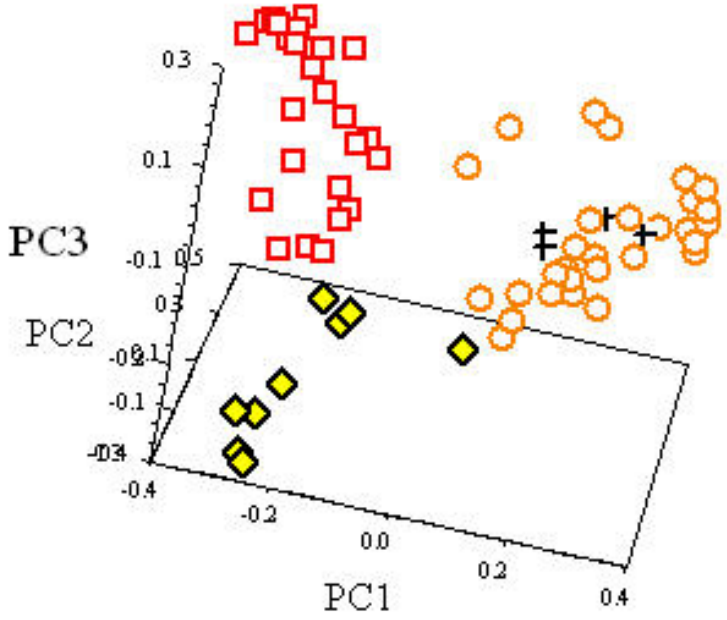


Figure 1.5. Principal Coordinate Analysis of all golden trout group individuals, including hybridized Upper Trout Creek (x) and Upper Coyote Creek (+) samples in comparison to hatchery and wild rainbow trout. Symbols as given in Figure 1.4.

a)



b)

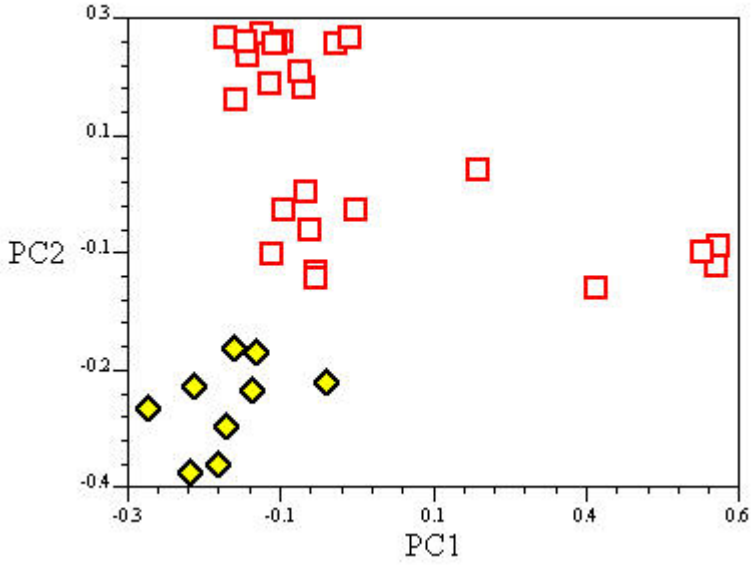


Figure 1.6. Principal Coordinate Analysis of all golden trout subspecies individuals, excluding Upper Trout Creek hybrids (a) and Kern River rainbow and Little Kern golden trout subspecies group comparison only (b). Symbols as given in Figure 1.4.

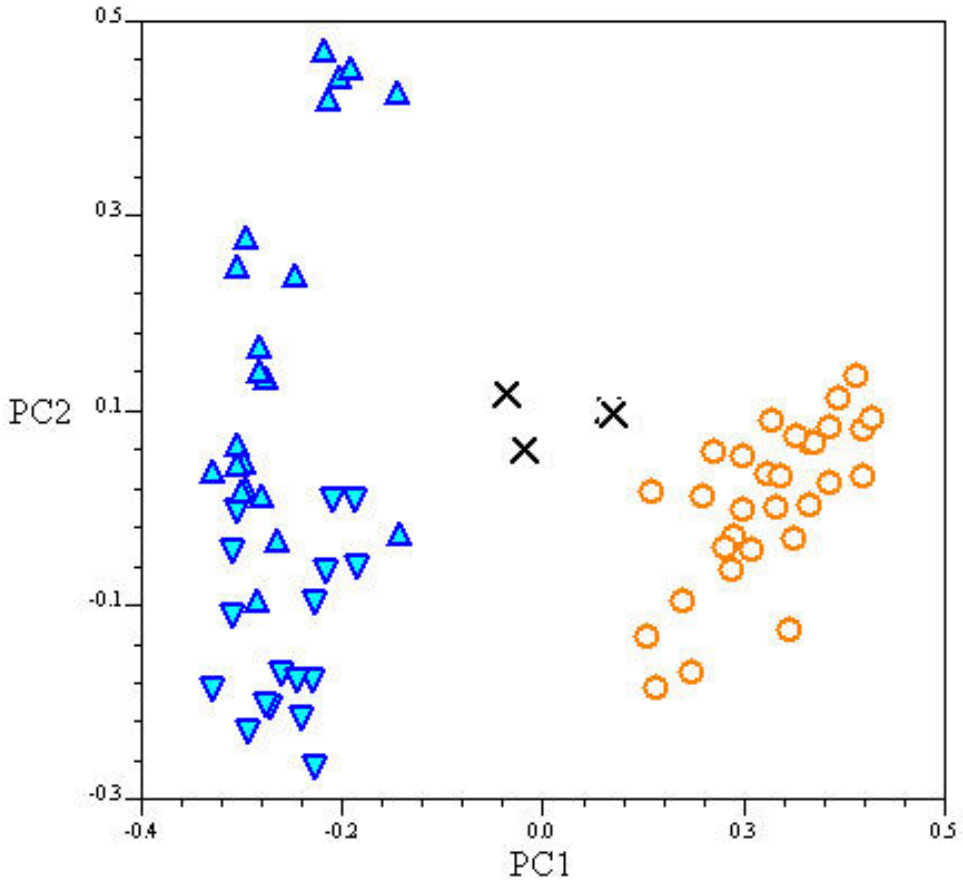


Figure 1.7. California golden trout, hatchery and wild rainbow trout, and known hybridized Upper Trout Creek population. Symbols as given in Figure 1.4.

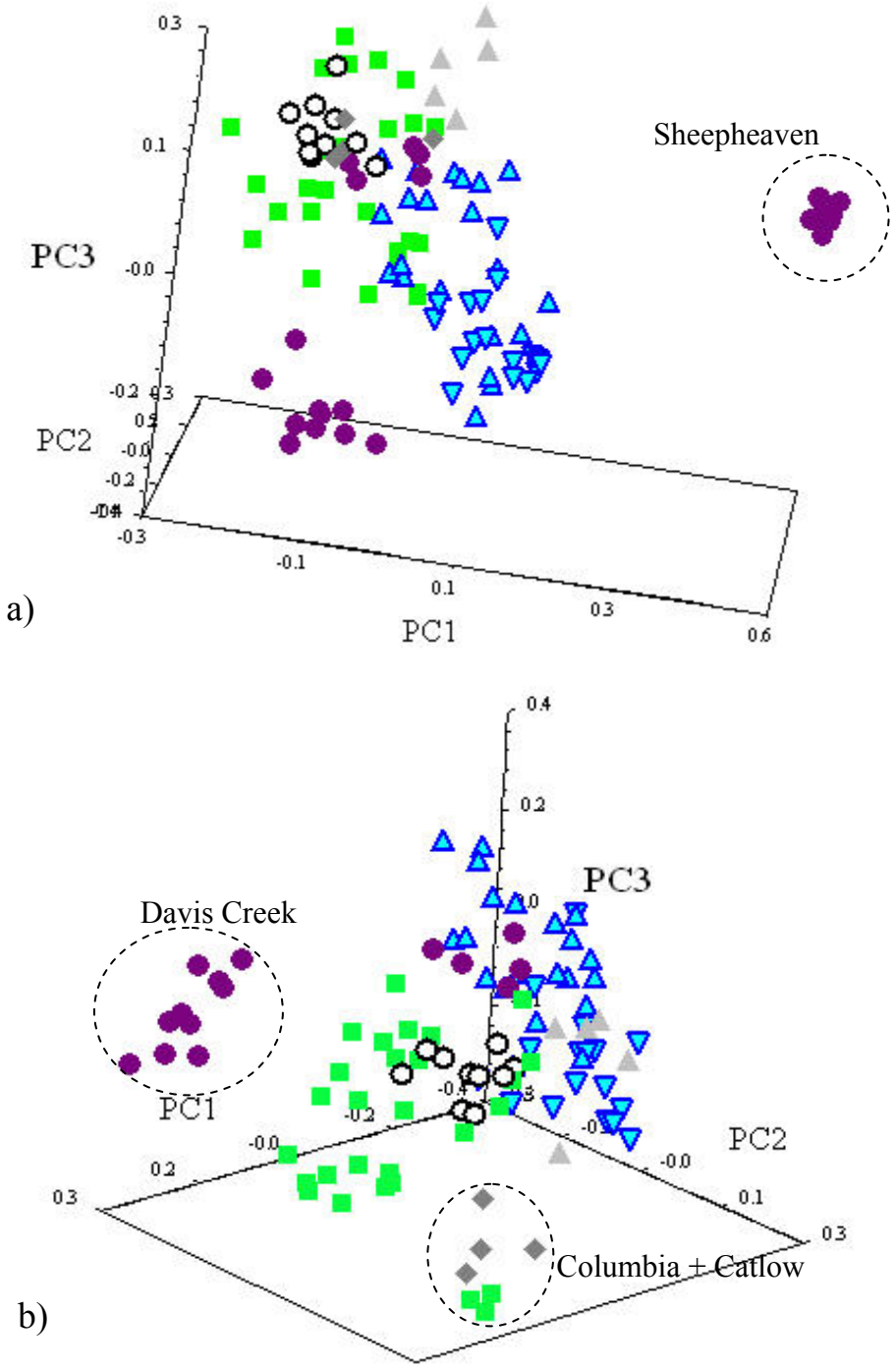


Figure 1.8. Principal Coordinate Analysis of all redband and rainbow trout populations including Sheepheaven Creek (circled) (a), and excluding the Sheepheaven redband population (b). Davis Creek redband cluster and Columbia and Catlow Valley (Upper Home and Home creek) individuals are circled for identification (not a statistical distribution).

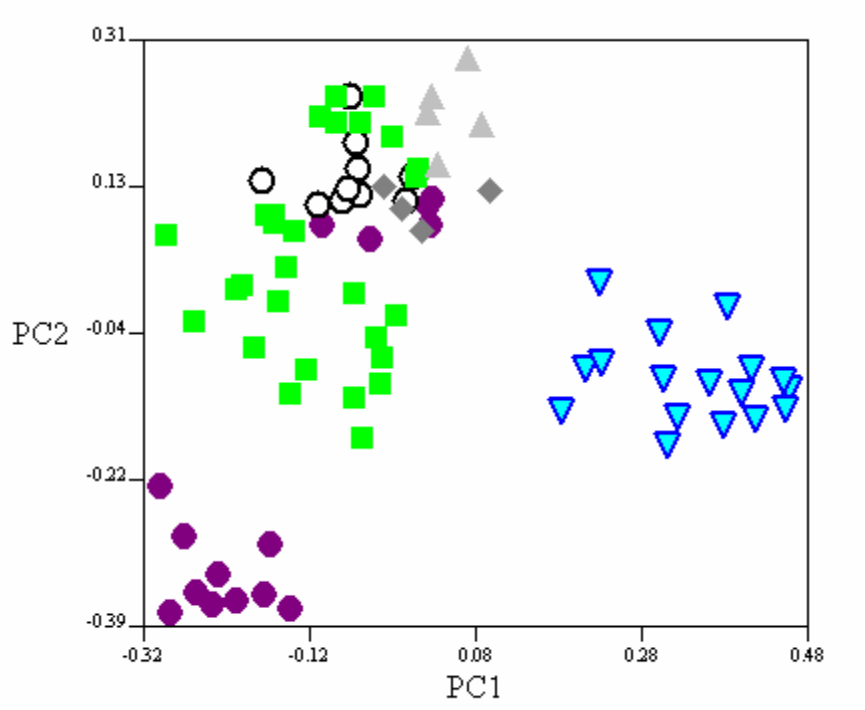


Figure 1.9. Principal Coordinate Analysis of all redband (excluding Sheepheaven Creek) and all native (non-hatchery) rainbow trout populations.

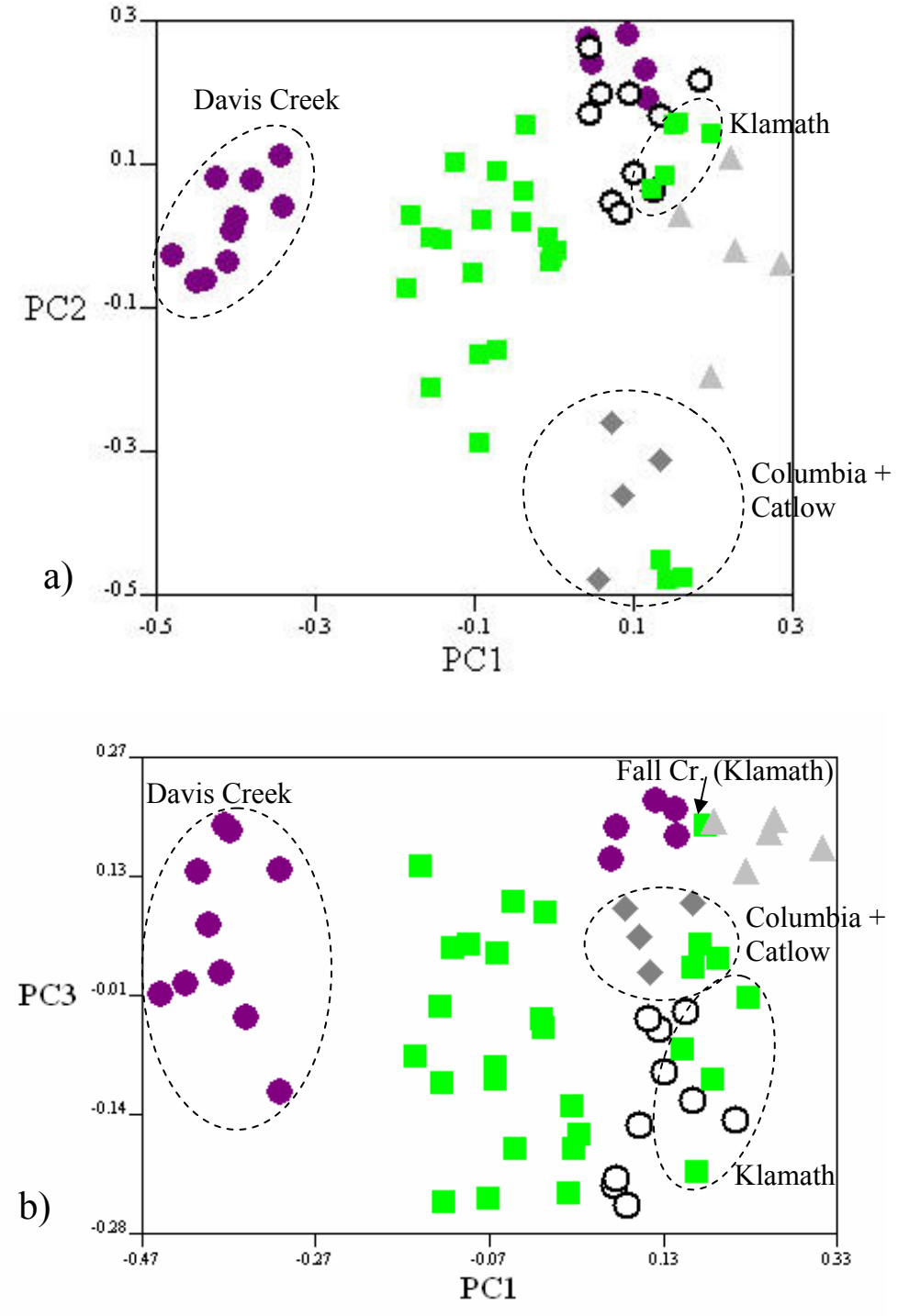


Figure 1.10. Principal Coordinate Analysis plots of all redband individuals, excluding Sheepheaven Creek and hatchery and wild rainbow trout populations. Plots show two different views of the same data set: Plot a shows principal coordinate 1 versus 2 and plot b shows principal coordinate 1 versus 3. Davis Creek, Columbia and Catlow Valley (Upper Home and Home creek), and Klamath populations are circled for identification, and the Fall Creek (Klamath) individual is also labeled in 1.10b.

Figure 1.11. STRUCTURE analyses of individual clustering. Initial division (a) between members of the "golden" subspecies groups and other "redband and rainbow" trout groups, with individuals grouped by q-value and group membership identified above. Vertical bars represent individual fish and Y-axis depicts the proportion of ancestry inferred to contribute to an individual's multilocus genome. Series b-d depicts subclustering analyses of the "golden" group using a value of K = 2 (b) and K=3 (c) under the admixture model and K=3 under the no-admixture model (admixture model not shown for K=2). Subspecies identity of individual clusters is given for the three unambiguously identified golden trout subspecies groups. The subclustering analysis of the "redband/rainbow" group is given in (e) for K=3, and named subspecies identified above. Population codes as given in Table 1.1.

Appendix 1.1. Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below) among populations.

	NFCC	SHP	EFN	DC	MC	CDC	CCL	CM	CX	BV	UTMC	WIL	HNY	DP	FLL	JC	JNS	HM	UHM	LNS	EBR	SFU	WLO
NFCC	****	0.48	0.51	0.48	0.51	0.53	0.47	0.47	0.47	0.48	0.54	0.48	0.49	0.46	0.47	0.50	0.51	0.48	0.46	0.49	0.48	0.50	0.47
SHP	0.74	****	0.80	0.81	0.78	0.80	0.80	0.74	0.72	0.72	0.83	0.79	0.80	0.72	0.78	0.80	0.78	0.74	0.76	0.71	0.73	0.75	0.75
EFN	0.68	0.22	****	0.87	0.89	0.90	0.90	0.87	0.84	0.84	0.88	0.90	0.90	0.83	0.92	0.89	0.87	0.82	0.82	0.78	0.81	0.81	0.84
DC	0.73	0.21	0.14	****	0.89	0.91	0.90	0.89	0.89	0.87	0.93	0.90	0.92	0.87	0.82	0.85	0.85	0.82	0.83	0.82	0.82	0.80	0.85
MC	0.67	0.25	0.11	0.11	****	0.95	0.91	0.90	0.87	0.86	0.91	0.92	0.91	0.89	0.86	0.92	0.91	0.84	0.83	0.80	0.81	0.82	0.87
CDC	0.64	0.23	0.11	0.10	0.05	****	0.91	0.91	0.89	0.88	0.93	0.92	0.94	0.90	0.88	0.92	0.89	0.88	0.89	0.83	0.86	0.85	0.89
CCL	0.75	0.22	0.11	0.10	0.09	0.09	****	0.92	0.87	0.89	0.91	0.90	0.93	0.86	0.86	0.90	0.87	0.86	0.86	0.81	0.85	0.82	0.85
CM	0.75	0.31	0.14	0.11	0.11	0.10	0.08	****	0.91	0.94	0.90	0.88	0.93	0.89	0.83	0.87	0.86	0.88	0.89	0.84	0.84	0.79	0.86
CX	0.75	0.32	0.17	0.12	0.14	0.12	0.14	0.10	****	0.90	0.89	0.88	0.91	0.87	0.80	0.84	0.83	0.84	0.86	0.84	0.84	0.81	0.85
BV	0.73	0.33	0.17	0.14	0.15	0.13	0.12	0.06	0.11	****	0.87	0.86	0.91	0.88	0.79	0.82	0.81	0.87	0.88	0.85	0.87	0.81	0.87
UTMC	0.62	0.19	0.13	0.08	0.10	0.08	0.10	0.10	0.12	0.14	****	0.90	0.92	0.87	0.85	0.88	0.87	0.86	0.85	0.80	0.83	0.81	0.86
WIL	0.73	0.23	0.11	0.11	0.08	0.08	0.11	0.12	0.13	0.15	0.11	****	0.95	0.90	0.89	0.88	0.86	0.86	0.85	0.82	0.84	0.80	0.87
HNY	0.72	0.23	0.10	0.08	0.09	0.07	0.07	0.08	0.09	0.10	0.09	0.05	****	0.92	0.88	0.86	0.87	0.87	0.88	0.84	0.85	0.83	0.86
DP	0.78	0.32	0.18	0.14	0.12	0.10	0.16	0.12	0.14	0.13	0.14	0.10	0.08	****	0.81	0.82	0.81	0.86	0.87	0.82	0.83	0.82	0.87
FLL	0.75	0.25	0.08	0.20	0.16	0.13	0.15	0.19	0.22	0.23	0.17	0.12	0.12	0.22	****	0.88	0.84	0.82	0.83	0.80	0.77	0.79	0.81
JC	0.69	0.22	0.12	0.17	0.09	0.08	0.11	0.14	0.18	0.20	0.12	0.13	0.15	0.20	0.12	****	0.95	0.86	0.83	0.80	0.84	0.82	0.85
JNS	0.68	0.24	0.14	0.17	0.10	0.11	0.14	0.16	0.18	0.20	0.14	0.15	0.14	0.20	0.17	0.05	****	0.85	0.84	0.80	0.81	0.80	0.83
HM	0.73	0.30	0.20	0.19	0.18	0.13	0.15	0.13	0.18	0.14	0.15	0.15	0.13	0.15	0.20	0.16	0.17	****	0.96	0.88	0.88	0.84	0.90
UHM	0.78	0.28	0.20	0.19	0.19	0.12	0.15	0.12	0.15	0.13	0.16	0.16	0.13	0.14	0.18	0.18	0.18	0.04	****	0.89	0.87	0.85	0.87
LNS	0.71	0.35	0.25	0.19	0.22	0.18	0.21	0.18	0.18	0.17	0.23	0.20	0.17	0.20	0.22	0.22	0.23	0.13	0.12	****	0.87	0.87	0.87
EBR	0.74	0.32	0.21	0.20	0.21	0.15	0.16	0.18	0.18	0.14	0.19	0.18	0.16	0.18	0.26	0.17	0.21	0.13	0.14	0.14	****	0.87	0.87
SFU	0.70	0.28	0.22	0.23	0.20	0.16	0.20	0.24	0.21	0.22	0.21	0.22	0.19	0.20	0.24	0.20	0.22	0.18	0.17	0.14	0.14	****	0.89
WLO	0.76	0.29	0.17	0.16	0.14	0.12	0.17	0.15	0.17	0.14	0.15	0.14	0.15	0.14	0.22	0.16	0.19	0.11	0.14	0.14	0.14	0.12	****
VC	0.57	0.30	0.21	0.22	0.19	0.16	0.20	0.22	0.23	0.20	0.16	0.25	0.19	0.26	0.25	0.19	0.21	0.19	0.22	0.23	0.27	0.25	0.25
VCLS	0.60	0.35	0.24	0.23	0.20	0.18	0.22	0.23	0.25	0.21	0.19	0.27	0.21	0.28	0.28	0.21	0.21	0.19	0.23	0.22	0.29	0.25	0.26
UMC	0.61	0.32	0.25	0.26	0.22	0.21	0.23	0.27	0.28	0.29	0.21	0.28	0.23	0.31	0.26	0.22	0.24	0.24	0.28	0.25	0.31	0.27	0.28
FCC	0.61	0.34	0.22	0.23	0.20	0.18	0.21	0.24	0.24	0.22	0.16	0.25	0.21	0.25	0.27	0.19	0.21	0.18	0.22	0.21	0.24	0.21	0.21
USFK	0.58	0.32	0.22	0.23	0.18	0.16	0.20	0.23	0.24	0.23	0.16	0.24	0.20	0.27	0.24	0.16	0.19	0.17	0.22	0.20	0.24	0.23	0.21
UT	0.62	0.26	0.18	0.16	0.14	0.11	0.14	0.20	0.19	0.20	0.12	0.19	0.16	0.26	0.21	0.13	0.18	0.18	0.21	0.18	0.20	0.20	0.19
AMC	0.62	0.34	0.23	0.26	0.20	0.20	0.24	0.26	0.27	0.27	0.20	0.27	0.23	0.29	0.27	0.20	0.23	0.21	0.28	0.24	0.30	0.26	0.24
UCC	0.58	0.29	0.18	0.21	0.14	0.12	0.16	0.20	0.20	0.20	0.15	0.20	0.16	0.22	0.20	0.16	0.19	0.17	0.19	0.19	0.25	0.21	0.20
UFC	0.70	0.32	0.25	0.26	0.22	0.19	0.23	0.29	0.30	0.28	0.23	0.26	0.22	0.29	0.28	0.22	0.21	0.24	0.25	0.23	0.29	0.27	0.26
SC	0.72	0.26	0.21	0.21	0.19	0.17	0.21	0.25	0.22	0.26	0.18	0.18	0.18	0.27	0.19	0.16	0.20	0.21	0.23	0.19	0.26	0.25	0.23
SSC	0.62	0.25	0.17	0.20	0.15	0.17	0.18	0.23	0.21	0.25	0.14	0.18	0.16	0.28	0.20	0.19	0.20	0.23	0.24	0.23	0.26	0.23	0.22
USSC	0.63	0.31	0.18	0.23	0.15	0.15	0.19	0.22	0.21	0.22	0.17	0.18	0.18	0.26	0.20	0.18	0.20	0.19	0.22	0.17	0.25	0.25	0.21
UWMC	0.70	0.28	0.23	0.25	0.20	0.21	0.27	0.28	0.23	0.26	0.20	0.23	0.22	0.29	0.27	0.25	0.27	0.26	0.27	0.24	0.32	0.27	0.25
UWC	0.67	0.29	0.21	0.23	0.19	0.18	0.25	0.28	0.24	0.28	0.19	0.21	0.22	0.27	0.21	0.21	0.24	0.23	0.25	0.22	0.31	0.27	0.25
GMC	0.78	0.34	0.30	0.30	0.27	0.25	0.28	0.36	0.36	0.35	0.26	0.28	0.25	0.37	0.32	0.29	0.26	0.32	0.33	0.30	0.34	0.30	0.33
CH	0.69	0.23	0.18	0.21	0.16	0.15	0.19	0.24	0.26	0.29	0.16	0.21	0.20	0.27	0.19	0.18	0.20	0.20	0.21	0.21	0.30	0.25	0.21
KK	0.60	0.25	0.18	0.20	0.17	0.15	0.22	0.23	0.24	0.22	0.15	0.21	0.19	0.27	0.21	0.19	0.19	0.19	0.22	0.18	0.27	0.21	0.20
UIC	0.69	0.22	0.19	0.20	0.18	0.14	0.18	0.24	0.20	0.24	0.14	0.19	0.16	0.22	0.21	0.18	0.19	0.20	0.20	0.22	0.22	0.18	0.18
UNF	0.63	0.21	0.17	0.17	0.15	0.12	0.18	0.24	0.23	0.22	0.14	0.18	0.16	0.21	0.20	0.16	0.19	0.23	0.23	0.23	0.22	0.19	0.18
NFAR	0.64	0.20	0.14	0.12	0.12	0.10	0.15	0.18	0.15	0.19	0.10	0.13	0.11	0.20	0.14	0.14	0.17	0.16	0.18	0.15	0.19	0.19	0.16
LC	0.62	0.28	0.16	0.18	0.14	0.10	0.16	0.18	0.17	0.22	0.13	0.17	0.14	0.22	0.20	0.16	0.21	0.19	0.22	0.18	0.22	0.21	0.18
WC	0.64	0.20	0.13	0.16	0.12	0.08	0.12	0.15	0.18	0.17	0.12	0.14	0.13	0.20	0.15	0.14	0.17	0.14	0.14	0.16	0.16	0.16	0.15
MSS	0.57	0.18	0.15	0.12	0.14	0.10	0.15	0.21	0.18	0.19	0.10	0.13	0.11	0.18	0.17	0.16	0.18	0.18	0.18	0.21	0.21	0.20	0.17
WH	0.71	0.34	0.20	0.20	0.18	0.13	0.21	0.23	0.24	0.23	0.19	0.19	0.20	0.26	0.22	0.21	0.24	0.23	0.24	0.26	0.24	0.28	0.22
CS	0.70	0.18	0.13	0.13	0.13	0.10	0.14	0.16	0.20	0.17	0.11	0.13	0.11	0.15	0.15	0.11	0.16	0.17	0.18	0.22	0.18	0.19	0.14
PS	0.76	0.23	0.14	0.12	0.14	0.13	0.18	0.16	0.18	0.19	0.12	0.12	0.12	0.20	0.17	0.16	0.17	0.20	0.21	0.20	0.21	0.26	0.20
EL	0.59	0.20	0.16	0.18	0.12	0.09	0.12	0.16	0.19	0.20	0.12	0.14	0.13	0.20	0.15	0.10	0.17	0.13	0.15	0.20	0.18	0.19	0.17

Appendix 1.1, continued

	VC	VCLS	UMC	FCC	USFK	UT	AMC	UCC	UFC	SC	SSC	USSC	UWMC	UWC	GMC	CH	KK
NFCC	0.56	0.55	0.54	0.55	0.56	0.54	0.54	0.56	0.50	0.49	0.54	0.53	0.50	0.51	0.46	0.50	0.55
SHP	0.74	0.70	0.72	0.72	0.73	0.77	0.71	0.75	0.72	0.77	0.78	0.73	0.76	0.75	0.71	0.79	0.78
EFN	0.81	0.79	0.78	0.80	0.80	0.83	0.79	0.84	0.78	0.81	0.85	0.83	0.79	0.81	0.74	0.84	0.83
DC	0.80	0.79	0.77	0.80	0.80	0.85	0.77	0.81	0.77	0.81	0.82	0.80	0.78	0.79	0.74	0.81	0.82
MC	0.83	0.82	0.80	0.82	0.84	0.87	0.82	0.87	0.80	0.83	0.86	0.86	0.82	0.83	0.76	0.85	0.85
CDC	0.86	0.83	0.81	0.84	0.86	0.89	0.82	0.89	0.82	0.85	0.85	0.86	0.81	0.83	0.78	0.86	0.86
CCL	0.82	0.80	0.79	0.81	0.82	0.87	0.79	0.85	0.79	0.81	0.84	0.82	0.76	0.78	0.76	0.82	0.81
CM	0.80	0.79	0.76	0.78	0.79	0.82	0.77	0.82	0.74	0.78	0.79	0.80	0.75	0.76	0.70	0.79	0.79
CX	0.79	0.78	0.76	0.78	0.78	0.83	0.77	0.82	0.74	0.80	0.81	0.81	0.79	0.79	0.70	0.77	0.79
BV	0.82	0.81	0.75	0.80	0.80	0.82	0.77	0.82	0.75	0.77	0.78	0.80	0.77	0.76	0.70	0.75	0.80
UTMC	0.85	0.82	0.81	0.85	0.85	0.88	0.82	0.86	0.80	0.83	0.87	0.85	0.82	0.83	0.77	0.85	0.86
WIL	0.78	0.76	0.76	0.78	0.79	0.83	0.76	0.82	0.77	0.83	0.84	0.84	0.80	0.81	0.75	0.81	0.81
HNY	0.83	0.81	0.79	0.81	0.82	0.85	0.80	0.85	0.81	0.83	0.85	0.83	0.80	0.81	0.78	0.82	0.82
DP	0.77	0.75	0.74	0.78	0.77	0.77	0.75	0.81	0.75	0.76	0.76	0.77	0.75	0.76	0.69	0.76	0.77
FLL	0.78	0.76	0.77	0.77	0.79	0.81	0.77	0.82	0.75	0.82	0.82	0.82	0.76	0.81	0.72	0.83	0.81
JC	0.82	0.81	0.80	0.83	0.85	0.88	0.82	0.85	0.80	0.85	0.83	0.83	0.78	0.81	0.75	0.84	0.83
JNS	0.81	0.81	0.79	0.81	0.82	0.84	0.79	0.83	0.81	0.82	0.82	0.82	0.76	0.79	0.77	0.82	0.82
HM	0.83	0.83	0.79	0.84	0.84	0.84	0.81	0.84	0.79	0.81	0.80	0.83	0.77	0.79	0.72	0.82	0.83
UHM	0.80	0.80	0.76	0.80	0.80	0.81	0.76	0.83	0.78	0.79	0.78	0.81	0.76	0.78	0.72	0.81	0.80
LNS	0.79	0.80	0.78	0.81	0.82	0.84	0.79	0.82	0.79	0.82	0.80	0.84	0.79	0.81	0.74	0.81	0.83
EBR	0.76	0.75	0.73	0.79	0.78	0.82	0.74	0.78	0.75	0.77	0.77	0.78	0.72	0.74	0.71	0.74	0.76
SFU	0.78	0.78	0.77	0.81	0.80	0.81	0.77	0.81	0.76	0.78	0.79	0.78	0.76	0.76	0.74	0.78	0.81
WLO	0.78	0.77	0.76	0.81	0.81	0.83	0.78	0.82	0.77	0.80	0.80	0.81	0.78	0.78	0.72	0.81	0.82
VC	****	0.97	0.89	0.94	0.96	0.89	0.92	0.95	0.86	0.86	0.86	0.87	0.83	0.85	0.80	0.82	0.88
VCLS	0.03	****	0.90	0.95	0.96	0.89	0.91	0.94	0.87	0.84	0.85	0.88	0.83	0.85	0.82	0.82	0.89
UMC	0.12	0.10	****	0.93	0.93	0.88	0.95	0.92	0.84	0.86	0.87	0.86	0.84	0.88	0.81	0.86	0.87
FCC	0.06	0.05	0.07	****	0.96	0.91	0.94	0.92	0.86	0.88	0.89	0.89	0.85	0.89	0.81	0.88	0.91
USFK	0.05	0.04	0.08	0.04	****	0.94	0.96	0.95	0.87	0.88	0.88	0.90	0.86	0.89	0.82	0.88	0.91
UT	0.11	0.12	0.13	0.09	0.06	****	0.91	0.93	0.87	0.91	0.90	0.94	0.89	0.91	0.84	0.90	0.91
AMC	0.09	0.09	0.05	0.06	0.05	0.09	****	0.94	0.83	0.88	0.86	0.88	0.86	0.90	0.77	0.85	0.87
UCC	0.05	0.06	0.09	0.08	0.06	0.08	0.06	****	0.88	0.87	0.89	0.91	0.86	0.90	0.83	0.86	0.90
UFC	0.16	0.14	0.18	0.15	0.14	0.14	0.18	0.13	****	0.89	0.89	0.90	0.88	0.88	0.92	0.84	0.86
SC	0.15	0.17	0.15	0.13	0.12	0.10	0.13	0.14	0.12	****	0.92	0.93	0.93	0.95	0.83	0.87	0.87
SSC	0.15	0.16	0.14	0.11	0.13	0.11	0.15	0.12	0.12	0.09	****	0.93	0.91	0.92	0.87	0.92	0.89
USSC	0.14	0.13	0.16	0.12	0.10	0.06	0.13	0.10	0.11	0.08	0.07	****	0.91	0.94	0.85	0.91	0.91
UWMC	0.18	0.19	0.17	0.16	0.15	0.12	0.15	0.15	0.12	0.08	0.09	0.10	****	0.95	0.83	0.86	0.87
UWC	0.16	0.16	0.13	0.12	0.12	0.09	0.10	0.11	0.13	0.05	0.08	0.06	0.05	****	0.83	0.90	0.90
GMC	0.22	0.20	0.21	0.21	0.20	0.18	0.26	0.19	0.08	0.19	0.14	0.16	0.19	0.19	****	0.82	0.82
CH	0.20	0.19	0.15	0.12	0.13	0.11	0.17	0.15	0.17	0.14	0.08	0.09	0.15	0.10	0.20	****	0.92
KK	0.13	0.11	0.14	0.09	0.10	0.10	0.13	0.11	0.15	0.13	0.11	0.10	0.14	0.10	0.20	0.08	****
UIC	0.17	0.22	0.19	0.19	0.17	0.14	0.20	0.15	0.18	0.15	0.13	0.16	0.16	0.17	0.20	0.17	0.16
UNF	0.18	0.22	0.20	0.18	0.16	0.13	0.21	0.16	0.18	0.15	0.14	0.16	0.17	0.17	0.21	0.16	0.14
NFAR	0.17	0.19	0.14	0.15	0.13	0.08	0.15	0.13	0.14	0.10	0.10	0.09	0.12	0.10	0.17	0.10	0.10
LC	0.15	0.16	0.12	0.13	0.11	0.08	0.13	0.12	0.16	0.12	0.13	0.11	0.15	0.13	0.22	0.12	0.12
WC	0.15	0.18	0.16	0.16	0.14	0.09	0.16	0.13	0.20	0.13	0.14	0.11	0.17	0.15	0.24	0.13	0.12
MSS	0.18	0.20	0.20	0.18	0.16	0.12	0.21	0.13	0.18	0.17	0.14	0.16	0.17	0.18	0.21	0.13	0.13
WH	0.23	0.25	0.24	0.23	0.18	0.12	0.22	0.20	0.23	0.19	0.23	0.18	0.20	0.21	0.29	0.20	0.18
CS	0.21	0.22	0.23	0.20	0.19	0.11	0.21	0.18	0.21	0.15	0.18	0.16	0.18	0.17	0.24	0.17	0.16
PS	0.21	0.22	0.25	0.20	0.18	0.13	0.22	0.21	0.22	0.14	0.18	0.15	0.20	0.17	0.26	0.19	0.16
EL	0.16	0.16	0.19	0.17	0.15	0.11	0.19	0.12	0.18	0.14	0.17	0.13	0.19	0.18	0.23	0.16	0.15

Appendix 1.1, continued

	UIC	UNF	NFAR	LC	WC	MSS	WH	CS	PS	EL
NFCC	0.50	0.53	0.53	0.54	0.53	0.56	0.49	0.50	0.47	0.55
SHP	0.80	0.81	0.82	0.75	0.82	0.83	0.71	0.84	0.80	0.82
EFN	0.83	0.84	0.87	0.85	0.88	0.86	0.82	0.88	0.87	0.85
DC	0.82	0.84	0.89	0.84	0.86	0.88	0.82	0.88	0.89	0.84
MC	0.84	0.86	0.88	0.87	0.89	0.87	0.84	0.88	0.87	0.89
CDC	0.87	0.89	0.90	0.90	0.92	0.91	0.88	0.90	0.88	0.91
CCL	0.84	0.83	0.86	0.85	0.88	0.86	0.81	0.87	0.84	0.88
CM	0.78	0.79	0.83	0.84	0.86	0.81	0.79	0.85	0.85	0.86
CX	0.82	0.79	0.86	0.84	0.83	0.84	0.79	0.82	0.84	0.83
BV	0.78	0.80	0.83	0.80	0.84	0.83	0.80	0.84	0.83	0.82
UTMC	0.87	0.87	0.91	0.88	0.89	0.91	0.83	0.90	0.89	0.88
WIL	0.83	0.83	0.88	0.84	0.87	0.88	0.82	0.88	0.89	0.87
HNY	0.85	0.85	0.90	0.87	0.88	0.89	0.82	0.90	0.89	0.88
DP	0.80	0.81	0.82	0.80	0.82	0.84	0.77	0.86	0.82	0.82
FLL	0.81	0.82	0.87	0.82	0.86	0.85	0.80	0.86	0.85	0.86
JC	0.84	0.85	0.87	0.85	0.87	0.85	0.81	0.90	0.85	0.91
JNS	0.83	0.83	0.85	0.81	0.84	0.83	0.78	0.85	0.85	0.85
HM	0.82	0.79	0.85	0.83	0.87	0.83	0.80	0.85	0.82	0.88
UHM	0.82	0.79	0.84	0.81	0.87	0.83	0.79	0.84	0.81	0.86
LNS	0.80	0.79	0.86	0.83	0.85	0.81	0.77	0.80	0.82	0.82
EBR	0.81	0.80	0.82	0.80	0.85	0.81	0.79	0.84	0.81	0.84
SFU	0.83	0.83	0.83	0.81	0.85	0.82	0.75	0.82	0.77	0.83
WLO	0.84	0.83	0.86	0.84	0.86	0.84	0.80	0.87	0.82	0.84
VC	0.84	0.84	0.85	0.86	0.86	0.84	0.79	0.81	0.81	0.85
VCLS	0.80	0.80	0.83	0.86	0.83	0.82	0.78	0.80	0.81	0.85
UMC	0.83	0.82	0.87	0.89	0.85	0.82	0.79	0.80	0.78	0.83
FCC	0.83	0.83	0.86	0.88	0.85	0.84	0.80	0.82	0.82	0.84
USFK	0.85	0.85	0.88	0.90	0.87	0.85	0.84	0.83	0.83	0.86
UT	0.87	0.87	0.92	0.93	0.91	0.88	0.88	0.89	0.88	0.90
AMC	0.82	0.81	0.86	0.88	0.85	0.81	0.80	0.81	0.80	0.83
UCC	0.86	0.85	0.88	0.89	0.88	0.88	0.82	0.83	0.81	0.88
UFC	0.83	0.83	0.87	0.85	0.82	0.84	0.80	0.81	0.80	0.83
SC	0.86	0.86	0.91	0.89	0.88	0.84	0.83	0.86	0.87	0.87
SSC	0.87	0.87	0.91	0.87	0.87	0.87	0.80	0.84	0.84	0.85
USSC	0.85	0.85	0.91	0.90	0.90	0.85	0.83	0.85	0.86	0.88
UWMC	0.86	0.84	0.89	0.86	0.85	0.84	0.82	0.83	0.82	0.83
UWC	0.84	0.85	0.90	0.87	0.86	0.84	0.81	0.84	0.84	0.83
GMC	0.82	0.81	0.84	0.80	0.79	0.81	0.75	0.79	0.77	0.79
CH	0.85	0.85	0.91	0.88	0.88	0.87	0.82	0.84	0.83	0.85
KK	0.85	0.87	0.91	0.89	0.89	0.88	0.83	0.85	0.85	0.86
UIC	****	0.96	0.93	0.87	0.87	0.91	0.84	0.88	0.81	0.84
UNF	0.05	****	0.92	0.86	0.87	0.91	0.86	0.90	0.84	0.84
NFAR	0.07	0.09	****	0.93	0.91	0.92	0.87	0.90	0.88	0.88
LC	0.14	0.15	0.07	****	0.92	0.88	0.87	0.87	0.85	0.90
WC	0.14	0.13	0.09	0.08	****	0.91	0.88	0.89	0.88	0.93
MSS	0.10	0.09	0.08	0.13	0.10	****	0.86	0.91	0.87	0.88
WH	0.17	0.15	0.14	0.14	0.13	0.15	****	0.83	0.82	0.82
CS	0.12	0.10	0.10	0.13	0.11	0.10	0.19	****	0.91	0.91
PS	0.21	0.18	0.12	0.16	0.13	0.14	0.20	0.09	****	0.86
EL	0.17	0.17	0.12	0.10	0.07	0.12	0.20	0.09	0.15	****

Chapter 2: Comparative analysis of SNP and microsatellite estimates of rainbow trout introgression in native California golden trout

Abstract

Hybridization between native and introduced rainbow trout is known to negatively impact native trout species. The California golden trout (*Oncorhynchus mykiss aguabonita;* "CAGT"), a Sierra Nevada endemic, persists in its non-hybridized form in a relatively minor portion of its native range. To maintain the historic golden trout genotype, management of this subspecies requires genetic monitoring techniques that are accurate, sensitive, and in keeping with current technological advances. This study demonstrates the utility of Single Nucleotide Polymorphism (SNP) and insertion-deletion markers in detecting and quantifying introgression with introduced rainbow trout. Patterns and levels of introgression detected by SNP estimates of introgression corresponded both in pattern and magnitude in the majority of populations with previously generated microsatellite and minisatellite estimates for the same populations and showed the prevalence of rainbow trout introgression in CAGT populations throughout their native range, particularly in the South Fork Kern River. Some non-hybridized populations persist in Golden Trout Creek and transplanted localities outside of the native CAGT range. The standardized nature of SNP data and ease of data collection make SNP markers more amenable to the task of tracking introgression levels over time for genetic monitoring studies for this subspecies and can be applied to other studies of introgression in species of concern.

Introduction

Hybridization is recognized as a natural, creative evolutionary force that can introduce novel adaptations, generate new species, infuse genetic diversity into existing populations, and persist in a stable zone over time, (Arnold 1997; 1999; Barton 2001; Dowling and Secor 1997). However, anthropogenically-induced hybridization between otherwise distinct taxa threatens the persistence and evolutionary legacy of many native species and can lead to subsequent "genomic extinction," or "extinction by hybridization" (Rhymer and Simberloff 1996), sometimes over a relatively short time period (Wolf et al. 2001). Such loss can occur through dilution or disruption of the native genome or maladaptation of hybrid or subsequent generation taxa (e.g., outbreeding depression and gametic wastage, Allendorf 2001). Hybridization is an increasingly common threat to native species (Levin 2002; Moyle and Light 1996) and has been documented as a conservation concern in a wide variety of taxa, ranging from bison (Halbert and Derr 2007) and red wolves (Adams et al. 2007; Miller et al. 2003), to owls (Haig et al. 2004), amphibians (Fitzpatrick and Shaffer 2007), and various plant groups (Wolf et al. 2001).

This phenomenon is particularly prevalent and well-studied in native North American trout of the genus *Oncorhynchus*, which are frequently threatened within their native ranges by hybridization with closely related introduced species: examples include Paiute cutthroat trout, *O. c. seleniris* (Cordes et al. 2004), Lahontan cutthroat trout, *O. c. henshawi* (Peacock and Kirchoff 2004); Yellowstone cutthroat trout, *O. c. bouvieri* (Gunnell et al. 2007); westslope cutthroat trout, *O. c. lewisi* (Rubidge et al. 2001; Rubidge and Taylor 2005), and Rio Grande cutthroat, *O. c. virginalis* (Pritchard et al. 2007), California golden trout, *O. m. aguabonita* (Cordes et al. 2006), Gila trout, *O. g.*

gilae (Wares et al. 2004), and Apache trout, *O. g. apache* (Porath and Nielsen 2003). At the subspecies level, introgressive hybridization poses challenges in that the hybridizing groups frequently lack isolating pre- or post-zygotic reproductive barriers or apparent selection against hybrid or backcross individuals; in some cases, hybridized taxa actually exhibit an increase in fitness (hybrid vigor) or possess adaptive advantages over parental types in intermediate, anthropogenically disrupted, or novel environments (Fitzpatrick and Shaffer 2007). Consequently, hybridization presents a serious conservation dilemma through loss of distinct, native, or potentially adaptive genetic components or lineages.

The technical difficulties associated with quantifying and monitoring introgression at lower taxonomic levels can be significant. While genetic analyses of introgression frequently employ diagnostic loci and reference populations in determining the genetic integrity of native populations, completely diagnostic loci may be unavailable, particularly in comparisons between closely related subspecies or between domesticated stocks and wild counterparts of the same subspecies. Furthermore, the use of reference populations may require unrealistic assumptions regarding populations of uncertain ancestry or questionable genetic "purity." Long-term genetic monitoring of focal species requires standardization of data over time and often across research laboratories (Smith et al. 2005; Welsh and May 2006). Such coordination efforts can be expensive, yet essential to better characterize extent to which hybridization functions as a threat to species persistence -- that is, whether introgression is likely to increase, decrease, or remain stable over time or under particular environmental conditions.

Single Nucleotide Polymorphism (SNP) markers have been applied successfully in population genetic studies of model organisms, and more recently, in several non-

model organisms. A SNP is a single base substitution, insertion, or deletion at a specific locus within the genome of a population of interest. SNPs are inherited in Mendelian fashion, are bi-allelic, co-dominant and may be less susceptible to homoplasy than microsatellites (Schlotterer 2004). SNP markers have several advantages over other marker types, including high reproducibility both within and across studies over time and among laboratories; genotyping of samples is amenable to high throughput methods (Melton 2003), and the resultant sequence-based data are comparable without requiring subsequent standardization. Recent SNP marker development efforts for selected rainbow trout subspecies ascertained highly informative SNP loci for the detection of introgression with introduced rainbow trout (Sprowles et al. 2006). Several SNP loci and insertion/deletions (indels) exhibited diagnostic or nearly diagnostic frequency differentials between hatchery and wild rainbow trout (*O. mykiss spp.,* and *O. m. irideus*, respectively) and several native *O. mykiss* subspecies.

The California golden trout (*O. m. aguabonita*), exemplifies many of the abovementioned challenges to studying hybridization. Introgression with introduced rainbow trout currently threatens the persistence of the endemic California golden trout (hereafter, "CAGT") to varying degrees throughout most of its native range (Cordes et al. 2006; Cordes et al. *in press*). A subspecies of rainbow trout, the CAGT is a California Species of Special Concern, a U.S. Forest Service Sensitive Species, and may warrant listing under the federal Endangered Species Act (Federal Register 2002, final decision on 12-month rule pending status review). The subspecies is currently the focus of a multi-agency conservation effort to prevent further loss and improve its status within its native range. Given the relatively shallow differentiation of *O. mykiss* subspecies from

one another and the heavy degree of anthropogenic influence on this particular subspecies, evaluating introgression in this group poses challenges more often associated with examining admixture between hatchery and wild fish stocks of the same subspecies.

Recent studies of CAGT in the two major drainage systems that comprise their native range -- Golden Trout Creek and the South Fork Kern River -- used a single copy nuclear (scn) DNA marker and a suite of six microsatellite loci to evaluate levels of introgression in populations both within and outside of the native range. These studies identified low-level, localized introgression in the Golden Trout Creek drainage, specifically in headwater lakes (and associated tributaries) that had been previously stocked with hybridized CAGT (Cordes et al. 2006); conversely, the South Fork Kern River exhibited a hybridization gradient, with relatively high levels of introgression (50-80%) in the lowest reaches of the mainstem South Fork Kern River and decreasing levels (1-13%) further upstream to headwater populations (Cordes et al. *in press*)

This study had three primary objectives: 1) to compare the abilities of SNP and microsatellite markers to detect hybridization among taxa, 2) to determine the degree of hybridization between golden trout and rainbow trout throughout the native range of CAGT and in transplanted populations, and 3) to evaluate the usefulness of genetic methodologies for making management decisions regarding golden trout.

Methods

Sampling and DNA extraction

Samples from previous microsatellite studies (Cordes et al. 2006; Cordes et al. *in press*) were used for SNP data analysis along with newly collected samples and temporal resampling from several locations during 2003-2006 (Table 2.1). California Department

of Fish and Game personnel (except where otherwise noted in table 1) collected fin clip samples from various locations in the South Fork Kern River, Golden Trout Creek, and a number of out-of-basin transplant populations. For comparison, fin clip samples were collected from three strains of hatchery rainbow trout, as well as from wild populations of rainbow trout in the North Fork American River and steelhead trout from the North Fork Navarro River (Table 2.1). Samples were preserved as dry fin clips or stored in either DMSO storage buffer (20% DMSO, 0.25 M EDTA, NaCl to saturation, pH 7.8), 95% ethanol, or as dry fin clips. Whole genomic DNA was extracted using the QIAGEN DNeasy™ Tissue Kit. Extracted DNA samples were stored at –30 °C until needed for Polymerase Chain Reaction (PCR) amplification of the SNP marker loci.

Data collection

SNP and insertion-deletion (indel) markers developed specifically for the study of hybridization in native trout subspecies (Sprowles et al. 2006) were used to generate genotypes for the populations given above. Three additional SNP loci were adapted from previous research (Bagley and Gall 1998), including two from the mtDNA control region (locus RTDL 316 and RTDL695) and one anonymous single copy nuclear DNA locus (A1A8_94). The 15 nuclear SNPs exist in 12 loci among which the degree of physical linkage is unknown, except in instances in which SNPs reside within the same locus (recombination activating gene loci R0917 230, R1175 137, and R1564 272; RAPD intergenic sequence loci RAPD 132 and RAPD 167). A total of 17 TaqMan assays consisting of forward and reverse primers and VIC- and FAM-labeled allele-specific probes were developed for each locus using either Applied Biosystems, Inc. Assays by Design or PrimerSelect software for use in 5'-nuclease reaction (Holland et al. 1991).

Each probe bore a minor groove binder and nonfluorescent quencher on the 3' end. Assay reactions were optimized on the individuals used in SNP marker discovery (Sprowles et al. 2006), including individuals of known genotype based on sequencing data. Known homozygotes, heterozygotes, and "composite" heterozygotes (generated by combining DNA from known homozygotes in ratios of 3:1, 1:1, and 1:3) were included as positive controls on every plate of samples analyzed, along with one no-template negative control. Reactions were carried out in 96-well microplates at a 5 µl volume. The majority of assays utilized 2X TaqMan Universal Master Mix (Applied Biosystems), 540nM each primer, 120nM each probe, and 10-20ng template DNA. Promega reagents were used for selected loci (see Table 2.2) at the following concentrations: 20u/ml Taq Polymerase, 0.2mM each dNTP, 5mM MgCl, 50mM KCl, 10mM Tris-HCl, 0.1% Triton® X-100, and concentrations of primers, probes, and template as given above. Reactions were performed using the Chromo4™ Real-Time PCR Detector (MJ Research/BioRad Laboratories, Inc.) and the following general thermal cycling protocol: initial denaturation of 94 degrees for 5 minutes, followed by 40 cycles of 92 degrees for 15 sec and an annealing temperatures ranging from 55-63.5 degrees (see Table 2.2) for 1 minute. Any individuals that failed to amplify using these initial conditions were reamplified using 2ul of template in 10ul reactions with the same reagent concentrations given above. Genotypes were scored using MJ Opticon Monitor analysis software (version 3.1, Bio-Rad Laboratories, Inc.) to visualize plots of endpoint fluoresence, subtracting baseline fluorescence averaged over the 10-20 cycle range, and identifying clusters of fluorescence corresponding with each probe. Genotyping results were confirmed for consistency with positive controls.

Data analyses

Allele frequencies and descriptive statistics for microsatellite data are reported elsewhere (Cordes et al. 2006; Cordes et al. *in press*). We analyzed SNP data in two phases: first, genotypes for all 17 loci were examined for allele frequencies, conformance to Hardy-Weinberg equilibrium and evidence of linkage disequilibrium using a subset of populations from the South Fork Kern River drainage and all rainbow trout populations. The best loci were selected from this panel for further data collection for all populations (detailed below). SNP data files were converted using Transformer-3 program (Caujape-Castells and Baccarani-Rosas 2005). Descriptive statistics for nuclear SNP data including observed and unbiased expected heterozygosities (Nei 1978) and inbreeding coefficients (F_{IS}; Weir and Cockerham) were calculated for all loci in each population sample using Genetix 4.05 (Belkhir et al. 1996-2004). Tests for departure from Hardy-Weinberg expectations due to heterozygote deficiencies were performed by permuting alleles among individuals within samples; tests of significance were performed in FSTAT (Goudet 1995) by determining the number of times F_{IS} values of the permuted samples exceeded the F_{IS} for the actual sample. Exact tests implemented in FSTAT were used to evaluate linkage equilibrium over all locus pairs in each population and also for overall linkage across populations. Statistical significances were computed using the Markov chain method to obtain unbiased estimates of Fisher's exact test based on 10,000 iterations (Guo and Thompson 1992). All significance values resulting from multiple comparisons were corrected for Type I error using Bonferroni correction as described by Rice (1989). GENEPOP on the Web (Raymond and Rousset 1995) was used to estimate

FST for each locus across populations following the methods of Weir and Cockerham (1984).

We employed a delta statistic (Smith et al. 2001) as a means of assessing marker efficiency for detecting differences between golden and rainbow trout subspecies groups. The estimate of δ for each SNP locus was calculated as the absolute value of the allelic frequency difference between two populations. The value of δ was calculated to determine the frequency differential for between populations where population "A" represented Volcano Creek and population "B" represented either Mt. Shasta Strain (δ_{V-M}) or North Fork American River (δ_{V-N}). Delta values range from 0 to 1, with 1 indicating fixed (diagnostic) differences in allele frequencies between the populations being compared. The Volcano Creek population was selected to represent CAGT because of its high degree of isolation and lack of apparent rainbow trout introgression. Lastly, the maximum delta value observed between any two populations (Maxδq) was calculated for all loci.

The Bayesian clustering program STRUCTURE, version 2.2 (Pritchard et al. 2000a) was used to determine the number of detectable genetic clusters (K) and to calculate posterior distributions of the admixture coefficient (q), or the proportional contribution of the observed groups to each individual's genotype, using an algorithm that defines groups by maximizing Hardy-Weinberg equilibrium within and minimizing linkage disequilibrium between groups. Mitochondrial loci were included in SNP STRUCTURE analyses as diploidized genotypes with one missing allele at each locus, as recommended in the program manual. We employed the admixture model and assumed correlated allele frequencies, with a burn-in-period of 30,000 and 100,000 MCMC

iterations for five runs of each K for K=1-5. STRUCTURE assumes K populations contribute to the gene pool of the sample population. For our analyses, no prior information on population of origin was employed; rather the program was allowed to determine admixture proportions independent of assumptions about which populations represented "pure" golden trout or "pure" rainbow trout. The most likely number of genetic clusters was determined by finding the K with the largest second-order rate of change in negative log-likelihood values (Evanno et al. 2005), and also confirmed by locating the asymptote of the negative log-likelihood values for all runs of K and examining the distribution of q-values in individuals as recommended by the authors (Pritchard 2000). Both the microsatellite and SNP data sets were each analyzed in the same manner, with data consisting of multilocus genotypes from individuals of all populations of both golden and rainbow trout. The program distruct (version 1.0, Rosenberg 2004) was used to visualize individual membership coefficients grouped by population code. Admixture estimates for the microsatellite data set were also compared to previously generated (Cordes et al. *in press*) admixture estimates based on likelihood estimation as implemented in LEADMIX 1.0 (Wang 2003).

Results

The majority of loci retained highly informative frequency differentials between representative rainbow and golden trout populations, as shown by high proportions for delta values and high overall F_{ST} values for individual loci. In general, delta values were high for the 8 loci ultimately used in the full data analysis of all populations, ranging from 0.5 to 1.0 for comparisons between Volcano Creek and Mt. Shasta Strain ($\delta q_{V\text{-}M}$) and 0.85 to 1.0 for comparisons to North Fork American River full populations (Table

2.2). A few loci had low frequency differentials: in preliminary analysis of the partial (South Fork Kern River) dataset, loci LDH 156 and RAPD 132 exhibited low delta values in comparisons of Volcano Creek golden trout and North Fork American River (wild) rainbow trout ($\delta q_{V\text{-}N}$=0.05; Table 2.2). Locus RAPD 167 exhibited low delta values in comparisons between Volcano Creek golden trout and Mount Shasta strain (hatchery) rainbow trout ($\delta q_{V\text{-}M}$=0.05; Table 2.2). Locus B9 164 had low delta values for both golden-rainbow comparisons, as reflected in low values for maxδq and F_{ST} (0.187 and 0.038, respectively; Table 2.2). Finally, locus URO 373 possessed lower delta values than other available loci. Locus B1 266 was not included for data collection on the full data set and could be of potential utility, given its moderate delta values ($\delta q_{V\text{-}M}$ = 0.69, $\delta q_{V\text{-}N.}$=0.52). Remaining loci were included in data analysis on the full set of populations for this study.

Allele frequencies, H_O, H_E, and F_{IS} values are reported for the 8 loci selected for final data analysis of all populations in Table 2.3 (descriptive statistics for other loci are also reported separately in Appendix 2.1). None of the F_{IS} values were significant (α=0.05/409 pairwise comparisons, $p < 0.000122$). Out of 1,810 possible pairwise comparisons, 49 revealed significant linkage disequilibrium after Bonferroni correction (α= 0.05/1,810; $p<0.00002762$): 47 deviations were due to linkage between R0917_230, R1175_137 and R1564_272 loci in multiple populations and one deviation was observed between RAPD 132 and 167, an expected result, given the close proximity of these SNP loci to one another within their respective genes. Several other comparisons within these locus-combinations were marginally significant in additional populations for the same locus combinations (data not shown). In comparisons of loci across all populations, five

locus combinations (R0917_230 x R1175_137, R0917_230 x R1564_272 R1175_137 x R1564_272, RAPD 132 x RAPD 167, and B9 164 x B9 388 deviated significantly out of a possible 105 locus combinations (α= 0.05/105, p = 0.00048) RAG9_230, RAG11_137, RAPD 132 were excluded from further analysis, though their utility as part of a haplotype block could be explored in future analyses. Similarly, RTDL_316 was selected to represent the mtDNA locus because of its higher δ value, relative to the RTDL 695 locus.

STRUCTURE analyses yielded the highest log-likelihood and delta K values for K = 2 for both the SNP and the microsatellite data sets, with all five runs converging on the same solution of two major groups corresponding to "golden" and "rainbow" trout (Figure 2.2). Log likelihood values differed in pattern between data sets, with the SNP data set reaching an asymptote more rapidly than the microsatellite data, which had log likelihood values that continued to increase incrementally with increasing values of K. Hybridized individuals were characterized by intermediate values of q ($0.1<q<0.9$; Figure 2.3). The division of groups for K=3 in the microsatellite data set corresponded somewhat with a division between South Fork Kern and all other California golden trout populations; however, several populations had multiple individuals that were fractionally assigned to each of the three groups at this value of K (data not shown).

Overall proportions of rainbow trout introgression for each CAGT population ranged from 0.01 to 0.92 for SNP data and 0.01 to 0.91 for microsatellite data, with the highest levels existing in the lower South Fork Kern River at Kennedy Meadow for both data sets (Table 2.4, Figure 2.3). The distribution of SNP q-values differed between major drainages, with rainbow trout introgression levels ranging from 0.01-0.03 for Golden Trout Creek populations, 0.04-0.91 for South Fork Kern River populations, 0.01-

0.03 for Cottonwood Basin transplanted populations, and 0.01 for the Wyoming transplanted populations (Table 2.4). The majority of individuals in the South Fork Kern River showed some level of introgression, demonstrating a cline of introgression that is high in the lower reaches and lower in populations located in headwater populations.

Estimates of the proportion of rainbow trout introgression for populations with two sampled time points were identical for four localities (LWMo 2001 and LWMn 2005 q= 0.02; SLCo 2000 and SLCn 2005, q= 0.01; GCo 2000 and GCn 2005, q= 0.03; MBSo 1999 and MBSn 2005, q = 0.04), and varied only slightly between MSSo 1999 and MSSn 2005 (0.03 and 0.02, respectively) and ASB 2002 and ASB 2004 (q=0.33 and 0.35, respectively).

Previous maximum likelihood-derived estimates (Cordes et al. 2006) for admixture were generally higher for Golden Trout Creek and transplanted lake populations derived from Golden Trout Creek, specifically for CSL, CL2, and CL4, which had negligible introgression values ($q<0.02$) based on STRUCTURE analyses, but significantly higher estimates of rainbow trout proportions (0.19-0.3) in maximum likelihood analyses.

Discussion

This study illustrates the utility of highly informative SNP and indel markers for detecting and quantifying introgression between introduced and native species of conservation concern. Understanding the dynamics of introgression over time is critical to elucidating the pattern and process of genetic change that occurs as a result of anthropogenic alteration of natural populations and their associated native habitats. Our panel of SNP and indel markers detected introgression in the majority of CAGT

populations with a sensitivity comparable to that of microsatellite markers, and will allow for better genetic monitoring into the future for this species. We have developed a powerful suite of markers that will allow for the collection of standardized genotype data utilizing the most current available technology. The validity of this technique has been verified through comparison to standard microsatellite data analyses and will greatly enhance our ability to conduct genetic monitoring of introgression in native golden trout populations over time, an important aspect to their continued management and conservation.

Use of SNP markers to study introgression

The expected theoretical number of random SNPs necessary to generate the statistical power equivalent to microsatellites in population genetic studies is thought to be substantially larger, by up to an order of magnitude (Morin et al. 2004). However, research suggests that selective use of highly informative markers, or SNPs which display large frequency differentials between groups of interest, can achieve the same results with fewer loci (Shriver et al. 1997; Wang 2003; Yang et al. 2005). Our results are similar to other recent findings that suggest relatively small numbers of informative SNP loci can be used to address questions of population admixture (Rosenberg et al. 2003; Yang et al. 2005). An optimal marker for assessment of admixture would be completely fixed, or diagnostic, between the two groups being evaluated, markers with a frequency differential between groups of 0.45 or greater are generally considered to retain a high degree of informativeness for studies of admixture (Shriver et al. 1997). SNP loci, which most commonly have only two alleles, are inherently less variable than most microsatellite loci. However, this deficiency appears to be more than compensated by the

power generated from informative frequency differentials between the groups of interest, in this case, between “rainbow” and “golden” trout groups.

The overwhelming advantage of SNP markers lies in the instant standardization of genotypes which overcomes the need for planned coordination of markers and allele scoring and associated costs of such endeavors, which increase with the number of loci and laboratories involved (LaHood et al. 2002; Moran et al. 2006; Welsh and May 2006). Even differences in data collection platforms within a laboratory can hinder accurate data comparison in the case of long-term projects spanning multiple years or decades. Furthermore, in the study of more differentiated groups in particular, the issue of homoplasy in microsatellites is not trivial, but SNPs have the clear advantage of being less prone to homoplasy and have a better understood underlying model of mutation (Vignal et al. 2002).

The retention of loci from the SNP marker development phase to the final data analysis phase was successful, with nearly half of the developed assays ultimately used in this study and four additional loci having potential applications for data analysis of these populations. Concerns that population genetic structure not detected during SNP marker ascertainment could further reduce the available loci were not realized in the case of CAGT, probably partly because limited detectable geographic structure persists in this subspecies due to the extensive translocation of populations both within and outside of their native basins. Three loci (CRB2677 106 and CTSD 33, Sprowles et al. 2006; F17a 80, adapted from Bagley and Gall 1998) did exhibit potential issues with null alleles during SNP data collection (data not shown), with deviations from Hardy-Weinberg Equilibrium that may be attributable to amplification issues and possibly null alleles in

selected populations. SNP assay forward or reverse primers could potentially be shifted up- or downstream to eliminate null alleles at these loci and apply to future analyses.

Estimation of rainbow trout introgression in California golden trout populations

Bayesian analyses of SNP and microsatellite data yielded similar population admixture estimates. Both marker types detected low, localized hybridization in Golden Trout Creek populations and a hybridization cline of increasing rainbow trout influence in the middle and lower reaches of the South Fork Kern River (Table 2.4). Two notable exceptions were Upper Trout Creek (UTC) and Kern Peak left stringer (KPLS), where SNP and microsatellite STRUCTURE-based estimates differed by more than 10%. Microsatellite-based STRUCTURE estimates of introgression were also generally consistent with those detected in previous research on these CAGT populations using non-Bayesian methods (Cordes et al. 2006; Cordes et al. *in press*).

The prevalence of hybridization observed in the majority of the South Fork Kern River and associated tributaries is consistent with patterns observed in hybrid swarms or clines; however, such patterns can also persist in situations of reduced hybrid fitness. The risk of genetic swamping is strongly related to competitive ability and initial frequency of the native taxon and also the presence of prezygotic reproductive barriers and habitat differentiation between the hybridizing taxa (Wolf et al. 2001). Theoretical evidence also shows that parental taxa can go extinct, despite a fitness penalty for hybrids and with a rate of extinction dependent upon strength of fitness gradient and starting proportion of admixture. (Epifanio and Philipp 2000). Habitat disruption could potentially facilitate introgression in CAGT, particularly in the South Fork Kern River where cattle grazing has heavily impacted channel morphology and habitat. Golden trout are negatively

affected by the siltation that accompanies cattle grazing, which increases population densities and decreases individual growth rates (Knapp et al. 1998). Introgression patterns observed in this and other genetic studies of CAGT most likely reflect the stocking history of rainbow trout, with the spread of rainbow trout alleles in CAGT a function of distance from the source of introduction (Allendorf 2001; Cordes et al. 2006; Cordes et al. *in press;* Rubidge and Taylor 2005) and accessibility of upstream sites based on absence of effective barriers to dispersal. The fact that the cline of introgression persists in the South Fork Kern River populations, despite the presence of three artificially constructed barriers that have been in place for several decades, suggests either that the rainbow trout influence in this drainage predates barrier construction or that barriers have not been effective at halting upstream movement of the rainbow trout.

Though Bayesian (STRUCTURE) analyses of SNP and microsatellite data sets were generally concordant, some large differences existed between Bayesian and previous Maximum Likelihood (LEADMIX) estimates of introgression for microsatellite data sets (Cordes et al. 2006, shown in Table 4, this study), most notably in out-of-basin transplanted lake populations derived from Golden Trout Creek. These lake populations show phenotypic characteristics of rainbow trout (e.g. spotting, coloration) and high levels of genetic introgression as assessed by LEADMIX analysis of both microsatellite loci and a diagnostic SCN locus (Cordes et al. 2006); however, these same lake populations yield lower estimates of introgression in STRUCTURE analyses of both SNP and microsatellite data (this study). STRUCTURE and LEADMIX differ not only in their algorithms, but also in their fundamental assumptions in estimating admixture: LEADMIX estimates introgression based on parental reference groups, assuming that the

allele frequencies in parental reference samples represent those of the true parental population and that admixture occurred at a single time point. While the populations used as references in LEADMIX analyses were the best available extant populations (Cordes et al. 2006), they may not adequately represent the genetic composition of the true parental populations. Likewise, lake populations were hybridized early on (probably during the 1930-40s), and the sources of hatchery rainbow trout introgression may have drifted genetically from the contemporary hatchery rainbow trout reference populations used in this study. Lastly, lake populations may also have experienced multiple admixture events, violating the assumptions of the algorithm. The consistently lower estimates of introgression produced by STRUCTURE analyses may be due to the lack of reference groups used; however, STRUCTURE estimates of introgression are also sensitive to the choice of reference population (e.g. Pritchard et al. 2007) and known to have difficulty in estimating the proportion of ancestry when groups are heavily introgressed (Pritchard et al. 2000b). These results underscore the importance of using multiple methods in estimating introgression and the need for careful application of quantitative introgression estimates to species management. Our data do not allow unambiguous determination between low levels of introgression and genetically "pure" populations. These observations argue against the use of strict cutoffs in determining management categories.

Recent advancements in data analysis methodologies, particularly the advent of Bayesian algorithms that do not require prior definition of reference "golden" and "rainbow" populations, allow for reduced bias in quantifying rainbow trout introgression in golden trout populations. The presence of low (less than 1%) levels of introgression in

Volcano Creek and other native CAGT populations and (conversely in the detection of a similar proportion of "golden" influence in the rainbow trout hatchery and wild populations) is likely not an indication of actual introgression. Rather, it is more likely a result of the STRUCTURE algorithm, which is constrained to consider even small probabilities of possible membership in the rainbow trout group. The additive accumulation of these probabilities may result in an estimation of low levels of introgression where none may exist. Refining analyses to account for what appears to be an artifact of the analysis software and ultimately provide improved estimates of introgression is needed.

The observation of very similar estimates of introgression for sampled localities with multiple time-series data suggests either that proportions of introgression at these localities have stabilized. Alternatively, time span between sampling (ranging from 2-5 years) may be insufficient to detect any change in introgression, given the generation times of golden trout. Ongoing genetic monitoring will provide the samples needed to better resolve this question in the future.

Native fish populations have been shown to persist genetically intact outside of their native range (Nielsen et al. 2001). The utility of such populations for reintroductions in native range though, should be approached cautiously. The headwater Wind River populations were derived from shipments of California golden trout dating back to the 1920's, probably prior to the hybridization of the CAGT broodstock in the Cottonwood Lakes. Although the genetic integrity of Wind River, Wyoming CAGT populations appears to match that of even the Volcano Creek CAGT population, and furthermore do not appear to have been introgressed with cutthroat trout based on

mtDNA markers (M. Campbell, unpublished data), their diversity has not yet been evaluated and additional risks (disease, etc.) associated with movement back into their native range should be carefully considered.

Management implications

Hybridization poses challenges to determining and enforcing legal protections for species listed or considered for listing under the U.S. Endangered Species Act (ESA). Hybrids are not currently afforded legal protection under the ESA, and a draft intercross policy (Federal Register 1996) providing guidance on this issue has never been issued. Recent debate surrounding hybrids (see Allendorf et al. 2005; Allendorf et al. 2004; Campton and Kaeding 2005) generally involves characterizing the extent to which hybridized populations threaten native taxa versus the extent to which they represent a component of genetic diversity worthy of protected status (and whether such individuals ought to be included in species counts for ESA listing decisions). A related concern is the sometimes seemingly arbitrary nature of cutoffs for acceptable levels or proportions of detectable introgression in listed taxa; what detectable level of introgression precludes inclusion as part of the species, and by what methods (genetic, morphological)? Ours and other studies (e.g. Pritchard et al. 2007) illustrate the analysis-dependent nature of introgression estimates, cautioning against the strict use of management by numbers without an adequate understanding of the variance surrounding introgression estimates.

Researchers studying introgression between native and non-native taxa frequently correlate genetic data with available morphological data in an attempt to identify the extent to which morphologically or phenotypically detectable evidence of introgression reflect genetic assessments of introgression (e.g., Beaumont et al. 2001; Pritchard et al.

2007). If the conservation goal is to conserve an organism that minimally retains the appearance of the native taxon, using a particular cutoff value for introgression estimates may be sufficient; however, if the conservation goal is conserve a native taxon with the adaptive genetic variation that will allow it to persist in its natural or extant environment, conservation by appearance may not be adequate. The degree to which neutral genetic markers measuring admixture reflect the true proportion of non-native invasion of the native genome is not well-studied (Mallet 2005). Even in instances where comprehensive genetic and morphological data are available, interpretation of these complex issues will require a continuing role for value judgment and expert opinion, not unlike that required in the evaluation of subspecies listings under the ESA (Haig et al. 2006).

In recent years, hatchery managers have begun stocking triploid rainbow trout in the lower reaches of the South Fork Kern River in an attempt to reduce the genetic impact of stocking. The unknown genetic risks of stocking triploid fish that may not be 100% triploid, and the known ecological effects of stocking triploids (larger fish, possible strong competitors, aggressive, disruptive of dynamics) suggest that this strategy is not consistent with the goal of protecting CAGT. A better strategy in light of available genetic information is to focus on protecting existing populations of known genetic "purity" or near-purity, such as populations in Golden Trout Creek and upper reaches of the South Fork Kern. This approach has worked well in areas of Golden Trout Creek, where genetic data (Cordes et al. 2006) were used successfully to direct the targeted eradication of hybridized trout populations in lakes that threatened downstream non-hybridized CAGT populations. The needs in the South Fork Kern River are more

complex, and require cost-benefit analysis of a range of management action options including strategic chemical or physical removal of heavily hybridized populations, reliance on physical barriers, and cessation of rainbow trout stocking. Additional information regarding the integrity of physical barriers is needed to inform the extent to which physical barriers can be relied upon to maintain existing levels of introgression, regardless of whether such levels are acceptable from a conservation perspective. Preservation of populations with limited amounts of introgression in the upper South Fork Kern and tributaries may be warranted, particularly if they are found to contain a distinct genetic component (Campton and Kaeding 2005; Peacock and Kirchoff 2004). Although a few key information needs persist (see below), the genetic data available from this study provide a strong foundation for developing a genetic management plan for CAGT that weighs genetic costs and benefits associated with proposed management actions. Such a plan will require an adaptive management approach that allows for the incorporation of new information, including ongoing genetic monitoring data, into a decision making framework.

Further genetic research

Future research using simulations could clarify the variance surrounding introgression estimates (Hansen, Pritchard 07), which would be useful for setting population management priorities and understanding uncertainty associated with estimates. The use of haplotypes to represent data from multiple linked loci might also be beneficial in determining whether additional SNPs in these loci increase the information content and power of these markers at assessing introgression. Comparison of other approaches that use reference populations in analyses is needed, focusing on approaches

that do not require a priori definition of reference groups, given that our particular situation tends to violate many of the assumptions of such software (e.g., ADMIX approach, which assumes that reference samples from true parental populations are available and the admixture event occurred at a single point in time, neither of which is plausible for our golden trout data set). Additional work is also needed to determine the extent to which populations in the South Fork Kern that have lower levels of rainbow trout introgression may represent a unique genetic component distinct from Golden Trout Creek populations.

Tables and Figures

Table 2.1. California golden trout populations used for SNP comparative study. Individual localities are grouped by major drainage, transplanted region, or designated as wild (RT-wild) or hatchery (RT-hat) rainbow trout. Each locality number (PopID) corresponds with map localities given in Figure 2.1. Locality descriptions, sample collection years, and number of individuals used in SNP data collection (N) are given (corresponding microsatellite data sample sizes, n, given parenthetically).

Drainage	Pop ID	Locality	Code	Coll. Year	N (n)
Golden Trout Creek	1	Volcano Creek [b]	VC	2000	39 (29)
	2	Volcano Creek Left Stringer	VCLSn	2005	41
	3	Golden Trout Creek, below Little Whitney [b]	LWC	2001	38 (19)
	3a	Golden Trout Creek, below Little Whitney	LWMn	2005	40
	4	Salt Lick Creek	SLCo	2000	40
	4a	Salt Lick Creek	SLCn	2005	33
	5	Lower Johnson Creek 1999	LJC	1999	32
	6	Middle Johnson Creek	MJC	1999	30 (28)
	7	Johnson Creek	JC	2000	24
	8	Johnson Lake	JL	2000	39 (26)
	9	Groundhog Creek [b]	GC	2000	37 (23)
	9a	Groundhog Creek	GCn	2005	39
	10	Golden Trout Creek, below Barigan	BBS	1999	30
	11	Mouth Barigan Stringer	MBS	1999	30 (29)
	11a	Mouth Barigan Stringer	MBSn	2005	40
	12	Golden Trout Creek above Barigan Stringer	ABSn	2004	28
	13	Golden Trout Creek, below Stokes Stringer	BSS	1999	30
	14	Middle Stokes Stringer	MSSo	1999	29
	14a	Middle Stokes Stringer	MSSn	2005	40
	15	Upper Stokes Stringer	USS	1999	30 (28)
	16	Chicken Springs Lake	CSL	2000	34 (30)
	17	Big Whitney Meadow	BWM	2001	40
	18	Headwaters, Golden Trout Creek	HW	1999	29
	19	Horseshoe Creek	HC	1999	30
	20	Cottonwood Lakes 2 (lakes 1,2,3)	CL2	2000	48 (32)
	21	Cottonwood Lakes 4 (lakes 4,5)	CL4	2000	50 (30)
	22	Little Cottonwood Creek 1	LCC1	2000	19
	23	Little Cottonwood Creek 2	LCC2	2000	25
South Fork Kern	24	Upper South Fork Kern	USFK	2001	42 (30)
	25	South Fork Kern River, above Ramshaw	ARB	1999	30 (30)
	26	South Fork Kern River, below Ramshaw	BRB	2002	29 (30)
	27	Kern Peak Left Stringer	KPLS	2002	30 (30)
	28	Below Movie Stringer	BMS	2001	30 (30)
	29	South Fork Kern River, above Templeton	ATB	2002	30 (30)
	30	Upper Mulkey Creek	UMC	2001	30 (30)
	31	Four Canyons Creek	FCC	2002	30 (30)
	32	South Fork Kern River, below Templeton	BTB	2002	30 (30)
	33	Strawberry Creek	SCn	2004	40
	34	South Fork Kern River, above Schaeffer	ASB	2002	30 (30)
	34a	South Fork Kern River, above Schaeffer	ASBn	2004	40
	35	South Fork Kern River, below Schaeffer	BSBn	2004	40

Table 2.1, continued

Drainage	Pop ID	Locality	Code	Coll. Year	N (n)
	36	Monache Meadows	MM	2002	30 (30)
	37	Middle Fish Creek	MFC	2001	40 (30)
	38	Upper Trout Creek	UTC	2001	30 (30)
	39	Kennedy Meadows	AKM	2003	8 (8)
	40	Rockhouse Basin	RHB	2004	10
Out-of-basin,	41	"Golden Pond," Wind River, WY	GP	2003	11
	42	Upper Wind River Ranch, Wind River, WY	WR	2003	29
Wild rainbow	43	North Fork American River	NFAR	2000	20 (24)
	44	North Fork Navarro River[a]	NFNR	2000	31 (29)
Hatchery rainbow	45	Hot Creek Strain	HCS	2002	30 (29)
	46	Mount Shasta Strain	MSS	2001	31 (30)
	47	Mt. Whitney Strain	MWS	2002	30 (30)

[a] Collected by students and staff of the John Muir Institute for the Environment, University of

[b] Collected by United States Forest Service (USFS) personnel

Table 2.2. Twenty SNP assays developed for California golden trout. Marker names consist of the locus identifier and nucleotide position targeted. Oligonucleotide sequences for unlabeled primers (Forward and Reverse) and labeled probes for each allele are given for each marker, annealing temperature (Ta), number of individuals successfully genotyped (N), expected (H_E; assuming panmixia) and observed (H_O) heterozygosities. Difference in "rainbow" allele frequency between Volcano creek California golden trout versus Mount Shasta Strain rainbow trout (delta V-M) and versus North Fork American River rainbow trout (delta V-N) are also given, along with maximum difference in "rainbow" allele frequency between any two collections (δq) and F_{ST} estimates for each marker.

Locus name	Oligonucleotide sequences (5'–3')	Ta(°C)	Reagents	N	$\delta q_{V\text{-}M}$	$\delta q_{V\text{-}N}$	Maxδq	F_{ST}
loci used in full analysis								
A1A8_94[1]	F:GTGTTTTCACATGCAGAAGTGATTACT R:GGCCCTTCTCAATTGGAACAGTA VIC-CTATATCTACCTTCCTAATGAA FAM-CTATATCTACCTTCCTATATCTACCTTCTTAATGAATTAATGAA	60	MM	1602	1.00	1.00	1.00	0.473
B9_snp388[2]	F:CTCTCTTCTCCTCGTATGGTGACT R:GCACCTGGTCTGCACCT VIC-CCCCCATGGATGTGTAT FAM-CCCCATGGACGTGTAT	60	Promega	1646	0.95	0.95	1.00	0.392
ID1c 77[3]	F:CAGGCTTTTTTTTCTATCAGAATTAAGTC R:TGTATGCTAACTTGTAATTTGCTGTTGT VIC-AGTTAACAGTTAATGAGT FAM-AGGCAGTTAACGAGTC	58	MM	1673	0.98	1.00	1.00	0.517
Omy_f1 259-260[3]	F:CCACACACACAAACACACATACAC R:CAAGCATTCTTCTGTTAAATGTGGTCTA VIC-CACACACAAACAGCA FAM-ACACACACACACAGCA	60	MM	1692	0.50	0.85	0.85	0.471
Omy_g1 103[3]	F:CTCAGCAAAAAAGAAACGTCCCTTT R:AGTCGTGACAATGAGAAACAGTGTT VIC-CCTTTTACAATGAAGATC FAM-CTTTTACAGTGAAGATC	61.5	MM	1636	0.86	0.86	1.00	0.258
Omy_h1 170[3]	F:CTGCTGCCTCTGGGTATGG R:ATTCTCACCTTGGGAATGGACATC VIC-ACACTGCTACACTTCA FAM-ACACTGCTATACTTCA	60	Promega	1642	1.00	0.98	1.00	0.494

Table 2.2, continued

Locus name	Oligonucleotide sequences (5'–3')	Ta(°C)	Reagents	N	$\delta q_{V\text{-}M}$	$\delta q_{V\text{-}N}$	Maxδq	F_{ST}
R1564 272[3]	F:GGTTTATGTTATTACACCTGTGTGAACTG	60	MM	1646	0.97	0.98	1.00	0.619
	R:ACTGGCACAACTGTATGTAAACCT							
	VIC-ATATGTTATGATAAAAAAAATTACA							
	FAM-ATGTTATGATAAAAAAATTACA							
RTDL 316[1]	F:AACATACGGTGATTTTAACCCCTCAT	60	MM	1686	1.00	1.00	1.00	--
	R:GTAAAGACGGAGCCCGTGTTA							
	VIC-CTTGGATTTGTGCTGATGT							
	FAM-CTTGGATTTGTACTGATGT							
Locus not selected to represent gene								
RTDL 695[1]	F:AAGCCGGGCGTTCTCTTATATG	62	MM	1680	0.87	0.87	1.00	--
	R:GTTAGACTTCTTTGCTTGCACTTGT							
	VIC-CATAGGGTTCTCTTTTTT							
	FAM-ATAGGGTTCTCCTTTTT							
R0917 230[3]	F:CGAGTAAACAGGGAAGCAAGTGA	60	MM	1670	0.95	0.98	1.00	0.603
	R:ACAACTCAAAATGGTGTCATCAGAGA							
	VIC-AATAAACTATCAAATCATTTCAG							
	FAM-ATAAACTATCAAATAATTTCAG							
R1175 137[3]	F:ACTGTCATGACTTTAACCTGATGATGTAC	57	MM	1657	0.95	0.98	1.00	0.601
	R:ACATACCGTCATGTAAAACGTGATGT							
	VIC-AGATTTTCATAATGTATAATATT							
	FAM-ATTTTCATAATGTGTAATATT							
Locus not used in full analysis to due lower frequency differentials								
B1_266[2]	F:TCATGTGAACTTTAATTGACTAGGAAGTCG	62	MM	608	0.69	0.52	1.00	0.388
	R:GATATGAAAATATCTGAAGAGTTATATTTGGGAAATTGAC							
	VIC-TCTATAAACAACATTTTTC							
	FAM-TCTATAAACAAAATTTTTC							
B9_164[2]	F:GCACAGAACACAGCCAATATTAACA	63.5	MM	599	0.05	0.05	0.187	0.038
	R:GCCTTGACTCTCCCTTCATGAC							
	VIC-CCTACAACTTGATCTAACGTG							
	FAM-CCTACAACTTGATCTACGTG							

Table 2.2, continued

Locus name	Oligonucleotide sequences (5'–3')	Ta(°C)	Reagents	N	$\delta q_{V\text{-}M}$	$\delta q_{V\text{-}N}$	Maxδq	F_{ST}
RAPD 132[3]	F:ATCATTACCACGCCCAACGTTA	60	MM	644	0.92	0.05	0.95	0.709
	R:AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT							
	VIC-CATGTTGGGATATATGA							
	FAM-ATGTTGGGAAATATGA							
LDH 156[3]	F:GTTTTGAAACCAGTTTAAGGTTGATTGC	62	Promega	591	0.88	0.14	0.967	0.639
	R:ACGGCATAGTCTGGACAGAGAT							
	VIC-CCATTTAGACGTTTTTT							
	FAM-CCATTTAGATGTTTTTT							
RAPD 167[3]	F:CCCAACATGCTCTATTGCAGCTA	55	MM	608	0.00	0.55	0.87	0.437
	R:AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT							
	VIC-ATTAAAACAATCCCCCCCAAAA							
	FAM-TTAAAACAATCCCACCCAAAA							
URO 373[3]	F:ACATCTGTAAACAGATGCTGCTGAA	60	MM	608	0.45	0.45	0.97	0.338
	R:GCCAGAGTTTAAGTAAATCTGCAAGGA							
	VIC-TTATTGCCTATTGACATATAA							
	FAM-TTGCCTATTGAAATATAA							

[1] Bagley and Gall 1888

[2] R. Philips, unpublished data

[3] Sprowles et al. 2006

Table 2.3. Descriptive statistics for all loci for which data were collected in all or a subset of populations. Frequency of designated predominately "rainbow" allele (nucleotide given as adenine [A], guanine [G], cytosine [C], and thymine [T]) ":"= 1 bp deletion), number of individuals amplified for each assay (n). Observed (H_O) and unbiased expected (H_E) heterozygosites and F_{IS} values also shown. Descriptive statistics for loci not selected for collection on all populations due to lower observed frequency differentials are given in Table A1.

Table 2.3

Code	Pop	Loci									
		A1A8 94					B9 388				
		N	C(T)	H_E	H_O	F_{IS}	N	A(G)	H_E	H_O	F_{IS}
1	VC	38	0.00	0.00	0.00	–	38	0.05	0.10	0.11	-0.04
2	VCLSn	40	0.03	0.05	0.05	-0.01	41	0.05	0.09	0.10	-0.04
3	LWC	30	0.17	0.28	0.20	0.30	31	0.08	0.15	0.16	-0.07
4	LWMn	40	0.04	0.07	0.08	-0.03	40	0.09	0.16	0.13	0.23
5	SLCo	40	0.03	0.05	0.05	-0.01	40	0.13	0.22	0.20	0.10
6	SLCn	33	0.00	0.00	0.00	–	33	0.09	0.17	0.18	-0.09
7	LJC	32	0.06	0.12	0.13	-0.05	32	0.05	0.09	0.09	-0.03
8	MJC	30	0.00	0.00	0.00	–	30	0.15	0.26	0.23	0.10
9	JC	22	0.02	0.05	0.05	0.00	22	0.11	0.21	0.23	-0.11
10	JL	33	0.14	0.24	0.27	-0.14	37	0.07	0.13	0.14	-0.06
11	GC	36	0.07	0.13	0.14	-0.06	34	0.26	0.40	0.41	-0.04
12	GCn	38	0.05	0.10	0.05	0.48	37	0.23	0.36	0.30	0.17
13	BBS	30	0.12	0.21	0.17	0.21	28	0.21	0.34	0.29	0.17
14	MBS	30	0.15	0.26	0.30	-0.16	30	0.15	0.26	0.30	-0.16
15	MBSn	40	0.14	0.24	0.28	-0.15	39	0.18	0.30	0.26	0.14
16	ABSn	28	0.04	0.07	0.07	-0.02	28	0.25	0.38	0.29	0.26
17	BSS	31	0.21	0.34	0.42	-0.25	30	0.20	0.33	0.27	0.18
18	MSSo	26	0.10	0.18	0.19	-0.09	25	0.22	0.35	0.44	-0.26
19	MSSn	39	0.10	0.19	0.21	-0.10	39	0.12	0.21	0.18	0.13
20	USS	30	0.03	0.07	0.07	-0.02	30	0.08	0.16	0.17	-0.07
21	CSL	28	0.21	0.34	0.43	-0.26	32	0.22	0.35	0.44	-0.27
22	BWM	26	0.08	0.14	0.15	-0.06	39	0.22	0.35	0.38	-0.12
23	HW	29	0.03	0.07	0.07	-0.02	29	0.14	0.24	0.28	-0.14
24	HC	26	0.00	0.00	0.00	–	22	0.00	0.00	0.00	–
25	CL2	47	0.03	0.06	0.06	-0.02	47	0.07	0.14	0.15	-0.07
26	CL4	50	0.10	0.18	0.16	0.12	50	0.06	0.11	0.12	-0.05
27	LCC1	19	0.00	0.00	0.00	–	19	0.00	0.00	0.00	–
28	LCC2	25	0.00	0.00	0.00	–	25	0.00	0.00	0.00	–
29	USFK	38	0.37	0.47	0.47	-0.01	41	0.23	0.36	0.32	0.12
30	ARB	30	0.33	0.45	0.47	-0.03	29	0.29	0.42	0.24	0.43
31	BRB	28	0.39	0.49	0.50	-0.03	28	0.23	0.36	0.39	-0.08
32	KPLS	30	0.37	0.47	0.47	0.01	30	0.17	0.28	0.33	-0.18
33	BMS	30	0.50	0.51	0.67	-0.32	30	0.28	0.41	0.37	0.11
34	ATB	30	0.42	0.49	0.57	-0.15	30	0.20	0.33	0.33	-0.03
35	UMC	30	0.00	0.00	0.00	–	30	0.13	0.24	0.27	-0.14
36	FCC	30	0.23	0.36	0.33	0.09	30	0.32	0.44	0.57	-0.29
37	BTB	29	0.48	0.51	0.41	0.19	30	0.27	0.40	0.33	0.16
38	SCn	40	0.54	0.50	0.53	-0.04	40	0.45	0.50	0.50	0.00
39	ASB	30	0.62	0.48	0.57	-0.18	30	0.48	0.51	0.57	-0.12
40	ASBn	38	0.70	0.43	0.34	0.20	39	0.51	0.51	0.51	-0.01
41	BSBn	40	0.58	0.49	0.50	-0.01	40	0.54	0.50	0.33	0.36
42	MM	30	0.65	0.46	0.30	0.36	29	0.47	0.51	0.59	-0.16
43	MFC	36	0.56	0.50	0.67	-0.34	40	0.05	0.10	0.10	-0.04
44	UTC	21	0.26	0.40	0.43	-0.08	24	1.00	0.00	0.00	–
45	AKM	8	1.00	0.00	0.00	–	8	0.94	0.13	0.13	0.00
46	RHB	10	0.70	0.44	0.40	0.10	10	0.75	0.39	0.50	-0.29
47	GP	5	0.00	0.00	0.00	–	11	0.00	0.00	0.00	–
48	WR	12	0.00	0.00	0.00	–	29	0.00	0.00	0.00	–
49	NFAR	20	1.00	0.00	0.00	–	20	1.00	0.00	0.00	–
50	NFNR	31	1.00	0.00	0.00	–	31	0.98	0.03	0.03	0.00
51	HCS	30	1.00	0.00	0.00	–	30	0.93	0.13	0.13	-0.06
52	MSS	30	1.00	0.00	0.00	–	30	1.00	0.00	0.00	–
53	MWS	30	1.00	0.00	0.00	–	30	0.98	0.03	0.03	0.00

Table 2.3, continued

Code	Pop	Loci									
		ID1c 77-83					Omy_f1 259-260				
		N	AGTTAAT(:::::::)	H_E	H_O	F_{IS}	N	::(AA)$_n$	H_E	H_O	F_{IS}
1	VC	37	0.00	0.00	0.00	–	39	0.00	0.00	0.00	–
2	VCLSn	41	0.00	0.00	0.00	–	41	0.00	0.00	0.00	–
3	LWC	38	0.01	0.03	0.03	0.00	38	0.00	0.00	0.00	–
4	LWMn	40	0.01	0.03	0.03	0.00	40	0.00	0.00	0.00	–
5	SLCo	40	0.00	0.00	0.00	–	39	0.00	0.00	0.00	–
6	SLCn	33	0.02	0.03	0.03	0.00	33	0.00	0.00	0.00	–
7	LJC	32	0.00	0.00	0.00	–	32	0.00	0.00	0.00	–
8	MJC	30	0.00	0.00	0.00	–	30	0.02	0.03	0.03	0.00
9	JC	24	0.02	0.04	0.04	0.00	24	0.02	0.04	0.04	0.00
10	JL	39	0.15	0.26	0.31	-0.17	39	0.10	0.19	0.21	-0.10
11	GC	39	0.03	0.05	0.00	1.00	38	0.00	0.00	0.00	–
12	GCn	39	0.00	0.00	0.00	–	39	0.00	0.00	0.00	–
13	BBS	30	0.00	0.00	0.00	–	30	0.00	0.00	0.00	–
14	MBS	30	0.10	0.18	0.20	-0.09	30	0.13	0.24	0.27	-0.14
15	MBSn	40	0.01	0.03	0.03	0.00	40	0.01	0.03	0.03	0.00
16	ABSn	28	0.04	0.07	0.00	1.00	28	0.00	0.00	0.00	–
17	BSS	30	0.03	0.07	0.07	-0.02	30	0.00	0.00	0.00	–
18	MSSo	28	0.02	0.04	0.04	0.00	28	0.00	0.00	0.00	–
19	MSSn	39	0.00	0.00	0.00	–	39	0.00	0.00	0.00	–
20	USS	30	0.02	0.03	0.03	0.00	30	0.00	0.00	0.00	–
21	CSL	33	0.24	0.37	0.48	-0.31	34	0.00	0.00	0.00	–
22	BWM	40	0.04	0.07	0.08	-0.03	40	0.00	0.00	0.00	–
23	HW	29	0.02	0.03	0.03	0.00	29	0.00	0.00	0.00	–
24	HC	30	0.10	0.18	0.20	-0.09	30	0.00	0.00	0.00	–
25	CL2	48	0.08	0.15	0.17	-0.08	48	0.07	0.14	0.15	-0.07
26	CL4	50	0.10	0.18	0.20	-0.10	50	0.17	0.29	0.34	-0.20
27	LCC1	18	0.00	0.00	0.00	–	19	0.00	0.00	0.00	–
28	LCC2	25	0.00	0.00	0.00	–	25	0.00	0.00	0.00	–
29	USFK	41	0.16	0.27	0.22	0.19	42	0.02	0.05	0.05	-0.01
30	ARB	29	0.22	0.35	0.45	-0.27	30	0.00	0.00	0.00	–
31	BRB	28	0.13	0.22	0.25	-0.13	29	0.00	0.00	0.00	–
32	KPLS	30	0.15	0.26	0.30	-0.16	30	0.02	0.03	0.03	0.00
33	BMS	30	0.15	0.26	0.30	-0.16	30	0.00	0.00	0.00	–
34	ATB	30	0.17	0.28	0.20	0.30	30	0.00	0.00	0.00	–
35	UMC	30	0.10	0.18	0.20	-0.09	30	0.00	0.00	0.00	–
36	FCC	30	0.15	0.26	0.23	0.10	30	0.00	0.00	0.00	–
37	BTB	30	0.15	0.26	0.30	-0.16	30	0.00	0.00	0.00	–
38	SCn	40	0.26	0.39	0.43	-0.09	40	0.03	0.05	0.05	-0.01
39	ASB	30	0.32	0.44	0.43	0.02	30	0.02	0.03	0.03	0.00
40	ASBn	40	0.34	0.45	0.58	-0.27	40	0.10	0.18	0.20	-0.10
41	BSBn	40	0.34	0.45	0.38	0.17	40	0.08	0.14	0.10	0.29
42	MM	30	0.45	0.50	0.57	-0.13	30	0.03	0.07	0.07	-0.02
43	MFC	39	0.12	0.21	0.23	-0.12	40	0.01	0.03	0.03	0.00
44	UTC	30	0.13	0.24	0.27	-0.14	30	0.00	0.00	0.00	–
45	AKM	8	0.94	0.13	0.13	0.00	7	0.71	0.44	0.00	1.00
46	RHB	10	0.80	0.34	0.40	-0.20	10	0.05	0.10	0.10	0.00
47	GP	9	0.00	0.00	0.00	–	11	0.00	0.00	0.00	–
48	WR	23	0.00	0.00	0.00	–	29	0.00	0.00	0.00	–
49	NFAR	20	1.00	0.00	0.00	–	20	0.85	0.26	0.20	0.24
50	NFNR	29	1.00	0.00	0.00	–	31	0.11	0.20	0.16	0.21
51	HCS	30	1.00	0.00	0.00	–	30	0.57	0.50	0.53	-0.07
52	MSS	28	0.98	0.04	0.04	0.00	31	0.50	0.51	0.48	0.05
53	MWS	29	1.00	0.00	0.00	–	30	0.82	0.30	0.23	0.24

Table 2.3, continued

Code	Pop	Loci										
		Omy g1 103						Omy_h1 170				
		N	T(C)	H_E	H_O	F_{IS}		N	G(A)	H_E	H_O	F_{IS}
1	VC	39	0.14	0.25	0.28	-0.15		38	0.00	0.00	0.00	–
2	VCLSn	41	0.20	0.32	0.24	0.24		40	0.00	0.00	0.00	–
3	LWC	26	0.60	0.49	0.50	-0.02		31	0.05	0.09	0.10	-0.03
4	LWMn	40	0.55	0.50	0.45	0.10		40	0.03	0.05	0.05	-0.01
5	SLCo	40	0.44	0.50	0.48	0.05		40	0.00	0.00	0.00	–
6	SLCn	33	0.42	0.50	0.55	-0.10		33	0.00	0.00	0.00	–
7	LJC	32	0.47	0.51	0.44	0.14		32	0.05	0.09	0.09	-0.03
8	MJC	30	0.75	0.38	0.43	-0.14		30	0.00	0.00	0.00	–
9	JC	23	0.63	0.48	0.39	0.18		23	0.00	0.00	0.00	–
10	JL	33	0.18	0.30	0.36	-0.21		36	0.00	0.00	0.00	–
11	GC	34	0.50	0.51	0.71	-0.40		34	0.07	0.14	0.15	-0.07
12	GCn	38	0.61	0.48	0.47	0.02		38	0.04	0.08	0.08	-0.03
13	BBS	30	0.40	0.49	0.47	0.05		30	0.10	0.18	0.13	0.28
14	MBS	30	0.32	0.44	0.43	0.02		30	0.02	0.03	0.03	0.00
15	MBSn	40	0.46	0.50	0.63	-0.25		39	0.15	0.26	0.31	-0.17
16	ABSn	28	0.55	0.50	0.32	0.37		28	0.02	0.04	0.04	0.00
17	BSS	30	0.62	0.48	0.50	-0.04		30	0.03	0.07	0.07	-0.02
18	MSSo	27	0.61	0.48	0.48	0.01		23	0.02	0.04	0.04	0.00
19	MSSn	39	0.56	0.50	0.51	-0.03		38	0.01	0.03	0.03	0.00
20	USS	30	0.57	0.50	0.33	0.34		30	0.02	0.03	0.03	0.00
21	CSL	29	0.02	0.03	0.03	0.00		32	0.00	0.00	0.00	–
22	BWM	28	0.55	0.50	0.46	0.08		40	0.03	0.05	0.05	-0.01
23	HW	29	0.67	0.45	0.52	-0.16		29	0.05	0.10	0.10	-0.04
24	HC	26	0.13	0.24	0.27	-0.14		23	0.00	0.00	0.00	–
25	CL2	47	0.07	0.14	0.15	-0.07		47	0.01	0.02	0.02	0.00
26	CL4	50	0.11	0.20	0.22	-0.11		50	0.08	0.15	0.16	-0.08
27	LCC1	19	0.47	0.51	0.53	-0.03		19	0.00	0.00	0.00	–
28	LCC2	25	0.52	0.51	0.64	-0.26		25	0.00	0.00	0.00	–
29	USFK	38	0.30	0.43	0.45	-0.05		42	0.18	0.30	0.26	0.12
30	ARB	29	0.40	0.49	0.59	-0.21		30	0.28	0.41	0.23	0.44
31	BRB	29	0.53	0.51	0.45	0.12		28	0.29	0.42	0.36	0.14
32	KPLS	30	0.33	0.45	0.40	0.12		30	0.17	0.28	0.27	0.06
33	BMS	30	0.50	0.51	0.67	-0.32		30	0.22	0.35	0.30	0.13
34	ATB	30	0.50	0.51	0.53	-0.05		30	0.33	0.45	0.53	-0.18
35	UMC	30	0.13	0.24	0.27	-0.14		30	0.00	0.00	0.00	–
36	FCC	30	0.30	0.43	0.33	0.22		30	0.15	0.26	0.23	0.10
37	BTB	30	0.45	0.50	0.50	0.01		30	0.45	0.50	0.50	0.01
38	SCn	40	0.50	0.51	0.35	0.31		40	0.38	0.47	0.65	-0.38
39	ASB	30	0.45	0.50	0.37	0.28		30	0.37	0.47	0.40	0.16
40	ASBn	40	0.36	0.47	0.33	0.31		38	0.39	0.48	0.47	0.02
41	BSBn	39	0.37	0.47	0.44	0.08		38	0.29	0.42	0.42	-0.01
42	MM	30	0.32	0.44	0.37	0.17		29	0.29	0.42	0.52	-0.23
43	MFC	40	0.05	0.10	0.10	-0.04		40	0.40	0.49	0.45	0.08
44	UTC	27	0.13	0.23	0.04	0.84		22	0.00	0.00	0.00	–
45	AKM	8	0.94	0.13	0.13	0.00		8	0.94	0.13	0.13	0.00
46	RHB	10	0.35	0.48	0.30	0.39		10	0.60	0.51	0.80	-0.64
47	GP	11	0.00	0.00	0.00	–		11	0.00	0.00	0.00	–
48	WR	28	0.00	0.00	0.00	–		29	0.00	0.00	0.00	–
49	NFAR	20	1.00	0.00	0.00	–		20	0.98	0.05	0.05	0.00
50	NFNR	31	0.98	0.03	0.03	0.00		29	0.81	0.31	0.31	0.01
51	HCS	30	1.00	0.00	0.00	–		30	1.00	0.00	0.00	–
52	MSS	30	1.00	0.00	0.00	–		30	1.00	0.00	0.00	–
53	MWS	30	1.00	0.00	0.00	–		30	0.98	0.03	0.03	0.00

Table 2.3, continued

Code	Pop	Loci										
		RAG0917 230						R1175 137				
		N	G(T)	H_E	H_O	F_{IS}		N	A(G)	H_E	H_O	F_{IS}
1	VC	39	0.00	0.00	0.00	–		39	0.00	0.00	0.00	–
2	VCLSn	41	0.00	0.00	0.00	–		41	0.00	0.00	0.00	–
3	LWC	34	0.00	0.00	0.00	–		37	0.00	0.00	0.00	–
4	LWMn	40	0.00	0.00	0.00	–		40	0.00	0.00	0.00	–
5	SLCo	40	0.00	0.00	0.00	–		40	0.00	0.00	0.00	–
6	SLCn	33	0.00	0.00	0.00	–		33	0.00	0.00	0.00	–
7	LJC	32	0.00	0.00	0.00	–		32	0.00	0.00	0.00	–
8	MJC	30	0.00	0.00	0.00	–		30	0.00	0.00	0.00	–
9	JC	23	0.00	0.00	0.00	–		24	0.00	0.00	0.00	–
10	JL	34	0.13	0.23	0.26	-0.14		36	0.14	0.24	0.28	-0.15
11	GC	36	0.03	0.05	0.06	-0.01		37	0.03	0.05	0.05	-0.01
12	GCn	38	0.03	0.05	0.05	-0.01		38	0.03	0.05	0.05	-0.01
13	BBS	30	0.07	0.13	0.13	-0.06		30	0.07	0.13	0.13	-0.06
14	MBS	30	0.05	0.10	0.10	-0.04		30	0.05	0.10	0.10	-0.04
15	MBSn	40	0.06	0.12	0.13	-0.05		40	0.06	0.12	0.13	-0.05
16	ABSn	28	0.00	0.00	0.00	–		28	0.00	0.00	0.00	–
17	BSS	31	0.02	0.03	0.03	0.00		30	0.02	0.03	0.03	0.00
18	MSSo	29	0.00	0.00	0.00	–		28	0.00	0.00	0.00	–
19	MSSn	39	0.01	0.03	0.03	0.00		39	0.01	0.03	0.03	0.00
20	USS	30	0.00	0.00	0.00	–		30	0.00	0.00	0.00	–
21	CSL	32	0.00	0.00	0.00	–		32	0.00	0.00	0.00	–
22	BWM	37	0.00	0.00	0.00	–		36	0.00	0.00	0.00	–
23	HW	29	0.02	0.03	0.03	0.00		29	0.02	0.03	0.03	0.00
24	HC	27	0.00	0.00	0.00	–		28	0.00	0.00	0.00	–
25	CL2	47	0.12	0.21	0.19	0.08		48	0.11	0.21	0.19	0.09
26	CL4	50	0.07	0.13	0.14	-0.07		50	0.07	0.13	0.14	-0.07
27	LCC1	18	0.00	0.00	0.00	–		19	0.00	0.00	0.00	–
28	LCC2	25	0.00	0.00	0.00	–		25	0.00	0.00	0.00	–
29	USFK	42	0.04	0.07	0.07	-0.03		41	0.04	0.07	0.07	-0.03
30	ARB	30	0.05	0.10	0.10	-0.04		30	0.05	0.10	0.10	-0.04
31	BRB	29	0.17	0.29	0.21	0.29		30	0.17	0.28	0.20	0.30
32	KPLS	30	0.10	0.18	0.13	0.28		30	0.10	0.18	0.13	0.28
33	BMS	30	0.13	0.24	0.27	-0.14		30	0.15	0.26	0.23	0.10
34	ATB	30	0.13	0.24	0.27	-0.14		30	0.13	0.24	0.27	-0.14
35	UMC	30	0.00	0.00	0.00	–		30	0.00	0.00	0.00	–
36	FCC	30	0.12	0.21	0.17	0.21		30	0.12	0.21	0.17	0.21
37	BTB	30	0.10	0.18	0.20	-0.09		30	0.10	0.18	0.20	-0.09
38	SCn	40	0.36	0.47	0.53	-0.12		40	0.36	0.47	0.53	-0.12
39	ASB	30	0.47	0.51	0.40	0.21		30	0.45	0.50	0.43	0.14
40	ASBn	40	0.41	0.49	0.43	0.14		37	0.45	0.50	0.46	0.08
41	BSBn	40	0.33	0.44	0.55	-0.24		40	0.33	0.44	0.55	-0.24
42	MM	30	0.33	0.45	0.40	0.12		30	0.37	0.47	0.47	0.01
43	MFC	40	0.76	0.37	0.38	-0.02		40	0.76	0.37	0.38	-0.02
44	UTC	28	0.96	0.07	0.07	-0.02		28	0.96	0.07	0.07	-0.02
45	AKM	8	0.94	0.13	0.13	0.00		8	0.94	0.13	0.13	0.00
46	RHB	10	0.70	0.44	0.60	-0.39		10	0.70	0.44	0.60	-0.39
47	GP	11	0.00	0.00	0.00	–		6	0.00	0.00	0.00	–
48	WR	28	0.00	0.00	0.00	–		16	0.00	0.00	0.00	–
49	NFAR	20	0.98	0.05	0.05	0.00		20	0.98	0.05	0.05	0.00
50	NFNR	31	1.00	0.00	0.00	–		31	1.00	0.00	0.00	–
51	HCS	30	0.82	0.30	0.30	0.02		30	0.82	0.30	0.30	0.02
52	MSS	31	0.95	0.09	0.10	-0.03		31	0.95	0.09	0.10	-0.03
53	MWS	30	0.98	0.03	0.03	0.00		30	0.98	0.03	0.03	0.00

Table 2.3, continued

Code	Pop	Loci									
		R1564 272						RTDL 316		RTDL 695	
		N	T(:)	H_E	H_O	F_{IS}		N	C(T)	N	T(C)
1	VC	39	1.00	0.00	0.00	–		39	0.00	39	0.13
2	VCLSn	40	1.00	0.00	0.00	–		41	0.00	41	0.10
3	LWC	29	1.00	0.00	0.00	–		37	0.00	36	0.06
4	LWMn	40	1.00	0.00	0.00	–		40	0.00	40	0.05
5	SLCo	40	1.00	0.00	0.00	–		40	0.00	40	0.00
6	SLCn	33	1.00	0.00	0.00	–		33	0.00	33	0.00
7	LJC	32	1.00	0.00	0.00	–		32	0.00	32	0.09
8	MJC	30	1.00	0.00	0.00	–		30	0.00	28	0.00
9	JC	22	1.00	0.00	0.00	–		24	0.00	23	0.00
10	JL	37	0.86	0.24	0.27	-0.14		38	0.08	39	1.00
11	GC	35	0.97	0.06	0.06	-0.02		36	0.00	37	0.05
12	GCn	39	0.97	0.05	0.05	-0.01		39	0.00	39	0.03
13	BBS	30	0.93	0.13	0.13	-0.06		30	0.00	30	0.07
14	MBS	30	0.95	0.10	0.10	-0.04		30	0.00	30	0.30
15	MBSn	40	0.95	0.10	0.10	-0.04		40	0.00	40	0.20
16	ABSn	28	1.00	0.00	0.00	–		28	0.00	28	0.07
17	BSS	31	0.98	0.03	0.03	0.00		30	0.00	30	0.00
18	MSSo	28	1.00	0.00	0.00	–		29	0.00	29	0.00
19	MSSn	38	0.97	0.05	0.05	-0.01		40	0.00	39	0.00
20	USS	30	1.00	0.00	0.00	–		30	0.00	30	0.03
21	CSL	32	1.00	0.00	0.00	–		33	0.00	33	1.00
22	BWM	40	0.98	0.05	0.05	-0.01		40	0.00	38	0.05
23	HW	29	0.98	0.03	0.03	0.00		29	0.00	29	0.03
24	HC	28	1.00	0.00	0.00	–		30	0.00	30	1.00
25	CL2	47	0.88	0.21	0.19	0.08		47	0.13	48	0.94
26	CL4	50	0.92	0.15	0.16	-0.08		50	0.00	50	0.92
27	LCC1	19	1.00	0.00	0.00	–		19	0.00	19	0.00
28	LCC2	25	1.00	0.00	0.00	–		25	0.00	25	0.00
29	USFK	40	0.96	0.07	0.08	-0.03		42	0.00	42	0.45
30	ARB	28	0.93	0.14	0.14	-0.06		30	0.00	30	0.27
31	BRB	28	0.88	0.22	0.11	0.52		29	0.00	30	0.30
32	KPLS	30	0.90	0.18	0.13	0.28		30	0.00	30	0.27
33	BMS	30	0.85	0.26	0.23	0.10		30	0.00	30	0.27
34	ATB	30	0.87	0.24	0.27	-0.14		30	0.00	30	0.27
35	UMC	30	1.00	0.00	0.00	–		30	0.00	30	1.00
36	FCC	30	0.88	0.21	0.17	0.21		30	0.00	30	0.60
37	BTB	30	0.90	0.18	0.20	-0.09		30	0.00	30	0.43
38	SCn	40	0.64	0.47	0.53	-0.12		40	0.15	40	0.48
39	ASB	30	0.50	0.51	0.47	0.08		30	0.20	30	0.70
40	ASBn	38	0.50	0.51	0.42	0.17		40	0.18	39	0.67
41	BSBn	40	0.65	0.46	0.55	-0.20		40	0.15	40	0.85
42	MM	30	0.62	0.48	0.43	0.10		29	0.17	30	0.77
43	MFC	40	0.24	0.37	0.38	-0.02		40	0.00	39	0.00
44	UTC	23	0.04	0.09	0.09	-0.02		28	0.00	30	0.00
45	AKM	8	0.06	0.13	0.13	0.00		8	0.88	8	0.88
46	RHB	10	0.30	0.44	0.60	-0.39		10	0.50	10	0.70
47	GP	7	1.00	0.00	0.00	–		11	0.00	10	1.00
48	WR	22	1.00	0.00	0.00	–		29	0.00	28	1.00
49	NFAR	20	0.03	0.05	0.05	0.00		20	1.00	18	1.00
50	NFNR	31	0.00	0.00	0.00	–		30	1.00	30	1.00
51	HCS	30	0.00	0.00	0.00	–		30	1.00	30	1.00
52	MSS	30	0.03	0.07	0.07	-0.02		30	1.00	30	1.00
53	MWS	30	0.02	0.03	0.03	0.00		31	1.00	31	1.00

Table 2.4. Comparison of SNP and microsatellite (μsat) population estimates of rainbow trout introgression, as inferred by the program STRUCTURE and in comparison to previous ADMIX microsatellite and minisatellite estimates.

	Population	STRUCTURE		LEADMIX	
		SNP	μsat	minisatellite	μsat
Golden Trout Creek					
1	Volcano Creek	0.01	0.02	reference	reference
2	Volcano Creek Left Stringer	0.01			
3	Golden Trout Creek Below Little Whitney (2001)	0.02	0.05	reference	reference
3a	Golden Trout Creek Below Little Whitney (2005)	0.02			
4	Salt Lick Creek (2000)	0.01			
4a	Salt Lick Creek (2005)	0.01			
5	Lower Johnson Creek	0.02			
6	Middle Johnson Creek	0.02	0.02	0	0.08
7	Johnson Creek	0.02			
8	Johnson Lake	0.06	0.01	0	0.25
9	Groundhog Creek (2000)	0.03	0.01	reference	reference
9a	Groundhog Creek (2005)	0.03			
10	Golden Trout Creek, Below Barigan Stringer	0.04			
11	Mouth Barigan Stringer (1999)	0.04	0.02	0	0.06
11a	Mouth Barigan Stringer (2005)	0.04			
12	Golden Trout Creek, Above Barigan Stringer	0.03			
13	Golden Trout Creek, Below Stokes Stringer	0.03			
14	Middle Stokes Stringer (1999)	0.03			
14a	Middle Stokes Stringer (2005)	0.02			
15	Upper Stokes Stringer	0.02	0.01	0	0.05
16	Chicken Springs Lake	0.04	0.01	0	0.30
17	Big Whitney Meadow	0.02			
18	Headwaters Golden Trout Creek	0.03			
19	Horseshoe Creek	0.02			
20	Cottonwood Lakes 2	0.05	0.02	0.11	0.19
21	Cottonwood Lakes 4	0.05	0.01	0.11	0.25
22	Little Cottonwood Creek 1	0.01			
23	Little Cottonwood Creek 2	0.01			
South Fork Kern River					
24	Upper South Fork Kern	0.08	0.04	0.07	0.12
25	South Fork Kern River, above Ramshaw Barrier	0.12	0.07	0.04	0.13
26	South Fork Kern River, below Ramshaw Barrier	0.13	0.15	0.09	0.08
27	Kern Peak Left Stringer	0.08	0.13	0.08	0.12
28	Below Movie Stringer	0.13	0.05	0.09	0.08
29	South Fork Kern River, above Templeton Barrier	0.13	0.07	0.2	0.08
30	Upper Mulkey Creek	0.02	0.01	0	0.13
31	Four Canyons Creek	0.09	0.07	reference	reference
32	South Fork Kern River, below Templeton Barrier	0.17	0.07	0.24	0.08
33	Strawberry Creek	0.29	–	–	–
34	South Fork Kern River above Schaeffer Barrier (2002)	0.33	0.34	0.33	0.25
34a	South Fork Kern River above Schaeffer Barrier (2004)	0.35	–	–	–
35	South Fork Kern River below Schaeffer Barrier (2004)	0.29	–	–	–
36	Monache Meadows	0.32	0.22	0.26	0.15
37	Middle Fish Creek	0.22	0.08	0.48	0.25
38	Upper Trout Creek	0.29	0.99	0.83	0.37
39	Kennedy Meadows	0.94	0.95	0.88	0.75
40	Rockhouse Basin	0.61	–	–	–

Table 2.4, continued

Population		STRUCTURE		LEADMIX	
		SNP	μsat	minisatellite	μsat
Wyoming					
41	"Golden Pond," Wyoming	0.01	–	–	–
42	Wind River, Wyoming	0.01	–	–	–
wild rainbow					
43	North Fork American River	0.99	0.96	reference	reference
44	North Fork Navarro River	0.97	0.96	reference	reference
hatchery rainbow					
45	Hot Creek Strain	0.97	0.99	reference	reference
46	Mount Shasta Strain	0.98	0.99	reference	reference
47	Mt. Whitney Strain	0.99	0.98	reference	reference

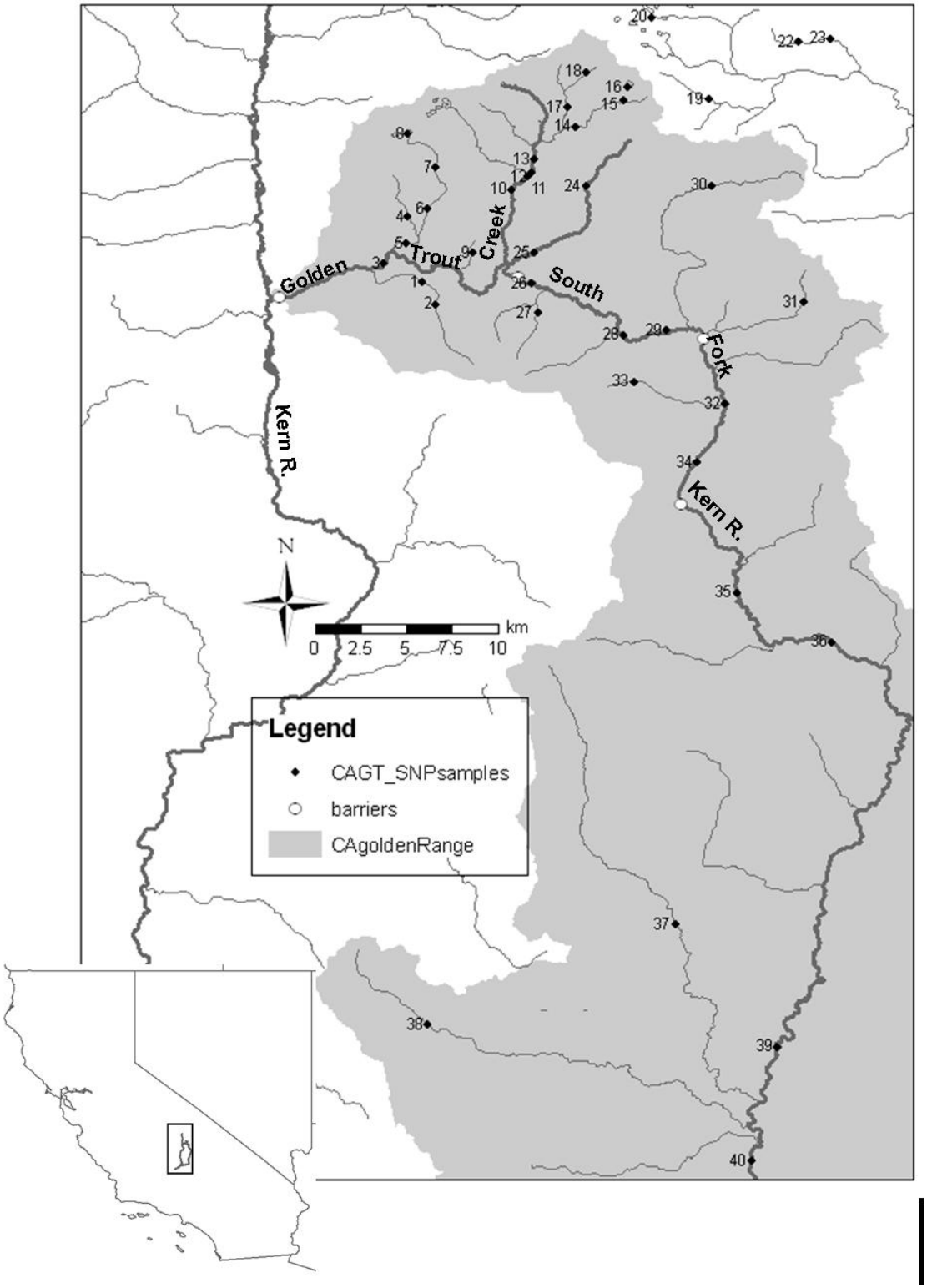


Figure 2.1. Map of sampling localities used in the study. Sampling locality numbers as given in Table 2.1.

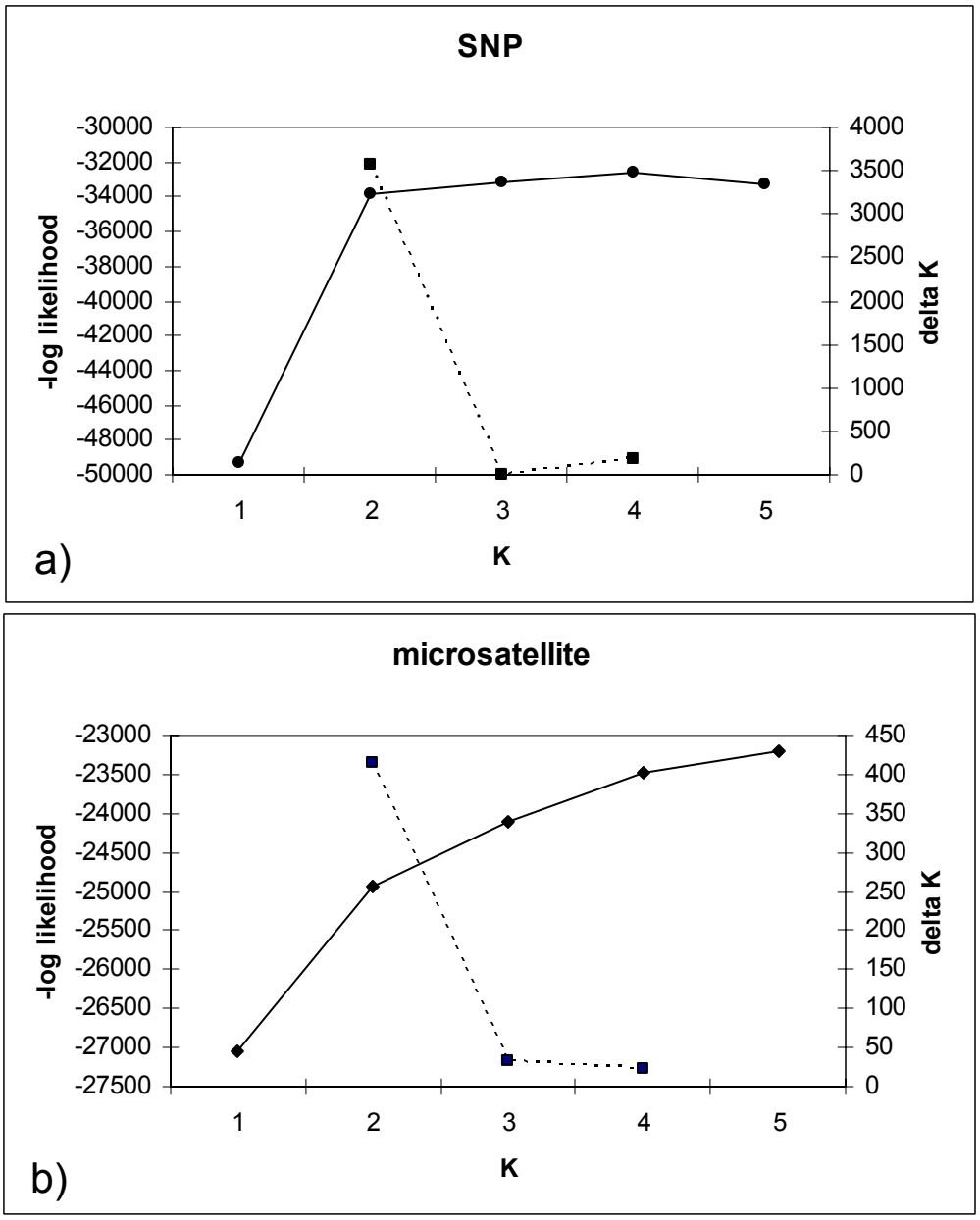


Figure 2.2. Plots of negative log-likelihood (primary x-axis) and delta K values (secondary x-axis) versus K for SNP (figure 2.2a) and microsatellite (Figure 2.2b) STRUCTURE data sets.

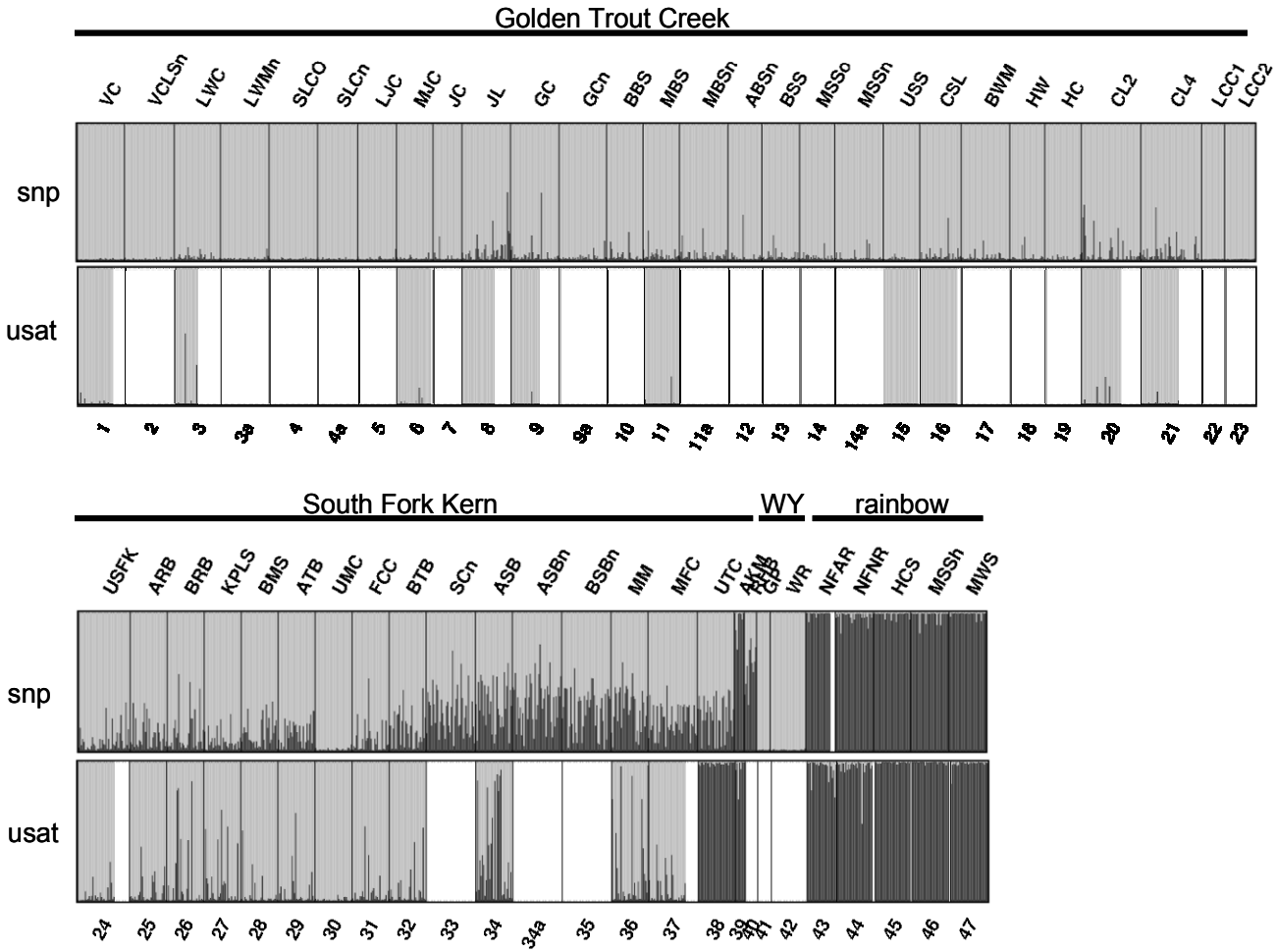


Figure 2.3. STRUCTURE representation of SNP (upper clusters) and microsatellite ("usat," lower clusters) data analyses. Each vertical bar represents an individual fish, with populations separated by black lines and grouped by population number (as given in Table 2.1) on the x-axis. Dark-gray bars indicate "rainbow trout" group membership, while light-gray denotes "golden" group membership. Open (white) bars indicate no data available for an individual or population. Populations are grouped by black bars overhead, indicating Golden Trout Creek, South Fork Kern River, Wyoming (WY), and rainbow trout populations

Appendix 2.1. Descriptive statistics for loci not selected for data analysis based on frequency differentials. Abbreviations as given in Table 2.1.

	Pop	Loci										
		B9 164						LDH156				
		N	A(:)	H_E	H_O	F_{IS}		N	T(C)	H_E	H_O	F_{IS}
1	VC	38	0.95	0.10	0.11	-0.04		36	0.00	0.00	0.00	–
2	VCLSn	0	–	–	0.00	–		0	–	–	0.00	–
3	LWC	0	–	–	0.00	–		0	–	–	0.00	–
4	LWMn	0	–	–	0.00	–		0	–	–	0.00	–
5	SLCo	0	–	–	0.00	–		0	–	–	0.00	–
6	SLCn	0	–	–	0.00	–		0	–	–	0.00	–
7	LJC	0	–	–	0.00	–		0	–	–	0.00	–
8	MJC	0	–	–	0.00	–		0	–	–	0.00	–
9	JC	0	–	–	0.00	–		0	–	–	0.00	–
10	JL	0	–	–	0.00	–		0	–	–	0.00	–
11	GC	0	–	–	0.00	–		0	–	–	0.00	–
12	GCn	0	–	–	0.00	–		0	–	–	0.00	–
13	BBS	0	–	–	0.00	–		0	–	–	0.00	–
14	MBS	0	–	–	0.00	–		0	–	–	0.00	–
15	MBSn	0	–	–	0.00	–		0	–	–	0.00	–
16	ABSn	0	–	–	0.00	–		0	–	–	0.00	–
17	BSS	0	–	–	0.00	–		0	–	–	0.00	–
18	MSSo	0	–	–	0.00	–		0	–	–	0.00	–
19	MSSn	0	–	–	0.00	–		0	–	–	0.00	–
20	USS	0	–	–	0.00	–		0	–	–	0.00	–
21	CSL	0	–	–	0.00	–		0	–	–	0.00	–
22	BWM	0	–	–	0.00	–		0	–	–	0.00	–
23	HW	0	–	–	0.00	–		0	–	–	0.00	–
24	HC	0	–	–	0.00	–		0	–	–	0.00	–
25	CL2	0	–	–	0.00	–		0	–	–	0.00	–
26	CL4	0	–	–	0.00	–		0	–	–	0.00	–
27	LCC1	0	–	–	0.00	–		0	–	–	0.00	–
28	LCC2	0	–	–	0.00	–		0	–	–	0.00	–
29	USFK	41	0.93	0.14	0.15	-0.07		42	0.00	0.00	0.00	–
30	ARB	30	0.85	0.26	0.23	0.10		29	0.00	0.00	0.00	–
31	BRB	29	0.97	0.07	0.07	-0.02		29	0.00	0.00	0.00	–
32	KPLS	30	0.93	0.13	0.13	-0.06		30	0.00	0.00	0.00	–
33	BMS	30	0.83	0.28	0.27	0.06		30	0.02	0.03	0.03	0.00
34	ATB	30	0.95	0.10	0.10	-0.04		30	0.00	0.00	0.00	–
35	UMC	30	1.00	0.00	0.00	–		30	0.00	0.00	0.00	–
36	FCC	30	0.85	0.26	0.30	-0.16		30	0.00	0.00	0.00	–
37	BTB	30	0.92	0.16	0.10	0.36		30	0.02	0.03	0.03	0.00
38	SCn	0	–	–	0.00	–		0	–	–	0.00	–
39	ASB	30	0.90	0.18	0.20	-0.09		29	0.07	0.13	0.14	-0.06
40	ASBn	0	–	–	0.00	–		0	–	–	0.00	–
41	BSBn	0	–	–	0.00	–		0	–	–	0.00	–
42	MM	30	0.92	0.16	0.10	0.36		29	0.07	0.13	0.14	-0.06
43	MFC	40	0.99	0.03	0.03	0.00		40	0.00	0.00	0.00	–
44	UTC	22	1.00	0.00	0.00	–		21	0.00	0.00	0.00	–
45	AKM	8	0.81	0.33	0.13	0.63		8	0.25	0.40	0.25	0.39
46	RHB	10	1.00	0.00	0.00	–		10	0.25	0.39	0.30	0.25
47	GP	0	–	–	0.00	–		0	–	–	0.00	–
48	WR	0	–	–	0.00	–		0	–	–	0.00	–
49	NFAR	20	1.00	0.00	0.00	–		18	0.14	0.25	0.28	-0.13
50	NFNR	31	0.92	0.15	0.16	-0.07		30	0.33	0.45	0.40	0.12
51	HCS	30	1.00	0.00	0.00	–		30	0.97	0.07	0.00	1.00
52	MSS	30	1.00	0.00	0.00	–		30	0.88	0.21	0.23	-0.12
53	MWS	30	1.00	0.00	0.00	–		30	0.48	0.51	0.50	0.02

Appendix 2.1, continued

	Pop	Loci									
		RAPD132					B1266				
		N	A(T)	H_E	H_O	F_{IS}	N	G(T)	F_{IS}	H_E	H_O
1	VC	39	0.00	0.00	0.00	–	39	0.31	-0.07	0.43	0.46
2	VCLSn	0	–	–	0.00	–	0	–	–	–	0.00
3	LWC	0	–	–	0.00	–	0	–	–	–	0.00
4	LWMn	0	–	–	0.00	–	0	–	–	–	0.00
5	SLCo	0	–	–	0.00	–	0	–	–	–	0.00
6	SLCn	0	–	–	0.00	–	0	–	–	–	0.00
7	LJC	0	–	–	0.00	–	0	–	–	–	0.00
8	MJC	0	–	–	0.00	–	0	–	–	–	0.00
9	JC	0	–	–	0.00	–	0	–	–	–	0.00
10	JL	0	–	–	0.00	–	0	–	–	–	0.00
11	GC	0	–	–	0.00	–	0	–	–	–	0.00
12	GCn	0	–	–	0.00	–	0	–	–	–	0.00
13	BBS	0	–	–	0.00	–	0	–	–	–	0.00
14	MBS	0	–	–	0.00	–	0	–	–	–	0.00
15	MBSn	0	–	–	0.00	–	0	–	–	–	0.00
16	ABSn	0	–	–	0.00	–	0	–	–	–	0.00
17	BSS	0	–	–	0.00	–	0	–	–	–	0.00
18	MSSo	0	–	–	0.00	–	0	–	–	–	0.00
19	MSSn	0	–	–	0.00	–	0	–	–	–	0.00
20	USS	0	–	–	0.00	–	0	–	–	–	0.00
21	CSL	0	–	–	0.00	–	0	–	–	–	0.00
22	BWM	0	–	–	0.00	–	0	–	–	–	0.00
23	HW	0	–	–	0.00	–	0	–	–	–	0.00
24	HC	0	–	–	0.00	–	0	–	–	–	0.00
25	CL2	0	–	–	0.00	–	0	–	–	–	0.00
26	CL4	0	–	–	0.00	–	0	–	–	–	0.00
27	LCC1	0	–	–	0.00	–	0	–	–	–	0.00
28	LCC2	0	–	–	0.00	–	0	–	–	–	0.00
29	USFK	42	0.02	0.05	0.05	-0.01	42	0.18	-0.21	0.30	0.36
30	ARB	30	0.00	0.00	0.00	–	30	0.30	-0.25	0.43	0.53
31	BRB	28	0.04	0.07	0.07	-0.02	30	0.27	-0.01	0.40	0.40
32	KPLS	30	0.02	0.03	0.03	0.00	30	0.23	-0.29	0.36	0.47
33	BMS	30	0.00	0.00	0.00	–	30	0.28	-0.22	0.41	0.50
34	ATB	30	0.00	0.00	0.00	–	30	0.15	-0.16	0.26	0.30
35	UMC	30	0.00	0.00	0.00	–	30	0.00	–	0.00	0.00
36	FCC	30	0.10	0.18	0.20	-0.09	30	0.18	-0.21	0.30	0.37
37	BTB	30	0.03	0.07	0.07	-0.02	30	0.27	-0.01	0.40	0.40
38	SCn	0	–	–	0.00	–	0	–	–	–	0.00
39	ASB	30	0.08	0.16	0.17	-0.07	30	0.43	-0.07	0.50	0.53
40	ASBn	0	–	–	0.00	–	0	–	–	–	0.00
41	BSBn	0	–	–	0.00	–	0	–	–	–	0.00
42	MM	30	0.05	0.10	0.10	-0.04	30	0.25	0.22	0.38	0.30
43	MFC	40	0.01	0.03	0.03	0.00	40	0.04	-0.03	0.07	0.08
44	UTC	28	0.00	0.00	0.00	–	28	0.27	0.74	0.40	0.11
45	AKM	8	0.13	0.23	0.25	-0.08	8	0.75	0.39	0.40	0.25
46	RHB	10	0.10	0.19	0.20	-0.06	10	0.25	0.76	0.39	0.10
47	GP	10	0.00	0.00	0.00	–	0	–	–	–	0.00
48	WR	28	0.00	0.00	0.00	–	0	–	–	–	0.00
49	NFAR	20	0.05	0.10	0.00	1.00	20	0.83	0.16	0.30	0.25
50	NFNR	31	0.00	0.00	0.00	–	31	0.74	0.17	0.39	0.32
51	HCS	30	0.95	0.10	0.10	-0.04	30	1.00	–	0.00	0.00
52	MSS	30	0.92	0.16	0.10	0.36	30	1.00	–	0.00	0.00
53	MWS	30	0.08	0.16	0.17	-0.07	30	0.85	-0.16	0.26	0.30

Appendix 2.1, continued

	Pop	Loci										
		RAPD167						URO 373				
		N	T(G)	H_E	H_O	F_{IS}		N	C(A)	H_E	H_O	F_{IS}
1	VC	39	0.00	0.00	0.00	–		39	0.55	0.50	0.59	-0.18
2	VCLSn	0	–	–	0.00	–		0	–	–	0.00	–
3	LWC	0	–	–	0.00	–		0	–	–	0.00	–
4	LWMn	0	–	–	0.00	–		0	–	–	0.00	–
5	SLCo	0	–	–	0.00	–		0	–	–	0.00	–
6	SLCn	0	–	–	0.00	–		0	–	–	0.00	–
7	LJC	0	–	–	0.00	–		0	–	–	0.00	–
8	MJC	0	–	–	0.00	–		0	–	–	0.00	–
9	JC	0	–	–	0.00	–		0	–	–	0.00	–
10	JL	0	–	–	0.00	–		0	–	–	0.00	–
11	GC	0	–	–	0.00	–		0	–	–	0.00	–
12	GCn	0	–	–	0.00	–		0	–	–	0.00	–
13	BBS	0	–	–	0.00	–		0	–	–	0.00	–
14	MBS	0	–	–	0.00	–		0	–	–	0.00	–
15	MBSn	0	–	–	0.00	–		0	–	–	0.00	–
16	ABSn	0	–	–	0.00	–		0	–	–	0.00	–
17	BSS	0	–	–	0.00	–		0	–	–	0.00	–
18	MSSo	0	–	–	0.00	–		0	–	–	0.00	–
19	MSSn	0	–	–	0.00	–		0	–	–	0.00	–
20	USS	0	–	–	0.00	–		0	–	–	0.00	–
21	CSL	0	–	–	0.00	–		0	–	–	0.00	–
22	BWM	0	–	–	0.00	–		0	–	–	0.00	–
23	HW	0	–	–	0.00	–		0	–	–	0.00	–
24	HC	0	–	–	0.00	–		0	–	–	0.00	–
25	CL2	0	–	–	0.00	–		0	–	–	0.00	–
26	CL4	0	–	–	0.00	–		0	–	–	0.00	–
27	LCC1	0	–	–	0.00	–		0	–	–	0.00	–
28	LCC2	0	–	–	0.00	–		0	–	–	0.00	–
29	USFK	41	0.04	0.07	0.02	0.66		42	0.35	0.46	0.60	-0.31
30	ARB	30	0.05	0.10	0.10	-0.04		30	0.27	0.40	0.47	-0.18
31	BRB	29	0.03	0.07	0.07	-0.02		28	0.39	0.49	0.71	-0.48
32	KPLS	30	0.13	0.24	0.13	0.44		30	0.40	0.49	0.53	-0.09
33	BMS	30	0.03	0.07	0.07	-0.02		30	0.48	0.51	0.50	0.02
34	ATB	30	0.05	0.10	0.10	-0.04		30	0.33	0.45	0.53	-0.18
35	UMC	30	0.00	0.00	0.00	–		30	0.03	0.07	0.07	-0.02
36	FCC	30	0.00	0.00	0.00	–		30	0.25	0.38	0.30	0.22
37	BTB	30	0.07	0.13	0.13	-0.06		30	0.43	0.50	0.60	-0.21
38	SCn	0	–	–	0.00	–		0	–	–	0.00	–
39	ASB	30	0.13	0.24	0.20	0.15		30	0.33	0.45	0.60	-0.34
40	ASBn	0	–	–	0.00	–		0	–	–	0.00	–
41	BSBn	0	–	–	0.00	–		0	–	–	0.00	–
42	MM	30	0.13	0.24	0.13	0.44		30	0.42	0.49	0.57	-0.15
43	MFC	40	0.00	0.00	0.00	–		40	0.45	0.50	0.50	0.00
44	UTC	30	0.00	0.00	0.00	–		30	0.63	0.47	0.33	0.30
45	AKM	8	0.75	0.40	0.50	-0.27		8	0.94	0.13	0.13	0.00
46	RHB	10	0.10	0.19	0.20	-0.06		10	0.75	0.39	0.30	0.25
47	GP	0	–	–	0.00	–		0	–	–	0.00	–
48	WR	0	–	–	0.00	–		0	–	–	0.00	–
49	NFAR	20	0.55	0.51	0.30	0.42		20	1.00	0.00	0.00	–
50	NFNR	31	0.31	0.43	0.48	-0.12		31	1.00	0.00	0.00	–
51	HCS	30	0.05	0.10	0.10	-0.04		30	1.00	0.00	0.00	–
52	MSS	30	0.00	0.00	0.00	–		30	1.00	0.00	0.00	–
53	MWS	30	0.87	0.24	0.20	0.15		30	1.00	0.00	0.00	–

Chapter 3: Little Kern golden trout – comparative analysis of SNP and microsatellite estimates of rainbow trout introgression

Abstract

Hybridization following the introduction of non-natives threatens the genetic integrity and persistence of many native taxa. The introduction of hatchery rainbow trout (*Oncorhynchus mykiss spp.)* into waters of the Little Kern Basin during the early 1900s resulted in widespread hybridization of native Little Kern golden trout (LKGT, *O. m. whitei*). Major restoration efforts, guided by allozyme genetic data, attempted to restore LKGT to its native range. We used 15 microsatellite loci to investigate population structure, genetic diversity and rainbow trout introgression in native LKGT. Furthermore, we validated the use of species-informative Single Nucleotide Polymorphism (SNP) markers in assessing rainbow trout introgression. Significant genetic structure exists throughout the native range of LKGT, with four major groupings found in both Bayesian and phylogenetic analyses that correspond to the known reintroduction history within this basin. However, evidence for strong differentiation among groups was accompanied by relatively low levels of heterozygosity for most and high levels of inbreeding for several Little Kern golden trout populations. Bayesian analyses of microsatellite and SNP data produced similar estimates for both the pattern and degree of rainbow trout introgression, giving confidence in the employment of SNP markers for assessing introgression in future genetic monitoring of these populations. The presence of several populations with high estimates of rainbow trout influence give

reason for concern regarding the genetic integrity of selected LKGT populations within the native basin and their potential influence on adjacent non-hybridized populations.

Introduction

Hybridization following the introduction of non-natives threatens the genetic integrity and persistence of many native taxa. The threat of extinction by hybridization (reviewed in Chapter II and Rhymer and Simberloff 1996) is particularly devastating for freshwater endemics in the western United States (Utter 2000), where widely introduced non-natives have yielded homogenizing consequences, particularly for closely related native taxa (Perry et al. 2002; Rahel 2000). The management and restoration of threatened and endangered native species impacted by hybridization requires effective eradication and restoration actions; however the removal of non-natives is complex, and hybrids may return or persist in portions of the native range despite eradication efforts (Cordes et al. 2004; Echelle and Echelle 1997; Shepard et al. 2005). We describe a case study in which the historical introduction of hatchery rainbow trout into the Little Kern River Basin nearly eradicated the endemic Little Kern golden trout (*Oncorhynchus mykiss whitei*). Restoration efforts guided by genetic information attempted to eliminate the threat posed by the non-native and introgressed forms. We therefore conducted a full genetic assessment of extant populations in order to evaluate the success of restoration efforts in eliminating non-native trout influence and retaining genetic diversity in the Little Kern Basin.

To determine whether restoration efforts eliminated rainbow trout introgression, we undertook a complete genetic assessment of Little Kern golden trout. We first used microsatellite DNA loci to detect and quantify rainbow trout hybridization in and to examine the geographic substructure, genetic diversity and demographic history of extant and restored Little Kern golden trout populations. Next, we used SNP markers to assess

hybridization and compared SNP and microsatellite estimates of rainbow trout introgression to validate the utility of a developed SNP marker panel. Genetic data from this updated assessment will provide critical information for the species' current and future federal ESA listing status and aid in conservation by guiding management decisions.

Genetic history of Little Kern golden trout

The Little Kern golden trout (hereafter, LKGT), is one of three forms of rainbow trout endemic to the Kern River Basin of the southern Sierra Nevada mountains of California and collectively known as the "golden trout." Both morphological and genetic characteristics differentiate LKGT and its sister taxon, the California golden trout (*O. m. aguabonita*) from one another and from other rainbow trout (Shreck and Behnke 1971, Gold and Gall 1975, Gold 1977, Bagley and Gall 1998). The taxonomic identity of the third form (Kern River rainbow trout , *O. m. gilberti*) remains somewhat elusive, though genetic analysis by Bagley and Gall (1998) showed significant nuclear and mitochondrial differences to differentiate all three golden trout subspecies. Overfishing during the late 19th and early 20th centuries greatly reduced the abundance of LKGT to the extent that stocking was deemed necessary to rescue the fishery. Subsequent introduction of hatchery rainbow trout (*O. m. spp.)* into waters of the Little Kern Basin during the 1930s and '40s (Dill 1941; Dill 1945; Dill 1950) and possibly earlier (cited in Christenson 1984; Ellis and Bryant 1920) resulted in hybridization between extant LKGT and the introduced rainbow trout. Habitat degradation and pollution from timber and mining extraction compounded the negative effects of rainbow trout stocking, leading to the eventual listing

of LKGT as threatened under the federal Endangered Species Act in 1978 and the designation of critical habitat in the Golden Trout Wilderness (Federal Register 1978).

Previous allozyme and meristic studies of population genetic structure, systematics, and hybridization status of Little Kern golden trout populations prior to chemical treatment found that only six non-hybridized populations remained in just ten out of 100 miles of stream in the native range of the subspecies (Gall 1976; Gall and May 1997). Deadman, Upper Soda Spring, Sheep, Willow, Wet Meadow, and Fish Creeks contained non-hybridized Little Kern golden trout, while downstream populations (below barriers) and other sampled localities throughout the basin showed evidence of rainbow trout hybridization. In addition, allozyme analysis of trout samples from Upper Coyote Creek and Little Crytes Lake, early out-of-basin transplanted populations originally established from Little Kern fish (Rifle Creek and possibly the Little Kern River) in the late in 1800's (Ellis and Bryant 1920), showed both samples to represent Little Kern golden trout (Gall 1973).

Intensive recovery efforts for LKGT between 1979 and 1995 involved the chemical removal of known and suspected hybridized populations. Populations were reestablished using the identified non-hybridized LKGT source stocks over the course of several years. Early allozyme studies were instrumental in guiding reintroduction of various LKGT into areas throughout the Little Kern and tributaries where non-native trout had been eliminated, focusing efforts on retaining genetic diversity and averting localized extinctions (Gall et al., unpublished reports). This genetic information was also used by the California Department of Fish and Game (CDFG) to select populations for LKGT broodstocks at the Kern River Planting Base hatchery facility. Wild-caught

LKGT were used to produce fingerlings, which were subsequently stocked into selected reclaimed waters. Some fish were transferred *in situ* from single sources, some populations were established from multiple sources, and some established from these hatchery-reared broodstocks. A schematic representation of stocking history in the basin is given in Figure 3.1, depicting the distribution of various LKGT sources into adjacent drainages within the Little Kern watershed (Christenson 1984; Gall and May 1997).

Allozyme studies of Little Kern populations following restoration efforts (Gall 1994, 1997, 1998, 1999) indicated that the majority of restored populations contained non-hybridized LKGT, but implicated some populations likely to be introgressed with rainbow trout including Middle Mountaineer, South Mountaineer, Little Kern River at Burnt Corral, Lower Clicks Creeks, and Maggie Lakes populations (summarized in Bagley et al. 1999). Evidence of a non-LKGT allele in the Lower Soda Spring Creek population (Bagley et al. 1999) and the discordant allele frequencies among different samples from Soda Spring creek was interpreted as heterogeneity rather than hybridization. The detection of rainbow trout alleles in the marked (hatchery) fish sampled in Upper Maggie Lake after chemical treatment supports the conclusion that the 1997 and possibly the 1996 Deadman Creek broodstocks used in restoring these lakes may have been genetically compromised. The persistence of introgression in other localities may likewise be attributable to unintentional introduction of hybridized stocks from the hatchery, incomplete chemical treatment or transfer of rainbow trout by non-CDFG personnel.

Methods

Sample Collection and DNA Extraction

CDFG personnel collected over 1,200 LKGT fin clip samples from locations throughout the Little Kern River watershed and from five LKGT broodstock samples used in reintroduction efforts (Table 3.1 , Figure 3.1). For comparison, samples were also collected from each of three strains of hatchery rainbow trout, two wild populations of rainbow trout in the North Fork American River, and steelhead trout from the North Fork Navarro River. All tissue was preserved in 95% ethanol, as dry fin clips or in DMSO storage buffer (20% DMSO, 0.25 M EDTA, NaCl to saturation, pH 7.8) and stored at room temperature. Whole genomic DNA was extracted from fin clips using the Promega Wizard Extraction Kit or QIAGEN DNeasy^{TM} Tissue Kit. Extracted DNA samples were stored at –20 °C.

Selection of loci and data collection

The high levels of variability inherent in microsatellite markers allow for increased sensitivity of introgression estimates and better characterization of genetic diversity and population structure in extant LKGT populations as compared to previous allozyme studies. Tetranucleotide microsatellite loci developed for other *Oncorhynchus* species (Palti et al. 2002; Rexroad III et al. 2002a; Rexroad III et al. 2002b; Spies et al. 2005; Williamson et al. 2002) were screened for variability in *O. m. whitei* and 19 loci were screened as genetic markers for this study (Table 3.2). PCR was performed in multiplexed reactions using a total volume of 10 µL and containing: 2 ng of template DNA, 2 mm MgCl2, 125 µm of each dNTP, 0.2 µm forward sequencing primer labeled with either VIC, 6-FAM or NED, 0.1 µm reverse primer, 0.01 µm forward primer and 2

U of Taq polymerase. The PCR thermal profile was as follows: 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 45min at 60 °C.

PCR products were electrophoresed and visualized on an MJ Research/Bio-Rad BaseStation automated genotyper using Genescan 400HD ROX-labeled size standard (Applied Biosystems, Inc.) in each lane to allow for accurate determination of fragment size. Cartographer software version 1.2.6 (MJ Research/Bio-Rad) was used to infer individual genotypes according to the fragment sizes of the PCR products relative to the internal size standard.

Ten TaqMan assays consisting of forward and reverse primers and VIC- and FAM-labeled allele-specific probes were developed for SNP and insertion-deletion (indel) loci using either Applied Biosystems, Inc. Assays by Design or PrimerSelect software for use in 5'-nuclease reaction (Holland et al. 1991, Table 3.3). Each probe bore a minor groove binder and nonfluorescent quencher on the 3' end. Assay reactions were optimized on the individuals used in SNP marker discovery (Sprowles et al. 2006), including individuals of known genotype based on sequencing data. Known homozygotes, heterozygotes, and "composite" heterozygotes (generated by combining DNA from known homozygotes in ratios of 3:1, 1:1, and 1:3) were included as positive controls on every plate of samples analyzed, along with one no-template negative control. Reactions were carried out in 96-well microplates at a 5 µl volume. The majority of assays utilized 2X TaqMan Universal Master Mix (Applied Biosystems), 540nM each primer, 120nM each probe, and 10-20ng template DNA. Promega reagents were used for locus LDH 156 (see Table 3.2) at the following concentrations: 20u/ml Taq Polymerase, 0.2mM each dNTP, 5mM MgCl, 50mM KCl, 10mM Tris-HCl, 0.1% Triton® X-100, and

concentrations of primers, probes, and template as given above. Reactions were performed using the Chromo4™ Real-Time PCR Detector (MJ Research/Bio-Rad Laboratories, Inc.) and the following general thermal cycling protocol: initial denaturation of 94 degrees for 5 minutes, followed by 40 cycles of 92 degrees for 15 sec and an annealing temperatures ranging from 55-63.5 degrees (see Table 3.2) for 1 minute. Any individuals that failed to amplify using these initial conditions were reamplified using 2ul of template in 10ul reactions with the same reagent concentrations given above. Genotypes were scored using MJ Opticon Monitor analysis software (version 3.1, Bio-Rad Laboratories, Inc.) to visualize plots of endpoint fluorescence, subtracting baseline fluorescence averaged over the 10-20 cycle range and identifying clusters of fluorescence corresponding with each probe. Genotyping results were confirmed for consistency with positive controls.

Microsatellite Data Analyses

Nei's (1987) unbiased gene diversity (expected heterozygosity), observed heterozygosity, and mean number of alleles per microsatellite locus were calculated using Microsatellite Toolkit (Park 2001). We calculated allelic richness, a measure of allelic variation that takes into account unequal sample sizes using rarefaction (Petit et al. 1998), and Weir & Cockerham's (1984) measure of F_{IS} using the program FSTAT (Goudet 1995). Tests of Hardy Weinberg equilibrium were performed for each locus in each population using Fisher's exact tests based on 10,000 permutations (Guo and Thompson 1992) as implemented in GDA (version 1.1, Lewis and Zaykin 2001) and over all loci in GENEPOP ver. 3.4 (Raymond and Rousset 1995). All locus-population combinations

were tested for linkage disequilibrium using exact tests in GDA. Significance of tests was corrected for multiple comparisons using Bonferroni correction (Rice 1989).

Population differentiation was assessed using pairwise comparisons of F_{ST} for individual populations, with significance was determined by 7,560 permutations in FSTAT and corrected alpha for multiple comparisons. Data were examined for evidence of genetic bottlenecks using the BOTTLENECK software (Cornuet and Luikart 1996) using the two-phase model (TPM) as recommended by the program authors and as appropriate for most microsatellite data sets (Di Rienzo et al. 1994). The TPM assumes that most mutations follow a stepwise mutation model (SMM, Ohta and Kimura 1973) but allows for a small portion of multistep changes. The proportion of alleles attributed to SMM under the TPM was 90%, with the default variance of 30 selected and 1000 simulations run. The Wilcoxon sign-rank test was used to determine whether a population exhibits a significant number of loci with gene diversity excess relative to the equilibrium gene diversity (H_{eq}, computed from the observed number of alleles), a signature of a recent genetic bottleneck. Estimations using the strict SMM and the Infinite Alleles Model (IAM, Kimura and Crow 1964) were also performed for comparison.

Both genetic trees (phenograms) and ordination of genetic data were used examine genetic relationships among samples. First, Cavalli-Sforza and Edwards's (1967) chord distances (D_{CE}) were calculated among samples using the GENDIST program of PHYLIP version 3.5c (Felsenstein 1995) and plotted as a neighbor-joining (NJ) phenogram (Saitou and Nei 1987) using NEIGHBOR. The original allele frequency matrix was then resampled 1,000 times using BOOTSTRAP and the chord distances among samples were estimated for each resulting matrix. A consensus UPGMA

phenogram was generated using CONSENSE, and all bootstrap values to indicate support for each node. Second, Factorial Correspondence Analysis (FCA) of genetic data using Genetix software version 4.05.2 (Belkhir et al. 1996-2004) was used to examine genetic relationships among individual samples for all data and for a subset containing only golden trout populations. Additionally, data from six loci (Ots3, Ots85, Ots249b, Ots423, OMM1082, and OMM1083) were combined with overlapping data from a previous study of California golden trout populations (Cordes et al., *in press*) to evaluate the relationship of the Coyote Creek sample to Little Kern golden trout, California golden trout, and rainbow trout populations simultaneously.

Bayesian estimation of admixture proportions for LKGT was performed in STRUCTURE, version 2.2 (Pritchard et al. 2000a), which uses an algorithm that defines groups by maximizing Hardy-Weinberg equilibrium and minimizing linkage disequilibrium between individual samples. Data consisted of multilocus genotypes from individuals in all populations of both golden and rainbow trout. We performed naïve clustering, whereby individuals are grouped according to their genetic similarity without any prior information on their population of origin. This allowed us to determine both the number of detectable genetic clusters (K) and the inferred proportion of ancestry for each individual in each cluster, which we use to describe proportion of admixture proportion (q) between the observed groups. We used the admixture model and correlated allele frequencies parameter, with a burn-in-period of 100,000 and 300,000 MCMC iterations for three runs of each K and values of K from 1-10. No prior information on population of origin was employed; rather the program was allowed to determine admixture proportions independent of assumptions about which populations represented “pure”

golden trout versus “pure” rainbow trout. This method was selected due to the admixed nature of the hatchery rainbow trout samples, which are known to be composed of several different rainbow trout groups (Busack and Gall 1980). The most likely number for K was determined by finding the K with the largest second-order rate of change in negative log-likelihood values (Evanno et al. 2005), effectively locating the sharpest rate of change in the negative log-likelihood values for all runs of K.

SNP Data Analyses

SNP data files were converted to GENEPOP format using the Transformer-3 program (Caujape-Castells and Baccarani-Rosas 2005). Descriptive statistics for nuclear SNP data including allele frequencies, observed and unbiased expected heterozygosities (H_O and H_E, Nei 1978) and inbreeding coefficients (FIS ,Weir and Cockerham 1984)) were calculated for all loci in each population sample using Genetix 4.05 (Belkhir et al. 1996-2004). Populations were tested for departures from Hardy-Weinberg equilibrium at each locus and for linkage disequilibrium between pairs of loci using Fisher's exact test in the Genetic Data Analysis program (GDA; Lewis and Zaykin 1999). We used an exact test based on the multinomial probability of the multilocus genotype, conditional on the single-locus genotypes (Zaykin et al. 1995). Significance was assessed by Monte Carlo simulation, by permuting the single-locus genotypes among individuals in the sample to simulate the null distribution. For each pair of SNPs, 3,200 replicate samples were simulated, to estimate the empirical p-value. A Bonferroni correction was applied for the multiple comparisons in the genotypic disequilibrium tests within each population sample (e.g., significance determined at P= 0.00138, α 0.05/36). Likewise, a Bonferroni correction was used within each population sample to test for deviations from Hardy-

Weinberg equilibrium across multiple loci (e.g., p = 0.0055, α 0.05/9). All statistical significances were computed using the Markov chain method to obtain unbiased estimates of Fisher's exact test based on 10,000 iterations (Guo and Thompson 1992). Significance values resulting from multiple comparisons were corrected for Type I error using sequential Bonferroni correction (Rice 1989). Allele frequencies for mtDNA locus were calculated manually.

The optimal marker for assessment of admixture would be completely fixed, or diagnostic, between the two groups being evaluated. However, markers with a frequency differential between groups of 0.5 or more are generally considered to retain a high degree of informativeness for studies of admixture (Shriver et al. 1997). We employed delta statistic (Smith et al. 2001) as a means of assessing marker efficiency for detecting differences between golden and rainbow trout subspecies groups. The estimate of δ for each SNP locus was calculated as the absolute value of the allelic frequency difference between two populations. The value of δ was calculated to determine the frequency differential for between Upper Soda Spring Creek (2002) golden trout and Mt. Shasta Strain ($\delta_{U\text{-}M}$), North Fork American River ($\delta_{U\text{-}N}$), and Hot Creek Strain ($\delta_{U\text{-}H}$) rainbow trout. Delta values range from 0 to 1, with 1 indicating fixed (diagnostic) differences in allele frequencies between the populations being compared. The Upper Soda Spring Creek population was selected to represent LKGT because of its high degree of isolation and lack of apparent rainbow trout introgression. Lastly, the maximum delta value observed between any two populations (Maxδq) was calculated for all loci. Because the inclusion of less informative markers may increase noise and worsen population

inference (Liu et al. 2005), only the most informative markers were targeted for data collection.

CONVERT (Glaubitz 2004) was used to create input files for Bayesian analysis in STRUCTURE. SNP data were analyzed in the same manner described for microsatellites above, but with fewer MCMC iterations (50,000 and 150,000 iterations for burn-in and data collection, respectively) required, given the greater stability of parameters. Data consisted of multilocus genotypes from individuals of all populations of both golden and rainbow trout. The mitochondrial locus was included by using diploidized genotypes with one missing allele at each locus, as recommended by the STRUCTURE authors for the inclusion of haploid data.

Results

Microsatellite data

Allele frequencies for all 18 microsatellite loci are reported in Appendix 3.1. Locus OMM1097 was dropped from analysis due to unscorable repeats. Deviations from Hardy-Weinberg equilibrium were observed in 25 out of 504 population-locus comparisons after Bonferroni correction (alpha = 0.05/28 = 0.0018): six populations for locus OMM1009 (CLK, CLN, FC, NFNR, NFAR, and UFC), seven populations for locus OtsG3 (DDM95, DDM96, CLN, CLK, SC, USSC02, and UWC), and fewer than two deviations per population for all remaining significant values, with no observable trends by population. Linkage disequilibrium was observed in 82 out of 4,284 comparisons after strict Bonferroni correction (alpha = 0.05/153=0.00033). The majority of these deviations were attributable to linkage involving three loci: OMM1058, OMM1078, and OMM1083. Consequently, OMM58 and OMM1078 were dropped from further analysis.

The linkage disequilibrium observed for these loci was expected, as they are found in the same linkage groups (Rodriguez et al. 2004; Guyomand et al. 2006). Ten populations exhibited significant linkage disequilibrium between OMM1046 and OMM1083. OMM1058, OMM1078 and OMM1046 were dropped from further analyses, but data may be utilized in future analyses that incorporate linked loci. Remaining deviations were observed in two or fewer populations for each locus combination.

Microsatellite estimates of genetic diversity and demographic history

The mean number of microsatellite alleles per locus ranged from 2.3 to 5.8 for Little Kern golden trout and LKGT broodstock populations, 9.9 for the Upper Coyote Creek transplant population, and 6.3 to 12.5 for rainbow trout populations, and allelic richness values ranged from 1.9-3.3, 4.4, and 3.9-5.4, for the same respective groups of populations (Table 3.4). Average observed and expected heterozygosities for all microsatellite loci are reported in Table 3.4. The rainbow trout samples were significantly more variable (both allelic richness and expected heterozygosity) than LKGT samples (permutation of observed differences in FSTAT, P=0.001).

Evidence of population bottlenecks was observed using all three methods. There was significant excess of heterozygosity relative to drift–mutation equilibrium in 12 of the 22 LKGT populations for the preferred TPM ($P \leq 0.01$): two broodstock, DDM97, WMC95; LKBC, MWMC, WMC, SL, LWC, SC, USSC02, CLN, UFC, and FC (Table 3.4). However, sample sizes of the DDM97 and UFC samples were smaller than recommended for accurate inference in this analysis.

Population differentiation

The UPGMA tree (Figure 3.2) depicts genetic relationships among Little Kern populations and wild and hatchery rainbow trout populations. LKGT broodstocks grouped both with their source and recipient populations, where applicable. Several major groups are well supported in this analysis. The first group contains Upper and Lower Willow Creeks, Sheep Creek, Rifle Creek, and Silver Lake. The second group contains all Wet Meadow Creek populations as well as the Little Kern at Broder's Cabin population. The third group contains all Fish Creek and Clicks Creek samples as well as Upper Mountaineer Creek. The fourth group contains all Soda Spring Creek populations. The fifth group contains all Deadman Creek broodstock populations and Upper Maggie Creek/Lakes. Upper Coyote Creek is not supported as a member of any LKGT groups; rather, this population exhibits an affinity for rainbow trout populations. The majority of F_{ST} values were significant in pairwise comparisons of populations with a few exceptions (Bonferroni correction $P < 0.0001$, Table 3.5). Though some nonsignificant comparisons were also recorded, several involved LKGT broodstocks with small sample sizes (Deadman 1997 n = 8 and Soda Spring 1995 n=10), and should be interpreted cautiously. The three Wet Meadow Creek populations also were not significantly different from one another.

The FCA displaying relationships among all individuals (Figure 3.3a) shows four major clusters: one Little Kern golden trout group "LKGTa" containing Sheep Creek, Lower and Upper Willow Creek, Rifle Creek, and Silver Lake, a second group "B" containing all other LKGT, a third cluster of hatchery and wild rainbow trout samples, and a fourth cluster of mainly Upper Coyote Creek individuals, positioned intermediately

between the rainbow and LKGT clusters. When rainbow trout populations and the Upper Coyote Creek population are removed from the FCA, a third clustering of Little Kern golden trout is evident, as the "LKGTb" group breaks into two clusters, one containing Wet Meadow Creek (Upper, Middle, and 1995 broodstock) and Broder's Cabin populations and "LKGTc" containing all remaining populations (Figure 3.3b). FCA based on the six microsatellite loci for which California golden trout data also exists (Figure 3.4) shows that the Coyote Creek population, when compared in the context of all trout subspecies, has a strong affinity for the California golden trout subspecies cluster.

Hybridization

STRUCTURE analysis of clustering for microsatellite data yielded the highest value of delta K for K=3 (Figure 3.5a), corresponding to one group containing all rainbow populations, and the second and third groups containing LKGT. This clustering differed from SNP data, for which 2 genetic clusters were detected (Figure 3.5b). Higher values of K revealed additional structure in the microsatellite data (summarized in Table 3.6) within the two LKGT groups, as evident in the gradual continuing increase in negative log likelihood values. All runs converged on the same solutions, with highly similar individual admixture estimates between replicate runs. Mean rainbow trout admixture coefficients (qRT, the proportion of ancestry attributable to the "rainbow trout" cluster) in the 22 Little Kern Basin populations ranged from 0.00-0.10, with 17/22 populations containing proportions $\leq$0.01, and 21/22 containing $\leq$0.04 rainbow trout introgression (Table 3.7). In the case of one LKGT broodstock population, Deadman 1996, the slightly higher mean rainbow trout coefficient (0.04) was attributable to a single individual with nearly 100% rainbow trout ancestry, as seen in the individual

admixture coefficients (Figure 3.6). Upper Mountaineer Creek contained the highest level of rainbow trout influence (10%) of all Little Kern Basin populations, while the out-of-basin Upper Coyote Creek population was estimated as 92% introgressed. Coefficients for the hatchery and wild rainbow trout samples ranged from 0.96-0.99 (Table 3.7).

SNP Data Results

Five comparisons (out of 270) were found to deviate from HWE expectations after correction for multiple comparisons: Omy_180 for DDM95, CHIT_80 for NFNR, and G6PD_103 for SSCPB, UMT, CLN, and FC04 populations. Genotypes for locus G6PD_103 were subsequently preserved for calculation of linkage disequilibrium. LD was detected at only 2 locus/population combinations after correction for multiple comparisons: Upper Coyote Creek for F5_306/OMY_180 and CHIT80/F5306.

Original delta values from SNP marker discovery ranged from 0.75-1.00 between Upper Soda Spring Creek (USSC) and Mt. Shasta strain (MSS) individuals and 0.13-1.00 for comparisons between USSC and NFAR individuals (data not shown). In general, delta values for most loci remained high, ranging from 0.25 to 1.00 for comparisons between USSC and MSS full populations, and 0.05 to 1.00 between USSC and NFAR populations (Table 3.3). Allele frequencies and heterozygosities are reported for SNP loci in Appendix 3.2.

STRUCTURE analysis identified two groups (K=2) for the SNP dataset, corresponding with LKGT and rainbow trout groupings (Figure 3.5b); all runs converged on the same solution, with similar individual admixture values generated between replicate runs. A comparison between SNP and microsatellite estimates of introgression

is presented in Table 3.7. Mean rainbow trout admixture coefficients in the 39 Little Kern Basin populations examined using SNP data ranged from 0.00-0.30, with 17/39 Little Kern Basin populations containing rainbow trout proportions (q) ≤0.01, and 32/39 containing ≤0.04. As in the microsatellite results, the slightly elevated mean rainbow trout introgression estimate (0.05) for the Deadman 1996 broodstock was due to a single individual of 100% rainbow ancestry. Upper Mountaineer Creek had a slightly lower SNP introgression estimate (0.06, compared to 0.10 for microsatellites). Several of the 2006 populations not examined using microsatellite data had estimates ≤10%: Alpine Creek, Jacobsen Creek, South Mountaineer Creek, Shotgun Creek. Both the Little Kern at Burnt Corral 2006 and 1998 samples had elevated introgression estimates (0.30 and 0.14, respectively). Lastly, the Upper Coyote Creek out-of-basin population had a coefficient of 0.17.

Discussion

The results of this study indicate significant genetic structuring throughout the native range of LKGT, with several major groupings found in both Bayesian and phylogenetic analyses that correspond to the known reintroduction history within this basin. Evidence for strong differentiation among these groups was accompanied by relatively low levels of heterozygosity for most, and significant inbreeding for several populations. Bayesian analyses of microsatellite and SNP data gave similar estimates for both the pattern and degree of rainbow trout introgression, validating the employment of SNP markers for assessing introgression in genetic monitoring of these populations. The presence of several populations with high estimates of rainbow trout influence give

reason for concern regarding the genetic purity of selected LKGT populations within the native basin and their potential influence on adjacent non-hybridized populations.

Rainbow trout introgression in LKGT

The general concordance between SNP and microsatellite markers in detecting and quantifying rainbow trout introgression demonstrates the utility of SNP markers for use in genetic monitoring of rainbow trout introgression in LKGT. Furthermore, the high-throughput nature of SNP data collection and improved amplification of poor-quality samples present major advantages in molecular genetic data collection. We were able to amplify DNA for SNP loci in several individuals from populations with low template quality that would not amplify at all for microsatellite loci: Little Kern River at Rifle Creek, Lower Deadman Creek, South Mountaineer Creek, Lower Mountaineer Creek, and Little Kern River at Burnt Corral 1998 (Table 3.1).

Our findings for the Little Kern golden trout contrast markedly with studies of rainbow trout introgression its sister taxon, the California golden trout, which is moderately to highly introgressed in large portions of its native range (Cordes et al. 2006; Cordes et al. *in press*) and with previous studies of LKGT prior to restoration efforts. Negligible levels of introgression, less than 1%, were found in 44% (17/39 total) of Little Kern Basin populations and the majority (33/39, or 85%) of populations showed less than 5% introgression, suggesting that reintroduction efforts were largely successful at eliminating rainbow trout influence in most areas the basin. These eradication efforts, however, were not without consequences for genetic diversity and population structure for the subspecies as a whole (see discussion below). Several localities did exhibit moderate mean estimates of rainbow trout influence (6-10%), with introgression

observed across multiple individuals in each population: Upper Mountaineer Creek, Alpine Creek, Jacobsen Creek, South Mountaineer Creek, and Shotgun Creek. Both 2006 and 1998 Little Kern at Burnt Corral samples had even higher SNP introgression estimates (0.30 and 0.14, respectively). Such levels are sufficiently high to warrant conservation concern for adjacent populations, particularly given the location of non-hybridized populations downstream from all of these hybridized localities (Figure 3.1). The relatively high levels of hybridization in the Shotgun Creek sample are somewhat perplexing, as other nearby populations (e.g. Rifle and Pistol creeks and Silver Lake) derived originally from the Coyote Creek and Crytes Lake stocks did not show evidence of introgression. A heavily used trail exists from the Kern River along Coyote Creek and over Coyote Pass into the Little Kern River basin. Trout may have historically, or recently been moved from the Kern River and planted in various waters, including upper Coyote Creek Big Crytes Lake, and Shotgun Creek.

The Burnt Corral population is located downstream relative to most other populations, and threats to other tributary populations will likely be reduced by isolating barrier falls protecting these tributaries. However, human transfer of trout in the Kern Basin and in other systems (Munro et al. 2005) is well documented; the presence hybridized populations therefore represents an ongoing threat to adjacent native LKGT populations.

The apparently conflicting introgression estimates for the Upper Coyote Creek out-of-basin population (0.17 and 0.92 for SNP and microsatellite data, respectively) likely reflects differences between marker types. The SNP data identify the California golden trout influence in this population as “golden,” that is, as Little Kern golden trout,

while the microsatellite data, analyzed without California golden trout groups as a reference in the analysis, identify this as "rainbow" influence. Regardless, this sample does not appear to represent LKGT, and likely contains other rainbow and golden influences. This appears to be localized hybridization, as the Lower Coyote Creek sample (n=40; qRT = 0.02) does not appear to be similarly admixed.

Comparison between the results of this and other post-restoration allozyme studies yields both similarities and discrepancies. Several Mountaineer Creek area populations and the Burnt Corral population were known from previous allozyme studies to be genetically compromised (Bagley et al. 1998; Bagley et al. 1999; Gall and May 1997), and the current study provides clearer estimates of the degree of rainbow trout influence. Introgression was not detected in allozyme analyses of Shotgun Creek, yet moderate levels were found in this study. Previous allozyme studies detected introgression in Soda Spring Creek and 1997 Deadman 1997 broodstock populations where none was detected in the current study.

The detection by both SNP and microsatellite data of a single rainbow trout individual in the Deadman Creek 1996 broodstock is of particular interest, given that it does not appear to be an admixed fish, but rather a "pure" rainbow trout. Inadvertent "contamination" of the Deadman Creek 1996 golden trout broodstock in the hatchery setting seems a likely explanation; given the ancestry coefficient of this particular individual, the data suggest this is a rainbow trout from the facility that was inadvertently mixed into the broodstock population, not a hybridized individual. This observation highlights the critical need for greater precaution and careful segregation of strains in the hatchery setting (or dedicated hatcheries for single strains) in order to prevent accidental

mixing or crossing in strains used for restoration. Deadman Creek hatchery broodstock (unidentified year) were planted into the following localities: Little Kern River at Burnt Corral, Little Kern River at Grey Meadow Creek, Little Kern River at Round Meadow, Little Kern River Horse Bridge area, Twin Lakes, Upper Alpine Creek, Little Kern River at Mountaineer Creek, Mountaineer Creek., Lower Maggie Lake, Middle Maggie Lake, Upper Maggie Lake, Maggie Creek, Pecks Canyon Creek, South Mountaineer Creek, and Soda Spring Creek. Of the populations examined with molecular data in the current study, three localities showed elevated introgression levels (Burnt Corral 2006 and 1998, Alpine Cr., and Upper Mountaineer). The fourth, Upper Maggie Lake, did not show evidence of introgression (SNP and indel markers recovered qRT = 0.01 for both SNP and microsatellite estimates, n=14 and 12, respectively), however the small sample size precludes any strong assurance that contaminated hatchery broodstock did not impact this reintroduced LKGT population. Genetic investigation of populations subsequently stocked with both Deadman 1996 and 1997 broodstock should be undertaken to determine what potential impacts these broodstock had on re-established populations.

Strong frequency differentials were maintained at the majority of the ten SNP loci, and results from the admixture analyses suggest that these loci have comparable power to microsatellite loci to detect introgression in LKGT. The SNP data set generated admixture estimates similar to those of microsatellite data sets. Structure analysis of the SNP data revealed a grouping of K=2 instead 3, as was observed in the microsatellite data set for the same samples. This difference is expected, given that the SNP data set was specifically designed to detect differences between golden versus rainbow trout, and not to detect geographic substructure inherent in the data set as the microsatellites.

Additional analyses (not shown) indicate that the presence of low delta value loci in analysis of SNP data may decrease the estimates for introgression levels, as evidenced by a two-fold increase in the estimate for Upper Mountaineer Creek when low-delta values (e.g., FGG_259 and G6PD_103) were excluded from STRUCTURE analysis. Further investigation is required before introgression estimates are applied to species management.

Population structure and genetic diversity

The overall relationships depicted in the NJ tree, FCA, and Bayesian clustering are highly consistent with one another. The intermediate nature of the Upper Coyote Creek population can be seen in the FCA (Figure 3.3a), attributable to either 1) rainbow introgression in this population creating an intermediate, introgressed population of LKGT or 2) natural or anthropogenically induced gene flow with either rainbow trout (Kern River or otherwise) or California golden trout.

The signature of restoration efforts is apparent in the genetic data, with the major groupings of populations in Bayesian, phylogenetic, and ordination analyses of microsatellite data showing a close correspondence between broodstocks or source populations with their recipient drainages. The FCA (Figure 3.3b) depicts the genetic distinction of three Little Kern groups: 1) Sheep, Rifle, and Willow Creek-derived populations, 2) Wet Meadow Creek-derived populations, and 3) Deadman/Soda Spring, and Fish Creek-derived populations. This is correspondingly supported by he STRUCTURE clustering (K=4). Both the STRUCTURE and the UPGMA dendrogram support the existence of an additional level of grouping containing Fish, Upper Mountaineer, and Clicks Creek populations (Figure 3.2, Table 3.6).

Genetic diversity represents the raw material for current and future evolution and serves as a proxy for a species' relative fitness and adaptability to current or future environmental (Reed and Frankham 2003). The significantly lower values for allelic richness and genetic diversity relative to rainbow trout populations and the prevalence of genetic bottlenecks and low F_{IS} values for several LKGT populations give reason for concern for continued LKGT persistence. In addition, some of the bottlenecked populations (e.g. Sheep and Fish Creeks) represent broodstock source localities, suggesting that populations could have already been bottlenecked prior to their use in restoration efforts. High F_{ST} values between LKGT populations imply limited gene flow in LKGT; this may be the natural movement of restored LKGT above and below barriers, or could represent a signature of founder effects and genetic drift in these populations.

Conservation and management implications

Species restoration, out of necessity, must often proceed in the face of imperfect information. Over three decades of effort to restore Little Kern golden trout to their native basin may represent the best attempt using available information to conserve what remained of the species, given the high degree of human disruption in this system. Without examining pre-restoration samples, it is impossible to determine whether the restoration efforts themselves caused reductions in genetic diversity or whether they simply rescued available remaining genetic diversity. Future conservation efforts will likely require ongoing assessment through genetic monitoring and possible intervention into the future.

Defining the goals of restoration allows researchers and resource managers to successfully conduct and evaluate restoration programs. The return of an ecosystem not

to its pre-degraded state, but rather to a close approximation of its pre-degraded state (to the extent capable in a rapidly changing natural system) represents a reasonable goal. Conservation efforts, therefore, should focus on not only preserving unique genetic lineages but also the environmental and evolutionary processes that generate diversity. Hybridization with non-native taxa has the potential to break down the evolutionary trajectory of native populations, particularly if hybrids enjoy any sort of selective advantage or higher fitness than one or both of their parental taxa (Rosenfield et al. 2004) or if a degraded environment facilitates hybrid invasion (Fitzpatrick and Shaffer 2007; Mallet 2005; Rieseberg et al. 2007). The paucity of information regarding how abiotic factors and species-environment interactions affect hybridization rates and patterns currently relegates management alternatives for nonnative trout to eradication through physical or chemical means or no action (Dunham et al. 2004). This presents an inherent dilemma in dealing with hybridized individuals and populations, which may simultaneously represent both a threat to the conservation of non-hybridized native populations and an important component of remaining genetic diversity, worthy of conservation (Allendorf 2001).

Several factors in addition to hybridization with rainbow trout were cited in the original decision to list the Little Kern golden trout under the ESA as a threatened species. These included habitat degradation from stream sedimentation (due to off-highway vehicles and logging related road conditions), stream pollution from mineral extraction and milling, recreation development in Mineral King and Jordan Peak, and livestock impacts. Although minor habitat improvements have been made, including some bank stabilization projects and reduction in grazing and off-highway vehicle use,

habitat conditions remain relatively unchanged since the listing and wilderness designation (C. McGuire, CDFG, personal communication).

Despite low levels of genetic diversity, the persistence of multiple populations of LKGT from four different lineages containing limited to no apparent rainbow trout influence gives reason for optimism regarding the persistence of this Sierra Nevada endemic subspecies. Focusing eradication efforts on the handful of hybridized sites will yield the largest returns in preventing the spread of introgression to additional localities. Clearly, if the use of hatchery-reared or hatchery-bred LKGT stocks in restoration is to continue in the future, establishing of a dedicated conservation hatchery and incorporating genetic monitoring of both hatchery-produced fish and restored populations would be a valuable means to prevent the accidental or deliberate planting of introgressed stocks.

Tables and Figures

Table 3.1. Details of Little Kern golden trout study populations including population number, locality, sample code, date of collection, number of individuals sampled for use in SNP and microsatellite analyses (NSNP and Nusat, respectively).

Pop ID	Code	Locality	Coll. Date	Stocking History	NSNP	Nusat
Little Kern broodstock					N	N
1	DDM95	Deadman Creek Stock 1995	1995	n/a	38	38
2	DDM96	Deadman Creek Stock 1996	1996	n/a	27	27
3	DDM97	Deadman Creek 1997[1]	1997	n/a	11	8
4	SSC95	Soda Springs Creek Stock 1995	1995	n/a	10	9
5	WMC95	Wet Meadow Creek Stock 1995	1995	n/a	29	29
Little Kern golden trout						
6	LKBC	Little Kern River above Broder's Cabin	2002	Wet Meadow	33	29
7	UWMC	Wet Meadow Creek, upper	2002	n/a	40	27
8	MWMC	Wet Meadow Creek, middle	2002	Wet Meadow	33	32
9	WMC	Wet Meadow Creek	2001	Wet Meadow	20	20
10	SL	Silver Lake	2002	Crytes	36	39
11	SHT06	Shotgun Creek	2006	Coyote	34	-
12	PST06	Pistol Creek	2006	Coyote	16	-
13	LKRC	Little Kern River above Rifle Creek	1999	Wet Meadow	29	-
14	RFC06	Rifle Creek	2006	Coyote	35	-
15	RC	Rifle Creek	1999	Coyote	30	28
16	TAM06	Tamarack Creek	2006	Willow	30	-
17	UWC	Willow Creek, Upper	2001	n/a	40	37
18	LWC	Willow Creek, Lower (below Sheep Creek)	2001	n/a	19	19
19	SC	Sheep Creek	2001	n/a	39	38
20	LIO06	Lion Creek	2006	Sheep	40	-
21	USSC02	Soda Spring Creek, Upper	2002	n/a	40	29
22	USSC01	Soda Spring Creek, Upper	2001	Soda Spring	28	16
23	SSCPB	Soda Spring Creek At Park Boundary	2001	Soda Spring	40	-
24	DDM06	Deadman Creek	2006		30	-
25	DDM03	Deadman03 (lower)	2003	Soda /Deadman	40	-
26	UML	Upper Maggie	1998	Deadman	14	12
27	ALP06	Alpine Creek	2006	Soda Spring	40	-
28	UMT	Mountaineer Creek, Upper (above good barrier)	1999	Deadman	30	30
29	SMC06	South Mountaineer Creek	2006	Deadman	40	-
30	SMC	South Mountaineer Creek	1999	Deadman	21	-
31	LMC	Lower Mountaineer Creek	1998	Deadman	37	-
32	JAC06	Jacobson Creek	2006	Deadman	40	-
33	CLN	Upper North Fork Clicks	2005	Fish	39	38
34	CLU	Clicks Creek, upper	2005	Fish	39	39
35	LKBRN06	Little Kern at Burnt Corral	2006	Fish	8	-
36	LKBRN	Little Kern River at Burnt Corral	1998	Fish	36	-
37	UFC	Fish Creek, Upper	2001	n/a	11	10
38	FC	Fish Creek	2004	n/a	40	38
39	TMC06	Trout Meadow Creek	2006	Fish	19	-

Table 3.1, continued

Pop ID	Code	Locality	Coll. Date	Stocking History	NSNP	Nusat
Kern River populations						
40	UCC	Coyote Creek, Upper	2001	n/a	30	28
41	LCC	Coyote Creek, Lower	2001	n/a	40	-
Wild rainbow trout						
42	NFAR	North Fork American River	2000	n/a	20	19
43	NFNR	North Fork Navarro River	2000	n/a	30	30
Hatchery rainbow strains						
44	HCS	Hot Creek Strain	2002	n/a	31	28
45	MSS	Mount Shasta Strain	2002	n/a	30	24
46	MWS	Mount Whitney Strain	2002	n/a	31	18

[1]held and collected in 1998

Table 3.2. Multiplexed primer combinations and primer sequence information, including multiplex number (multi), forward (f) and reverse (r) primer name, fluorescent label, primer sequence, concentration used in PCR, and original reference.

Multi	Primer	Label	Sequence 5'-3'	primer [μM]	Primer reference
1	OMM1037f	FAM	GCGACTGGATTTAATACTGC	0.1	(Rexroad III et al. 2002a)
	OMM1037r	–	TCCTCTGACTGCCATTACATC	0.1	
	OMM1036f	NED	TGTAGCAGGTGAGAATACCCA	0.1	(Rexroad III et al. 2002a)
	OMM1036r	–	CACCATCTCCATCCTAGGC	0.1	
	OMM1089f	VIC	GCAGCTCCTGTTTTCTATGTG	0.1	(Rexroad III et al. 2002b)
	OMM1089r	–	CTGAGATGCAGTGCCTTAGAC	0.1	
2	OtsG85f*	FAM	CCATGTCAGCACTGACTTAAT	0.2	(Williamson et al. 2002)
	OtsG85r	–	GGATGTTGTTCCTAATGTTTT	0.2	
	OMM1322f	NED	GCGCTCCTTTCATCTCTGATACAG	0.1	(Palti et al. 2002)
	OMM1322r	–	GGTGAATACTTTCGCAAGCC	0.1	
	OtsG423f*	VIC	AGGCCTGCCAGGCACTAAAGGTAT	0.1	(Williamson et al. 2002)
	OtsG423r	–	GCAAGCAAACATGTAGCTTCATGG	0.1	
3	OMM1082f*	FAM	CAAGAGCACTAACGACCATGT	0.1	(Rexroad III et al. 2002b)
	OMM1082r	–	CGCAAGCAAGCTAACACA	0.1	
	Omy1009UWf	NED	TGAGTAAAAAGGGGAAACAAGC	0.1	(Spies et al. 2005)
	Omy1009UWr	–	R: GCGAAAACACTCTGGCAAAT	0.1	
4	OMM1097f	VIC	CTAGCCATCCGAACACTG	0.1	(Rexroad III et al. 2002b)
	OMM1097r	–	AGAATAGGGTGCCTGTATCTC	0.1	
	OMM1046f	NED	CAGGCACTATAATGGCAC	0.1	(Rexroad III et al. 2002a)
	OMM1046r	–	GCCCACGAGTTACAAGA	0.1	
	OMM1078f	VIC	AACTCACGCCCTGACCAACCTAAC	0.1	(Rexroad III et al. 2002b)
	OMM1078r	–	GATTTCAGTATTGGTGCCGAGCC	0.1	
	OMM1051f	NED	CCTACAGTAGGGATTAACAGC	0.1	(Rexroad III et al. 2002a)
	OMM1051r	–	CATGCCCACACATTACTAC	0.1	
5	OtsG249bf*	FAM	ATGGCAGTTAAGAGAACAAAAGTT	0.1	(Williamson et al. 2002)
	OtsG249br	–	CCTACCCTTCTCATTCAAGACTAA	0.1	
	OMM1088f	FAM	CTACAGGCCAACACTACAATC	0.1	(Rexroad III et al. 2002b)
	OMM1088r	–	CTATAAAGGGAATAGGCACCT	0.1	
	Omy1011UWf	NED	AACTTGCTATGTGAATGTGC	0.1	(Spies et al. 2005)
	Omy1011UWr	–	GACAAAAGTGACTGGTTGGT	0.1	
6	OMM1058f	VIC	GTGTGTATGTGCGTTCAC	0.1	(Rexroad III et al. 2002b)
	OMM1058r	–	CCAATGAGAAGCGTTAC	0.1	
	OtsG3f*	FAM	GGACAGGAGCGTCTGCTAAATGACTG	0.1	(Williamson et al. 2002)
	OtsG3r	–	GGATGGATTGATGAATGGGTGGG	0.1	
	OMM1083f*	NED	GCCCTGACCAACCTAACACA	0.1	(Rexroad III et al. 2002b)
	OMM1083r	–	TGTCTGACATTCGGTTAGTAGTGG	0.2	
	OMM1081f	VIC	CCGTTGTATAACAATGACC	0.1	(Rexroad III et al. 2002b)
	OMM1081r	–	TCTTTACACAGAGGGTTCTAC	0.2	

* = locus used in California golden trout microsatellite studies (Cordes et al. 2006, Cordes et al. 2003)

Table 3.3. Ten SNP assays developed for Little Kern golden trout. Marker names consist of the locus identifier and the targeted nucleotide position. Oligonucleotide sequences for unlabeled primers (Forward and Reverse) and labeled probes for each allele are given for each marker, annealing temperature (Ta), number of individuals successfully genotyped (N), expected (H_E; assuming panmixia) and observed (H_O) heterozygosities. Difference in "rainbow" allele frequency between Upper Soda Spring Creek Little Kern golden trout versus Mount Shasta Strain rainbow trout (δ_{U-M}), North Fork American River rainbow trout (δ_{U-N}), and Hot Creek Hatchery strains (δ_{U-H}) are also given, along with maximum difference in "rainbow" allele frequency between any two collections (Maxδq) and FST estimates for each SNP marker.

Locus name	Oligonucleotide sequences (5'–3')	Ta (°C)	[primer mix]	N	δqU-N	δqU-M	δqU-H	Maxδq	*F*st
CHIT 80	F: GGCCTTATCAATTATGTCACGTGGAT	60	0.1ul	1383	0.63	0.72	0.73	0.98	0.59
	R: CCCTTTTCTCTCACAGTAACTTTCCA								
	FAM-CACCCTTCAATAACA								
	VIC-CACCCTTGAATAACA								
CRB2677 117	F: TCTGCCAAATTCACATGACAAAAGAC	60	0.1ul	1335	0.29	0.66	0.79	0.79	0.47
	R: ATTACAATGAAAGTACTTGAGTGTTTATGCAAA								
	FAM-TGCAACAGAGGGTTG								
	VIC-CATTGCAACATAGGGTTG								
F5 306	F: GAACACTTGGTGTGATTTGCATCAT	60	0.1ul	1379	0.44	0.69	0.69	1.00	0.44
	R: GCTGAGGAGAGAAAAGGAGAAATGA								
	FAM-CATTACACATTATTTTCT								
	VIC-CCATTACACATTCTTTTCT								
ID1c 77-83	F:CAGGCTTTTTTTTCTATCAGAATTAAGTC	58	0.125ul	1364	0.88	0.86	0.88	0.88	0.40
	R:TGTATGCTAACTTGTAATTTGCTGTTGT								
	VIC-AGTTAACAGTTAATGAGT								
	FAM-AGGCAGTTAACGAGTC								
OMY180	F: CTGGATGTGTAGTATCGGTGGAAAA	62	0.125ul	1347	0.68	0.72	0.75	0.75	0.18
	R: CACTGGGCACCTCTGATCTC								
	FAM-CTGTAGTAGTCCCCATTGT								
	VIC-CTGTAGTAGTCCGCATTGT								
B9_164[1]	F:GCACAGAACACAGCCAATATTAACA	63.5	0.1ul	1342	1.00	1.00	1.00	1.00	0.39
	R:GCCTTGACTCTCCCTTCATGAC								
	FAM-CCTACAACTTGATCTACGTG								
	VIC-CCTACAACTTGATCTAACGTG								
RAPD 132[1]	F:ATCATTACCACGCCCAACGTTA	60	0.1ul	1376	0.05	0.92	0.95	0.95	0.82
	R:AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT								
	FAM-ATGTTGGGAAATATGA								
	VIC-CATGTTGGGATATATGA								
Omy_f1 259-260	F: CCACACACACAAACACACATACAC	60	0.1ul	1232	0.40	0.05	0.12	0.85	0.26
	R: CAAGCATTCTTCTGTTAAATGTGGTCTA								
	FAM-ACACACACACACAGCA								
	VIC-CACACACAAACAGCA								

Table 3.3, continued

Locus name	Oligonucleotide sequences (5'–3')	Ta (°C)	[primer mix]	N	δqU-N	δqU-M	δqU-H	Maxδq	*F*st
LDH 156	F: GTTTTGAAACCAGTTTAAGGTTGATTGC R: ACGGCATAGTCTGGACAGAGAT FAM-CCATTTAGATGTTTTTT VIC-CCATTTAGACGTTTTTT	62	0.1ul[1]	1340	0.14	0.88	0.97	0.97	0.60
RTDL 695	F: AAGCCGGGCGTTCTCTTATATG R: GTTAGACTTCTTTGCTTGCACTTGT FAM-ATAGGGTTCTCCTTTTT VIC-CATAGGGTTCTCTTTTTT	62	0.1ul	1351	1.00	1.00	1.00	1.00	–

[1]used Promega reagents

Table 3.4. Summary statistics for 15 microsatellite loci, including number of individuals amplified for each population (N), observed (H_O) and expected (H_E) heterozygosities, standardized number of alleles (Na) and allelic richness (r). Inbreeding as detected by F_{IS} and significance of homozygote excess relative to Hardy-Weinberg equilibrium (H-W pval). BOTTLENECK test results are given for tests under the Infinite Alleles Model (IAM), Stepwise Mutation Model (SMM), and Two-phase Model (TPM), with significant values in bold ($P \leq 0.01$).

Population	N	H_E	H_O	Na	r	F_{IS}	H-W pval	IAM	SMM	TPM
Deadman Creek Stock 1995	38	0.645	0.635	4.3	3.1	0.02	0.580	**0.000**	0.076	0.024
Deadman Creek Stock 1996	27	0.662	0.625	5.8	3.3	0.06	0.018	0.013	0.402	0.180
Deadman Creek 1997	8	0.650	0.628	3.8	3.2	0.04	0.437	**0.000**	0.008	**0.007**
Soda Springs Creek Stock 1995	9	0.584	0.461	3.8	3.0	0.22	0.000*	0.007	0.196	0.052
Wet Meadow Creek Stock 1995	29	0.488	0.469	3.8	2.5	0.04	0.097	**0.000**	**0.009**	**0.004**
Little Kern R. above Broder's Cabin	29	0.555	0.550	4.1	2.9	0.01	0.665	**0.000**	0.054	**0.009**
Wet Meadow Creek, upper	27	0.513	0.537	3.5	2.7	-0.05	0.695	**0.000**	0.094	0.018
Wet Meadow Creek, middle	32	0.541	0.506	3.7	2.8	0.07	0.107	**0.000**	**0.007**	**0.000**
Wet Meadow Creek	20	0.522	0.527	3.7	2.7	-0.01	0.582	**0.000**	**0.006**	**0.001**
Silver Lake	39	0.399	0.392	3.6	2.3	0.02	0.745	**0.000**	0.077	**0.010**
Rifle Creek	28	0.534	0.467	3.9	2.8	0.13	0.000*	**0.000**	0.097	0.018
Willow Creek, Upper	37	0.510	0.475	4.9	2.8	0.07	0.042	**0.000**	0.211	0.047
Willow Creek, Lower	19	0.565	0.530	5.4	3.2	0.06	0.003	**0.000**	0.179	**0.015**
Sheep Creek	38	0.557	0.504	4.7	3.0	0.10	0.000*	**0.001**	0.077	**0.015**
Upper Soda Spring Creek 2002	29	0.338	0.282	2.3	1.9	0.17	0.001	**0.000**	**0.000**	**0.000**
Upper Soda Spring Creek 2001	16	0.435	0.360	3.5	2.5	0.18	0.000*	0.028	0.165	0.104
Upper Maggie	12	0.650	0.608	4.4	3.3	0.07	0.088	0.028	0.138	0.094
Mountaineer Creek, Upper	30	0.590	0.537	6.4	3.3	0.09	0.000*	0.165	0.924	0.577
Upper North Fork Clicks	38	0.592	0.562	4.0	3.0	0.05	0.020	**0.000**	**0.008**	**0.007**
Clicks Creek, upper	39	0.615	0.574	4.9	3.2	0.07	0.024	**0.000**	0.054	0.015
Fish Creek, Upper	10	0.509	0.460	3.3	2.7	0.10	0.064	**0.001**	**0.008**	**0.007**
Fish Creek	38	0.496	0.472	3.3	2.5	0.05	0.018	**0.000**	**0.000**	**0.000**
Coyote Creek, Upper	28	0.747	0.648	9.9	4.4	0.14	0.000*	0.032	0.972	0.906
North Fork American River	19	0.800	0.692	9.9	4.9	0.14	0.000*	0.126	0.999	0.972
North Fork Navarro River	28	0.857	0.793	12.5	5.4	0.08	0.000*	0.015	0.995	0.916
Hot Creek Strain	30	0.775	0.764	7.9	4.3	0.01	0.179	**0.000**	0.467	0.084
Mount Shasta Strain	24	0.731	0.665	7.1	3.9	0.09	0.001	**0.000**	0.511	0.104
Mount Whitney Strain	18	0.752	0.724	6.3	4.0	0.04	0.088	**0.001**	0.339	0.042

Table 3.5. Pairwise F_{ST} (below diagonal) and p-values (above diagonal) for pairwise comparisons of Little Kern golden trout populations. Non-significant values (5% nominal level) are indicated in boldface type.

	DDM95	DDM96	DDM97	UML	SSC95	USSC01	USSC02	UMT	CLN	CLK	FC	UFC	SC	LWC	UWC	RC	SL	WMC95	BCB	MWMC	UWMC	WMC	UCC	NFNR	NFAR	MWS	HCS	MSS
DDM95		**0.096**	0.000	**0.057**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DDM96	0.004		**0.112**	**0.589**	**0.003**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DDM97	0.063	0.047		**0.089**	**0.015**	**0.001**	**0.001**	**0.001**	**0.000**	0.000	**0.000**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.001**	**0.001**	**0.003**	**0.001**	**0.000**	0.000	**0.001**	**0.002**	**0.000**	**0.001**
UML	0.013	-0.005	0.058		**0.002**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SSC95	0.114	0.110	0.120	0.124		**0.003**	**0.001**	**0.001**	0.000	0.000	0.000	**0.001**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
USSC01	0.232	0.228	0.264	0.270	0.043		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
USSC02	0.299	0.304	0.348	0.356	0.097	0.058		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
UMT	0.162	0.174	0.171	0.175	0.064	0.131	0.180		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CLN	0.200	0.202	0.198	0.181	0.197	0.289	0.342	0.108		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CLK	0.187	0.196	0.194	0.179	0.162	0.233	0.295	0.074	0.049		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FC	0.299	0.310	0.307	0.295	0.322	0.401	0.456	0.197	0.111	0.069		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
UFC	0.273	0.285	0.288	0.274	0.290	0.391	0.468	0.158	0.060	0.037	0.045		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SC	0.260	0.259	0.259	0.274	0.224	0.283	0.337	0.180	0.251	0.225	0.315	0.298		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LWC	0.281	0.272	0.261	0.287	0.259	0.330	0.387	0.220	0.250	0.239	0.311	0.298	0.043		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
UWC	0.311	0.307	0.311	0.326	0.299	0.358	0.404	0.251	0.292	0.276	0.343	0.344	0.051	0.037		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RC	0.291	0.289	0.298	0.307	0.304	0.377	0.442	0.261	0.278	0.259	0.320	0.315	0.091	0.107	0.114		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SL	0.395	0.398	0.414	0.430	0.432	0.486	0.530	0.366	0.374	0.358	0.404	0.429	0.166	0.169	0.167	0.059		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
WMC95	0.250	0.267	0.307	0.297	0.251	0.372	0.441	0.256	0.310	0.282	0.400	0.376	0.267	0.331	0.349	0.324	0.437		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BCB	0.235	0.249	0.279	0.279	0.227	0.330	0.405	0.236	0.259	0.248	0.360	0.312	0.248	0.282	0.321	0.296	0.419	0.095		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MWMC	0.249	0.263	0.285	0.293	0.232	0.339	0.398	0.239	0.273	0.263	0.371	0.336	0.239	0.270	0.306	0.298	0.416	0.117	0.032		0.000	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000
UWMC	0.241	0.256	0.296	0.287	0.257	0.365	0.438	0.264	0.291	0.277	0.389	0.362	0.271	0.318	0.352	0.322	0.446	0.073	0.055	0.056		**0.002**	0.000	0.000	0.000	0.000	0.000	0.000
WMC	0.248	0.258	0.300	0.298	0.256	0.367	0.443	0.265	0.299	0.283	0.401	0.368	0.254	0.296	0.331	0.308	0.439	0.076	0.021	0.024	0.015		0.000	0.000	0.000	0.000	0.000	0.000
UCC	0.190	0.191	0.193	0.182	0.204	0.304	0.382	0.199	0.210	0.189	0.265	0.227	0.252	0.251	0.294	0.253	0.357	0.249	0.221	0.221	0.240	0.231		0.000	0.000	0.000	0.000	0.000
NFNR	0.181	0.180	0.178	0.180	0.196	0.277	0.356	0.200	0.225	0.202	0.271	0.225	0.224	0.219	0.259	0.235	0.333	0.241	0.200	0.210	0.228	0.213	0.106		0.000	0.000	0.000	0.000
NFAR	0.208	0.211	0.206	0.204	0.211	0.313	0.398	0.214	0.235	0.204	0.274	0.230	0.260	0.255	0.300	0.264	0.372	0.276	0.236	0.249	0.273	0.255	0.117	0.071		0.000	0.000	0.000
MWS	0.264	0.255	0.250	0.250	0.264	0.361	0.441	0.270	0.269	0.259	0.316	0.284	0.295	0.280	0.316	0.297	0.397	0.341	0.302	0.317	0.322	0.313	0.190	0.131	0.129		0.000	0.000
HCS	0.247	0.232	0.236	0.232	0.259	0.334	0.412	0.274	0.268	0.256	0.308	0.278	0.287	0.261	0.307	0.289	0.379	0.321	0.283	0.289	0.310	0.296	0.181	0.129	0.150	0.171		0.000
MSS	0.275	0.263	0.254	0.259	0.282	0.367	0.442	0.273	0.256	0.249	0.305	0.262	0.308	0.291	0.329	0.317	0.410	0.350	0.298	0.310	0.327	0.321	0.205	0.135	0.171	0.159	0.137	

Table 3.6. Summary of population clustering for additional levels of K from K=3-7. Populations assigned to group with the majority of the proportion of individuals clustered.

			K		
Population	K=3	K=4	K=5	K=6	K=7
DDMA	1	1	1	1	1
DDMB	1	1	1	1	1
DDMC	1	1	1	1	1
SSC95b	1	1	1	1	1
UM	1	1	1	1	1
UMT	1	1	1	1	1
USSC01	1	1	1	1	1
USSC02	1	1	1	1	1
CLK	1	1	5	5	5
CLN	1	1	5	5	5
FC	1	1	5	5	5
UFC	1	1	5	5	5
WMC95	2	2	2	2	2
BCB	2	2	2	2	2
UWMC	2	2	2	2	2
WMC	2	2	2	2	2
MWMC	2	2	2	2	2
LWC	2	4	4	4	4
RC	2	4	4	4	4
SC	2	4	4	4	4
SL	2	4	4	4	4
UWC	2	4	4	4	4
UCC	3	3	3	3	7
NFNR	3	3	3	3	3
NFAR	3	3	3	3	3
HCS	3	3	3	6	6
MSS	3	3	3	6	6
MWS	3	3	3	6	6

Table 3.7. Comparison of Little Kern golden trout inferred proportion of rainbow trout introgression for SNP and microsatellite data. Number of individuals (N) for SNP and microsatellites data sets and inferred proportion of membership in each cluster: rainbow trout (qRT), LKGT (qLK), LKGT cluster "a" (qLKa), and LKGT cluster "b" (qLKb). Locality codes as given in Table 3.1.

		microsatellite mean q values, K=3				SNP mean q values, K = 2		
Pop	Code	qRT	qLKa	qLKb	N	qRT	qLK	N
1	DDM95	0.00	0.01	0.99	38	0.01	0.99	38
2	DDM96	0.04	0.02	0.94	27	0.05	0.95	27
3	DDM97	0.02	0.01	0.98	8	0.01	0.99	11
4	SSC95	0.00	0.11	0.88	9	0.01	0.99	10
5	WMC95	0.00	0.96	0.04	29	0.02	0.98	29
6	LKBC	0.00	0.98	0.02	29	0.02	0.98	33
7	UWMC	0.00	0.99	0.01	27	0.01	0.99	40
8	MWMC	0.00	0.99	0.01	32	0.02	0.98	33
9	WMC	0.00	1.00	0.00	20	0.01	0.99	20
10	SL	0.01	0.99	0.00	39	0.02	0.99	36
11	SHT06	-	-	-	-	0.12	0.88	34
12	PST06	-	-	-	-	0.01	0.99	16
13	LKRC	-	-	-	-	0.01	0.99	29
14	RFC06	-	-	-	-	0.03	0.97	35
15	RC	0.01	0.98	0.02	28	0.01	0.99	30
16	TAM06	-	-	-	-	0.03	0.97	30
17	UWC	0.00	0.99	0.01	37	0.03	0.98	40
18	LWC	0.02	0.96	0.02	19	0.02	0.98	19
19	SC	0.00	0.99	0.01	38	0.03	0.97	39
20	LIO06	-	-	-	-	0.03	0.97	40
21	USSC02	0.00	0.00	1.00	29	0.01	0.99	40
22	USSC01	0.01	0.01	0.99	16	0.02	0.98	28
23	SSCPB	-	-	-	-	0.02	0.98	40
24	DDM06	-	-	-	-	0.01	0.99	30
25	DDM03	-	-	-	-	0.01	0.99	40
26	UML	0.01	0.01	0.98	12	0.01	0.99	14
27	ALP06	-	-	-	-	0.07	0.93	40
28	UMT	0.10	0.04	0.86	30	0.06	0.94	30
29	SMC06	-	-	-	-	0.10	0.90	40
30	SMC	-	-	-	-	0.04	0.97	21
31	LMC	-	-	-	-	0.01	0.99	37
32	JAC06	-	-	-	-	0.08	0.92	40
33	CLN	0.00	0.00	0.99	38	0.01	0.99	39
34	CLU	0.00	0.01	0.99	39	0.01	0.99	39
35	LKBRN06	-	-	-	-	0.30	0.70	8
36	LKBRN	-	-	-	-	0.14	0.86	36
37	UFC	0.03	0.04	0.94	10	0.03	0.97	11
38	FC	0.00	0.01	0.99	38	0.01	0.99	40
39	TMC06	-	-	-	-	0.01	0.99	19
40	UCC	0.92	0.05	0.03	28	0.17	0.83	30
41	LCC	-	-	-	-	0.02	0.98	40
42	NFAR	0.98	0.01	0.01	19	0.89	0.11	20
43	NFNR	0.99	0.00	0.00	30	0.99	0.01	30
44	HCS	0.96	0.03	0.01	28	0.98	0.02	31
45	MSS	0.99	0.00	0.00	24	0.99	0.01	30
46	MWS	0.99	0.00	0.00	18	0.77	0.23	31

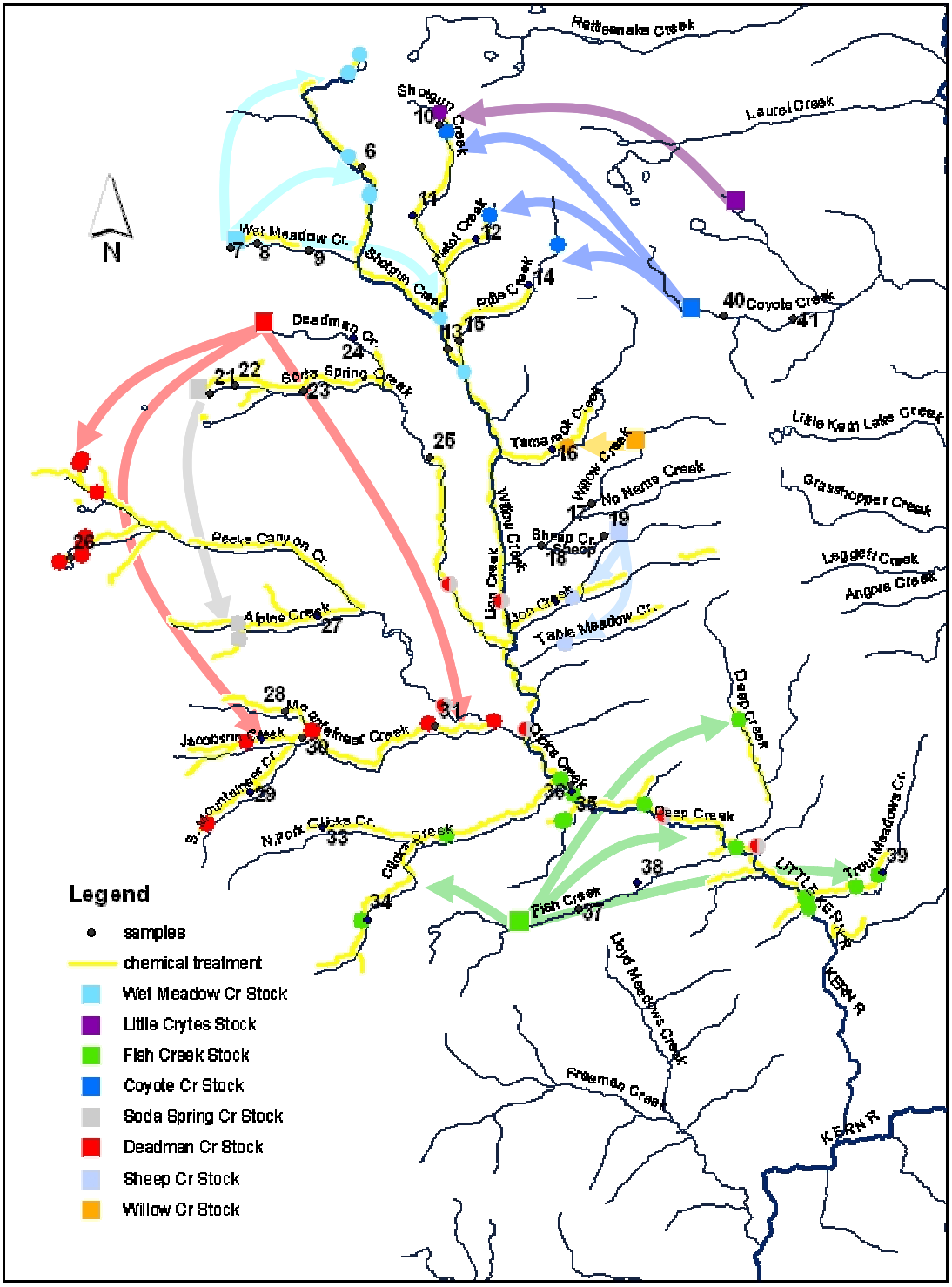


Figure 3.1. Chemical treatment, stocking history, and sample locations evaluated in the current study. Streams were treated from 1974-1995. Source populations for stocking efforts are represented by colored squares and recipient stocked localities represented by circles of the same color. Genetic sampling locations correspond with numbers given in Table 3.1, with 10 points not pictured: 1-5 identifying Little Kern golden trout broodstock populations and 42-46 identifying reference rainbow trout populations (see Table 3.1). Chemical treatments occurred between 1974 and 1995.

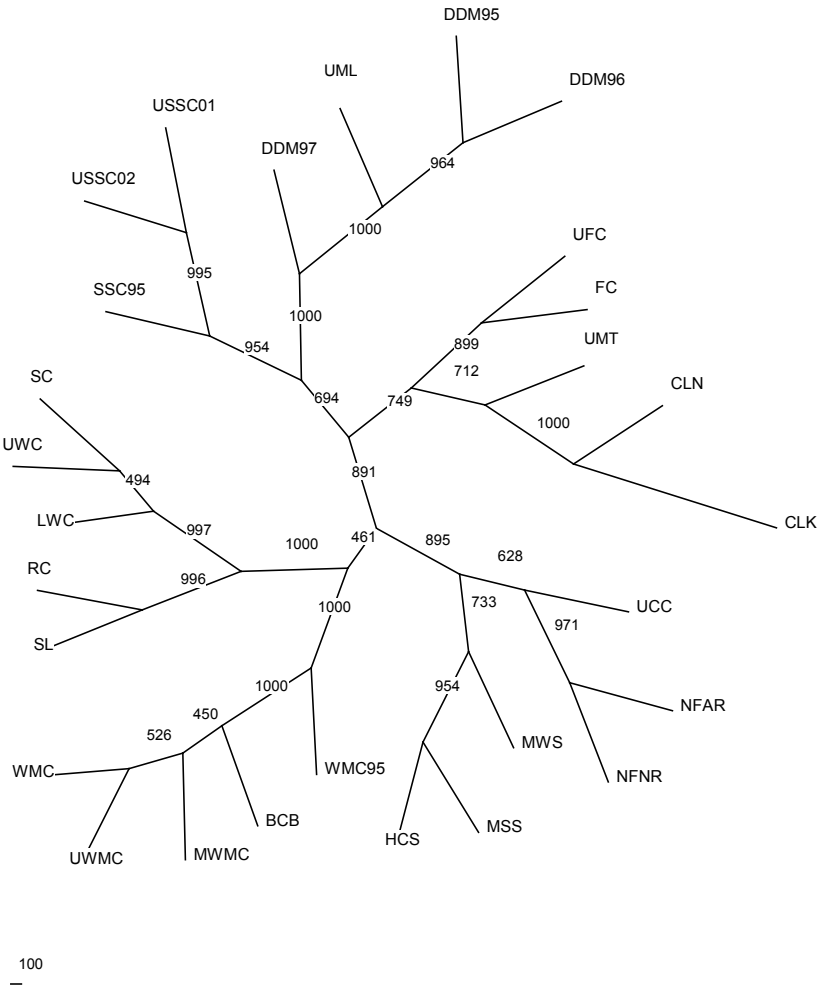


Figure 3.2. Unrooted UPGMA tree of genetic distances based on 15 microsatellite loci. Numbers at nodes represent support based on 1,000 bootstrapped pseudoreplicates. Population abbreviations as given in Table 3.1.

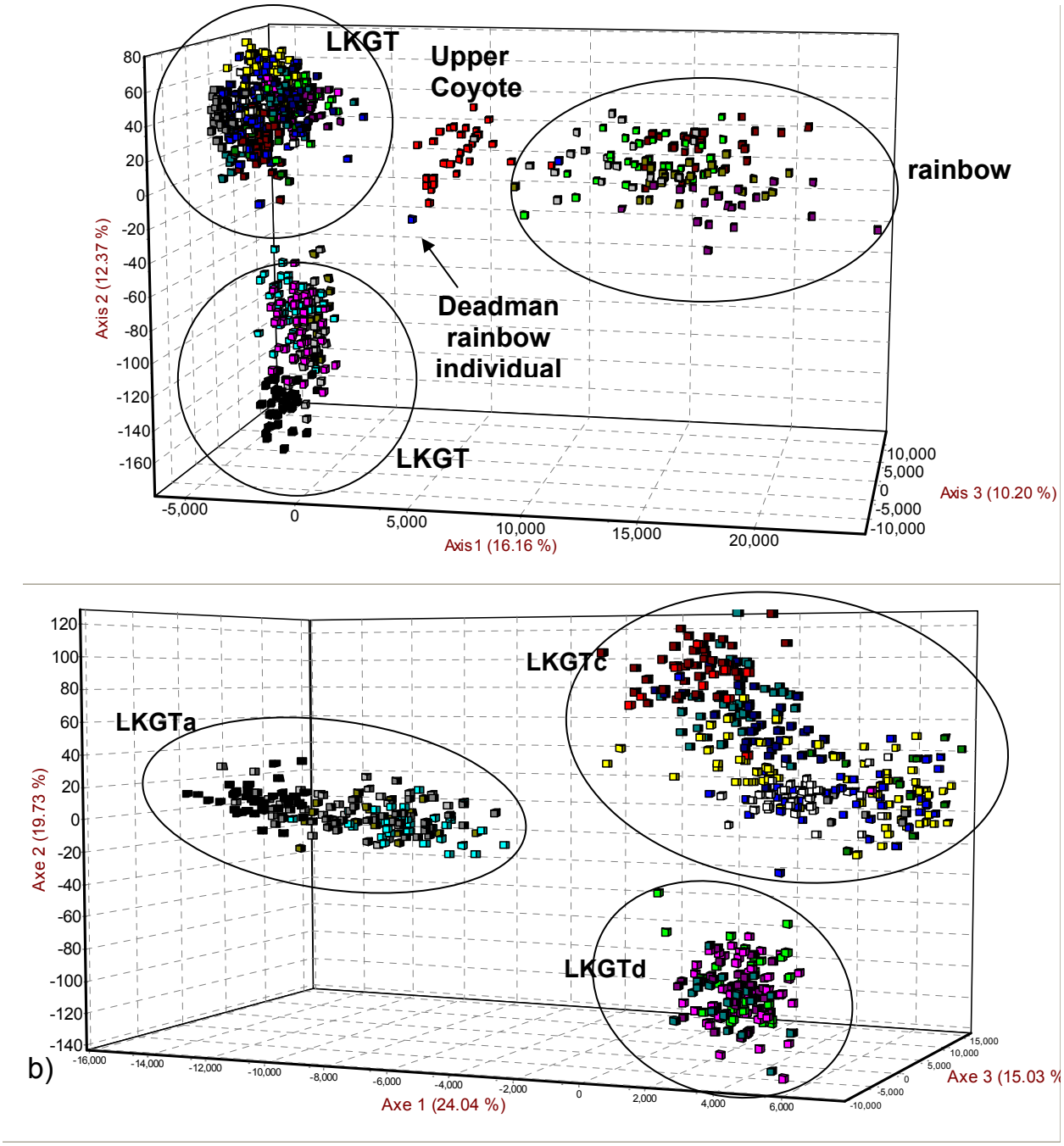


Figure 3.3. Individual-based FCA of microsatellite data for 3a) all Little Kern golden trout, Upper Coyote Creek trout, and rainbow trout reference samples. Red filled circles identify Upper Coyote Creek individuals. "LKGTa" group includes Little Kern golden trout from Lower Willow Creek, Upper Willow Creek, Rifle Creek, Sheep Creek, and Silver Lake. "LKGTb" group includes all other Little Kern golden trout populations. The "rainbow" group includes all rainbow trout populations sampled. Refer to text for discussion of other indicated groups. 3b) FCA for all Little Kern golden trout samples, excluding Coyote Creek population. "LKGTa" includes Little Kern golden trout from Lower Willow Creek, Upper Willow Creek, Rifle Creek, Sheep Creek, and Silver Lake. "LKGTc" includes WMC95, WMC, UWMC, MWMC, and BCB populations and "LKGTd" includes all remaining Little Kern golden trout populations.

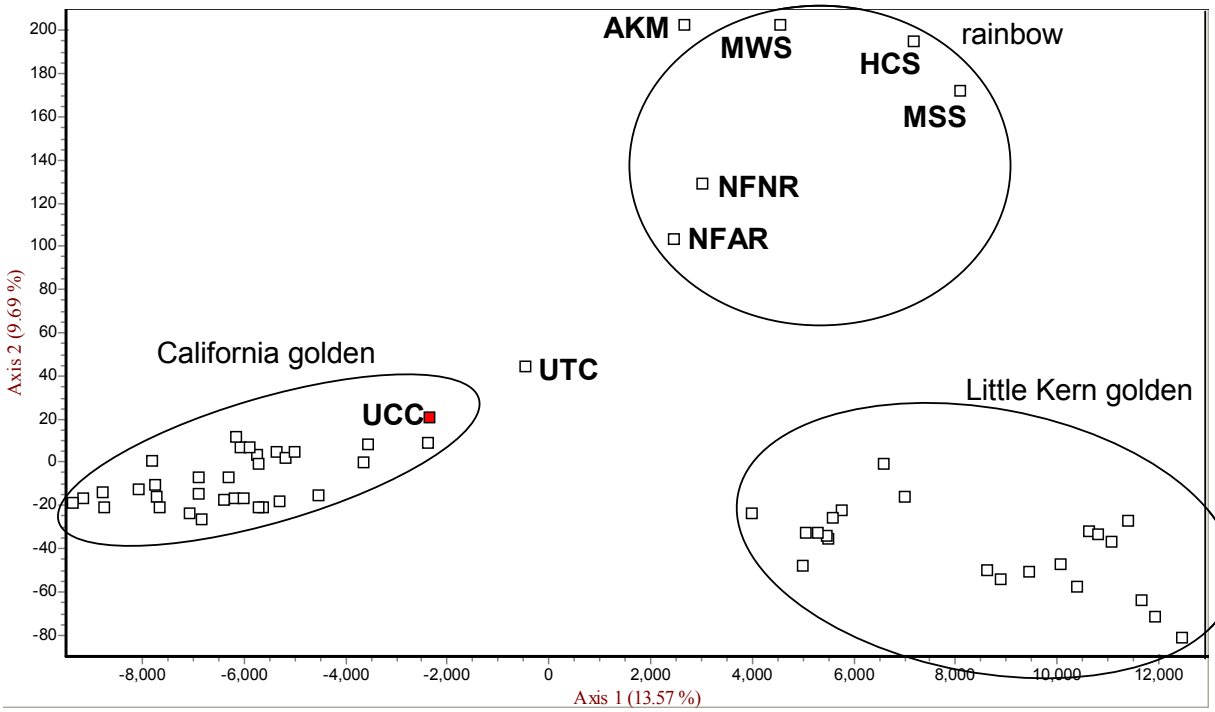


Figure 3.4. Population-based FCA of microsatellite data for 6 overlapping loci from previous study of California golden trout (Cordes et al. *in press*) and the current study. Three major groups are labeled: California golden trout, Little Kern golden trout, and "rainbow trout," which includes both hatchery and wild populations. Abbreviations as given in Table 3.1. AKM and UTC are California golden trout populations known to be heavily introgressed with introduced rainbow trout. The Upper Coyote Creek is designated UCC.

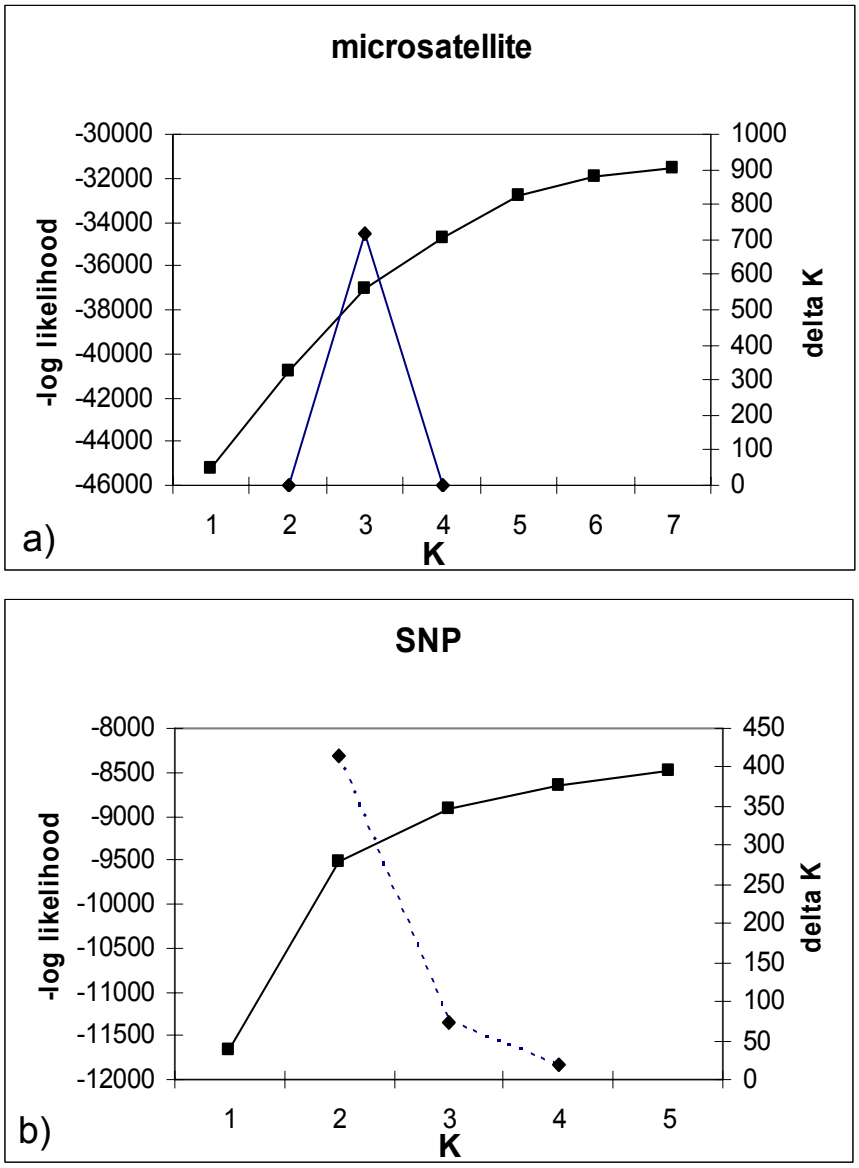


Figure 3.5. STRUCTURE results for clustering of rainbow and Little Kern individuals for microsatellite (5a) and SNP (5b) data sets. Negative log-likelihood values (solid lines) and delta K values (dashed) are plotted for given numbers of groups (K).

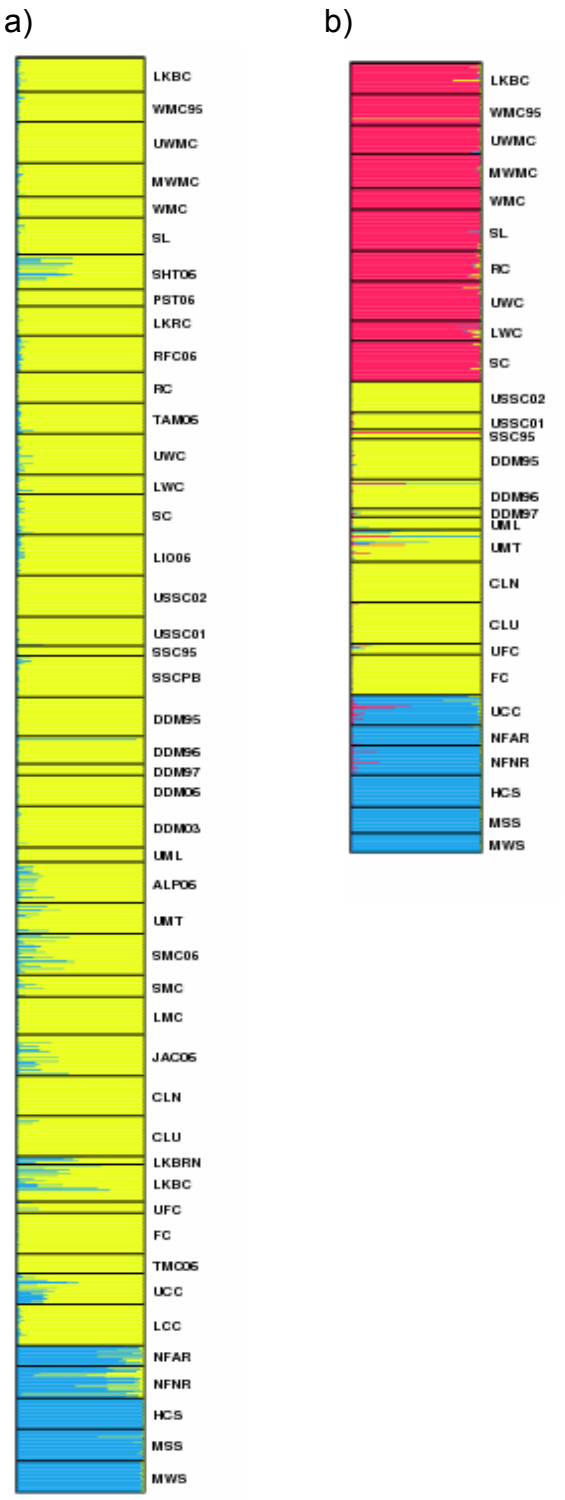


Figure 3.6. STRUCTURE analysis of LKGT SNP data (a), for which two genetic clusters were detected: blue represents rainbow trout and yellow represents golden trout. Each individual bar represents an individual fish, with the colors indicating the proportion of ancestry attributable to each cluster. Individuals are grouped by black bars denoting population affiliation (locality codes given in Table 3.1). Microsatellite data results (b) are given for comparison, with two genetic clusters of LKGT identified (shown in red and yellow) and the proportion of blue indicating the level of rainbow trout introgression in a given individual.

Appendix 3.1. Microsatellite allele frequencies at 18 loci used in the study of Little Kern golden trout and rainbow trout populations.

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
Locus:	1037																											
N	38	27	8	9	29	39	38	38	29	19	32	28	38	38	28	10	12	30	16	29	37	27	20	30	28	24	18	19
138	–	0.02	–	0.06	0.22	0.03	–	0.24	0.02	0.42	0.17	0.46	0.30	0.67	–	0.10	–	0.02	–	–	0.60	0.11	0.10	0.35	0.05	0.29	0.36	0.05
142	0.47	0.50	0.31	0.22	0.38	0.74	0.74	0.76	0.22	0.05	0.08	0.13	0.18	0.05	0.64	0.75	0.75	0.45	0.16	–	0.01	0.35	0.13	–	–	0.08	0.06	0.08
146	0.37	0.39	0.63	0.67	0.29	0.23	0.26	–	0.53	0.18	0.63	–	0.25	0.05	0.25	0.15	0.21	0.48	0.84	1.00	0.01	0.37	0.48	–	0.02	–	–	–
150	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	0.08
154	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
158	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	0.22	–	0.10	0.14	0.11
162	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.13
166	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
170	–	0.02	–	–	0.09	–	–	–	0.22	0.21	0.13	0.41	0.26	0.22	–	–	–	–	–	–	0.31	0.17	0.30	0.20	0.20	0.33	0.25	0.11
174	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	0.40
178	0.16	0.07	0.06	0.06	0.02	–	–	–	–	0.03	–	–	–	–	–	–	0.04	–	–	–	0.07	–	–	0.05	0.07	0.10	–	–
182	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	0.08	–	–
186	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.17	0.27	–	–	–
190	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	0.03	–
198	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.04	–	0.17	–
202	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
218	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
Locus:	1036																											
N	38	27	8	9	29	39	37	38	29	19	32	28	38	37	28	10	12	30	14	29	37	27	20	30	28	23	17	18
197	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–
201	–	–	–	–	–	–	–	–	0.24	–	0.25	0.07	–	–	–	–	–	–	–	–	–	0.28	0.23	–	–	–	–	–
205	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11
209	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	0.02	0.07	0.04	–	–
213	–	–	–	–	–	–	–	–	–	0.03	–	0.11	0.17	0.37	–	–	–	–	–	–	0.01	–	–	0.02	0.09	–	–	–
217	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–	–	–	–	–	–	–	–	–	0.07	–	–
221	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–	–	–	–	0.07	–	0.38	0.19
225	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	0.05	–	0.03	–	–	–	–	–	0.18	0.38	0.09	0.27	0.25
229	0.30	0.22	0.63	0.06	0.02	0.04	–	0.09	–	0.08	–	0.02	–	–	0.04	–	0.25	0.08	0.07	–	–	–	–	0.02	0.07	–	–	0.17
233	0.08	0.11	–	0.61	0.29	0.30	0.22	–	0.38	0.87	0.41	0.27	0.71	0.19	0.16	0.10	0.08	0.43	0.79	0.79	0.89	0.24	0.43	0.20	0.05	0.04	–	–
237	–	0.04	0.06	–	–	0.24	0.28	0.26	0.12	–	–	–	0.03	–	–	0.55	–	0.25	0.14	0.21	0.04	–	–	0.02	0.07	0.50	0.12	–
241	0.62	0.61	0.31	0.22	0.02	0.15	0.32	0.41	–	–	–	0.14	–	–	0.05	0.10	0.67	0.12	–	–	–	–	–	0.25	0.02	–	0.12	0.03
245	–	–	–	–	–	0.27	0.18	0.24	–	–	–	0.25	–	0.18	–	0.20	–	0.03	–	–	–	–	–	–	–	0.02	0.12	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
249	–	–	–	0.11	0.67	–	–	–	0.26	0.03	0.34	0.13	0.09	0.18	0.07	–	–	–	–	–	0.05	0.46	0.35	0.20	0.07	–	–	–
253	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	0.04	–	–	–	–	–	–	–	–	–	0.09	0.02	–	0.19
257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
261	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	0.02	–	–
265	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
269	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	0.03
273	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–	–	–	–	–	–	–	–
277	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
281	–	–	–	–	–	–	–	–	–	–	–	0.02	–	0.03	0.02	–	–	–	–	–	–	–	–	0.10	0.02	0.20	–	–
285	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–
297	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	0.03
Locus:	1089																											
N	38	27	8	9	29	39	38	37	29	19	32	28	38	38	28	10	12	30	16	29	37	26	20	30	28	24	18	19
87	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	0.02	–	–
91	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–
95	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–
99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.33	0.04	0.10	0.19	0.11
103	–	–	–	–	–	0.06	–	0.22	–	–	–	–	–	–	0.23	–	–	–	–	–	0.10	–	–	0.02	–	–	–	0.05
107	0.45	0.50	0.31	0.22	0.03	–	–	–	–	–	–	–	–	–	0.23	–	0.38	0.02	0.13	–	–	–	–	–	0.05	–	–	0.03
111	0.07	0.06	0.06	0.06	0.07	0.04	0.01	0.01	0.24	–	0.11	–	0.03	–	–	0.10	–	0.02	0.19	0.33	–	0.31	0.20	–	0.05	–	–	0.03
115	–	0.02	–	0.11	0.50	0.60	0.70	0.62	0.55	1.00	0.69	1.00	0.97	0.99	0.05	0.70	–	0.52	0.09	0.19	0.91	0.39	0.60	–	0.04	–	–	–
119	0.36	0.32	0.50	0.56	–	0.30	0.29	0.14	–	–	–	–	–	–	0.16	0.20	0.42	0.30	0.59	0.48	–	–	–	–	–	–	–	0.03
123	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	0.03	–	–	–	0.05
127	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	–	–	0.08	–	–	–	–	–	0.07	0.02	–	–	0.03
131	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.07	–	–	–	–	–	0.05	–	–	0.14	0.03
135	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.38	–	0.26
139	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08
143	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	0.39	0.03
147	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.15	–	–
151	0.13	0.11	0.13	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	–	–	–	–	–	–	–	0.05	0.06	0.03	0.03
155	–	–	–	–	–	–	–	–	0.07	–	0.09	–	–	–	0.02	–	–	–	–	–	–	0.12	–	–	0.02	0.27	–	0.03
159	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.30	–	–	0.16
163	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
167	–	–	–	0.06	0.40	–	–	–	0.14	–	0.11	–	–	–	0.04	–	–	–	–	–	–	0.19	0.20	0.23	–	0.02	0.22	0.08
171	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.27	–	–	–	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
179	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
183	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–
Locus:	85																											
N	38	27	7	8	28	39	37	36	29	19	31	26	38	39	28	10	12	30	15	26	37	18	20	29	28	23	18	19
127	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–	–	–	–	–	–	–
135	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	0.17	–
139	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	–	–	0.03
143	0.28	0.37	0.50	0.44	0.34	0.14	0.20	–	0.12	0.03	0.03	–	–	–	–	–	0.21	0.40	0.37	0.29	0.03	0.06	0.10	0.02	–	–	–	0.03
147	0.16	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.02	–	–	0.08	–	–	–	–	–	0.17	0.05
151	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18	–	–	–	–	–	–	–	–	–	–	0.11	–	0.18
155	–	–	–	–	–	–	–	–	–	0.24	–	0.10	0.08	–	–	–	–	–	–	–	0.04	–	–	–	–	0.04	0.25	0.32
159	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	0.03	–	0.02	–	–	0.16
163	0.08	0.15	–	0.06	0.02	–	–	–	–	–	–	0.17	–	–	0.36	–	0.25	–	–	–	–	–	–	–	0.11	–	–	–
167	0.13	0.06	–	0.06	–	–	–	–	–	0.11	–	0.65	0.20	0.73	0.04	–	0.13	0.03	0.10	–	0.30	–	–	0.03	0.09	–	0.03	0.11
171	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.05	–	–	0.07	–	–	–	–	–	–	0.02	–	0.06	–
175	–	–	–	–	0.05	–	–	–	0.26	–	0.18	–	0.08	–	–	–	–	0.02	–	–	0.15	0.03	0.10	–	0.09	0.13	–	0.05
179	–	–	–	0.38	–	0.24	0.11	0.25	0.09	0.03	0.32	–	0.09	–	–	0.10	0.08	0.17	0.50	0.71	–	0.22	0.10	–	–	–	–	–
183	0.09	0.07	0.43	0.06	–	0.27	0.12	0.42	–	–	–	–	–	–	0.02	0.35	0.08	0.08	–	–	–	–	–	–	0.02	–	0.03	–
187	–	–	–	–	–	0.35	0.47	0.33	–	0.03	–	0.06	0.04	0.09	–	0.55	–	0.12	0.03	–	–	–	–	–	–	–	–	–
191	–	–	–	–	–	–	0.10	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	0.02	0.05	–	–	0.03
195	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–	–	–	0.09	0.02	–	–	–
199	–	–	–	–	0.09	–	–	–	0.07	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.13	–	–	–
203	–	0.02	–	–	0.29	–	–	–	0.36	–	0.23	–	–	–	–	–	–	–	–	–	–	0.25	0.35	0.03	0.13	–	–	–
207	–	–	–	–	0.14	–	–	–	0.03	–	–	–	–	–	0.04	–	–	–	–	–	–	0.22	0.10	–	0.02	–	–	–
211	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–	–	–	0.21	0.04	0.15	0.08	0.05
215	–	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	0.05	0.04	0.52	–	–
219	–	–	–	–	–	–	–	–	–	0.11	–	–	0.04	–	0.04	–	–	0.02	–	–	0.16	–	–	–	0.02	–	–	–
223	0.09	0.11	–	–	–	–	–	–	–	0.47	–	–	0.21	0.10	0.02	–	0.04	0.02	–	–	0.15	–	0.03	0.36	–	–	–	–
227	–	–	–	–	–	–	–	–	0.02	–	0.13	–	–	–	–	–	–	–	–	–	–	0.11	0.10	0.17	–	–	–	–
231	–	0.02	–	–	–	–	–	–	–	–	–	0.02	0.04	–	0.02	–	–	–	–	–	–	–	0.03	0.02	–	0.04	0.22	–
235	–	–	–	–	0.07	–	–	–	0.05	–	0.03	–	0.22	0.04	–	–	–	–	–	–	0.04	0.11	0.08	–	–	–	–	–
249	0.17	0.15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.17	–	–	–	–	–	–	–	–	–	–	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
Locus:	1322																											
N	38	27	8	9	28	39	37	38	29	19	32	28	38	39	28	10	11	30	16	28	37	27	20	29	28	23	18	19
169	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.26	–	–	–	0.03
173	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03
181	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–
185	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	0.25	0.05
189	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.33	–	–
193	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	–	–	–	–	–	–	–	–	0.09	–	–	–	0.03
197	–	–	–	–	–	–	–	–	–	0.03	–	–	0.04	–	0.04	–	–	–	–	–	–	–	–	0.03	0.04	–	–	–
201	0.47	0.56	0.50	0.22	0.68	0.17	0.23	–	0.67	0.03	0.83	0.13	–	–	0.36	–	0.50	0.02	0.03	–	–	0.96	0.98	0.09	0.11	–	–	–
205	0.53	0.43	0.50	0.78	0.32	0.83	0.76	1.00	0.19	0.76	0.17	0.88	0.83	1.00	0.39	0.95	0.46	0.90	0.97	1.00	0.96	0.04	0.03	0.12	0.11	–	0.19	0.37
209	–	–	–	–	–	–	0.01	–	0.14	0.11	–	–	0.13	–	–	0.05	–	–	–	–	0.04	–	–	0.02	0.20	0.22	–	–
213	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	0.21
217	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	0.04	–	–	0.05
221	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.09	0.20	–	0.03
225	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
229	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	0.26	0.14	–
237	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	0.11	–	0.08	0.18
241	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–
245	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–
249	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	–	–	–	–
253	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	0.19	0.03
257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–
365	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–
369	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	–
Locus:	423																											
N	38	27	8	9	28	39	38	37	27	19	30	28	38	37	27	10	12	30	16	25	37	16	20	29	28	23	18	18
79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.19	0.02	–	–	–
83	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.04	0.04	–	0.03
87	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	0.08
91	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	0.04	–	–
95	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.47	0.11	0.04	–	–
99	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	0.26	0.36	0.17
103	–	–	–	–	0.14	–	–	–	0.24	0.11	0.55	–	0.03	–	0.48	–	–	–	–	–	–	0.38	0.33	–	0.21	0.04	–	0.22
107	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	0.17	–	–	–	–	–	0.05	–	–	0.12	0.02	–	–	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
111	–	–	0.06	–	–	0.03	–	0.16	–	0.03	–	0.13	–	–	–	–	–	–	–	–	–	–	–	0.03	0.14	0.07	–	–
115	0.04	0.11	0.13	0.06	0.02	–	–	–	–	0.37	–	0.25	0.26	0.53	–	–	0.21	0.05	0.03	0.06	0.49	–	–	–	0.02	0.07	0.17	0.06
119	0.57	0.46	0.25	0.78	0.82	0.31	0.16	0.01	0.72	0.05	0.40	0.11	0.21	–	–	0.20	0.46	0.45	0.81	0.92	0.03	0.56	0.63	–	0.04	–	–	0.17
123	0.08	0.15	0.06	0.17	–	–	–	–	–	–	–	–	0.01	–	–	–	0.04	0.02	0.09	–	–	–	–	0.02	0.14	0.04	–	0.14
127	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.04	0.36	0.06
131	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.03
135	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.05	–	–	–
139	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–
143	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	0.02	–	–	–	–	–	–	0.02	–	–	–
147	–	–	–	–	–	–	–	–	0.02	0.03	0.05	0.23	0.17	0.01	0.02	–	–	0.05	–	–	0.39	0.06	0.05	–	0.04	–	–	–
151	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
155	–	–	–	–	–	–	–	–	–	0.21	–	0.25	0.18	0.39	0.04	–	–	–	–	–	0.04	–	–	–	0.07	–	–	–
159	–	–	–	–	–	–	–	–	–	0.08	–	0.04	–	0.05	0.09	–	–	–	–	–	–	–	–	–	0.02	–	0.11	–
163	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
167	–	–	–	–	–	–	–	–	–	0.03	–	–	0.04	–	0.06	–	–	–	–	–	–	–	–	–	0.02	–	–	–
171	–	–	–	–	–	–	0.01	–	–	–	–	–	0.09	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
175	0.09	0.06	0.25	–	–	0.08	0.13	–	–	–	–	–	–	–	–	–	0.13	0.02	–	–	–	–	–	–	–	–	–	–
179	0.15	0.15	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	0.04	0.03	–	0.02	–	–	–	–	–	–	–	–
183	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.15	–	0.05	–	–	–	–	–	–	–	–	–	0.06
187	0.08	0.06	0.25	–	–	0.05	0.17	0.04	–	–	–	–	–	–	–	0.05	0.13	0.03	–	–	–	–	–	–	–	0.02	–	–
191	–	–	–	–	–	0.04	0.11	0.22	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–	–	–	–	0.04	–	–
195	–	–	–	–	–	0.31	0.13	0.45	–	–	–	–	–	–	–	0.20	–	0.12	–	–	–	–	–	–	–	0.20	–	–
199	–	–	–	–	–	0.09	–	0.10	–	–	–	–	–	–	–	0.15	–	0.03	–	–	–	–	–	–	–	–	–	–
203	–	–	–	–	–	0.10	0.29	0.03	–	–	–	–	–	–	–	0.25	–	0.03	0.06	–	–	–	–	–	–	0.02	–	–
Locus:	1082																											
N	38	27	8	9	29	39	38	38	29	19	32	27	38	39	26	10	12	30	16	29	37	27	20	30	28	24	18	17
176	0.34	0.22	0.38	0.33	0.14	0.64	0.88	0.84	0.28	0.47	0.38	0.13	0.24	–	0.10	1.00	0.42	0.55	0.06	0.36	0.34	0.28	0.18	0.08	0.02	0.35	0.03	–
180	–	0.04	–	–	–	–	–	–	–	–	–	0.20	–	–	0.42	–	–	–	–	–	–	–	–	0.28	–	0.04	0.36	0.09
184	0.36	0.41	0.50	0.17	–	0.03	–	–	0.09	0.11	0.14	–	0.21	–	0.06	–	0.33	0.15	–	–	0.19	–	0.15	0.08	0.04	0.25	0.03	0.09
188	0.08	0.09	0.06	–	–	–	–	–	–	–	–	–	–	–	0.10	–	0.04	–	–	–	–	–	–	0.33	0.02	0.04	0.03	0.47
192	0.22	0.24	0.06	0.50	0.64	0.31	0.12	–	0.62	0.13	0.42	0.17	0.34	0.10	0.02	–	0.21	0.30	0.94	0.64	0.15	0.72	0.63	–	0.07	–	0.03	–
196	–	–	–	–	0.22	0.03	–	0.16	0.02	0.29	0.06	0.50	0.21	0.90	0.23	–	–	–	–	–	0.32	–	0.05	0.10	0.05	–	–	0.03
200	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	0.11	–	0.33	0.12
204	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.11	–	0.11	0.09
208	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.04	0.02	–	0.03

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
212	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	0.07	0.29	–	0.06
216	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.23	–	–	0.03
220	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–
224	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	–
232	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.03	–
236	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18	–	–	–
240	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
Locus:	1009																											
N	38	27	8	9	29	36	31	31	29	19	32	26	38	39	28	10	12	30	16	29	37	27	20	30	28	24	18	17
162	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	–	0.27	–	–
166	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.23	–	0.44	0.44	0.06
170	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–	–	–	–	–	–	–	–
174	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	0.22	–
178	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–
186	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	0.06	0.15
190	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.08	–
194	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	0.06
198	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	0.02	–	–	–
202	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	0.07	–	–	–
206	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–	–
210	–	0.02	–	–	–	–	–	–	–	–	0.02	–	–	–	0.02	–	–	–	–	–	–	–	–	0.10	0.02	0.02	–	–
214	–	0.04	–	–	–	–	–	–	0.21	0.13	0.16	0.08	–	0.04	–	–	–	–	0.13	–	0.05	0.06	0.18	0.20	0.05	0.08	–	–
218	0.75	0.67	0.69	0.61	0.83	0.31	0.29	0.03	0.26	0.21	0.45	0.14	0.66	0.10	0.11	–	0.63	0.77	0.69	0.72	0.39	0.63	0.50	–	0.21	–	–	–
222	–	0.02	–	–	–	–	–	–	0.02	0.42	–	0.23	0.24	0.31	0.05	–	–	0.03	–	–	0.38	–	–	0.02	0.16	–	–	–
226	0.25	0.26	0.25	0.06	–	–	–	–	–	–	–	0.08	–	–	0.18	–	0.38	–	–	–	0.04	–	–	0.05	–	–	–	–
230	–	–	–	0.33	–	0.01	–	–	–	0.03	–	–	–	–	0.07	–	–	0.02	0.03	0.28	–	–	–	–	–	–	–	0.21
234	–	–	–	–	–	0.07	–	0.36	–	–	–	–	–	–	0.14	–	–	–	–	–	–	–	–	0.12	–	–	0.19	0.15
238	–	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	0.02	–	–	0.24
242	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.11	0.10	–	–
246	–	–	–	–	0.17	0.13	0.47	0.16	0.52	–	0.38	–	–	–	–	0.40	–	0.05	–	–	0.03	0.32	0.33	0.03	–	–	–	–
250	–	–	–	–	–	0.44	0.16	0.45	–	–	–	0.02	–	–	–	0.45	–	–	0.03	–	–	–	–	0.08	–	–	–	0.03
254	–	–	–	–	–	0.01	–	–	–	0.18	–	0.46	0.11	0.55	–	0.15	–	–	–	–	0.05	–	–	–	0.02	–	–	0.09
258	–	–	–	–	–	0.03	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
262	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
266	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	0.02	–	–	0.03

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
270	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
274	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.02	–	–	–
278	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.20	–	–	–	–	–	–	–	–	–	–	–	–	–
282	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	–
286	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
Locus:	1046																											
N	38	27	7	9	29	39	38	38	29	19	31	28	38	36	28	10	11	30	15	29	37	27	20	30	28	24	17	19
96	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	
100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	
104	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	0.52	0.20	–	–	–
108	–	–	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	0.07	0.05	0.08	0.06	0.03
112	–	–	–	–	–	–	–	–	–	0.63	–	0.09	0.51	0.39	–	–	–	–	–	–	0.54	–	–	0.05	0.05	0.08	–	0.03
116	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	0.16	0.06	–	–
120	–	–	–	–	–	–	–	–	–	–	–	0.18	–	–	–	–	–	0.02	–	–	–	–	–	0.22	–	0.52	0.18	0.03
124	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	0.05	–	0.24	0.03
128	–	–	–	–	–	–	–	–	–	–	–	0.27	–	0.17	–	–	–	–	–	–	–	–	–	0.12	0.02	0.23	0.09	0.16
132	0.04	0.04	–	0.06	0.64	0.51	0.50	0.90	0.31	0.03	0.23	0.05	0.05	0.04	0.02	0.60	–	0.23	0.13	–	0.03	0.39	0.25	–	0.07	–	0.03	0.29
136	0.01	0.06	0.07	0.67	0.10	0.39	0.30	0.09	0.22	0.21	0.34	–	0.21	0.14	0.16	0.25	0.05	0.52	0.73	0.98	0.32	0.17	0.23	–	0.04	0.02	0.41	0.16
140	0.57	0.56	0.71	0.22	0.22	0.03	–	–	0.47	0.11	0.44	0.11	0.22	0.26	0.11	–	0.41	0.17	–	0.02	0.11	0.44	0.53	–	0.04	–	–	–
144	0.38	0.32	0.14	0.06	0.03	0.08	0.20	–	–	0.03	–	–	–	–	0.30	–	0.55	0.05	0.13	–	–	–	–	–	0.02	–	–	0.21
148	–	0.02	0.07	–	–	–	–	–	–	–	–	0.20	–	–	0.09	–	–	–	–	–	–	–	–	–	0.09	–	–	–
152	–	0.02	–	–	–	–	–	0.01	–	–	–	–	–	–	0.07	0.10	–	–	–	–	–	–	–	–	0.07	–	–	0.03
156	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	0.05
160	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	–	–	–	–	–	–	–	–	–	0.05	–	–	–
Locus:	1078																											
N	38	27	6	9	29	39	38	37	27	19	30	27	38	38	27	10	11	27	15	28	37	24	20	30	28	24	17	19
198	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
202	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.29	–
206	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
210	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.20	–	–	–
214	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.32	–	–	0.04	–	–	–	–	–	–	0.14	–	–	0.03
222	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.05	0.13	0.09	0.08
226	0.01	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	0.02	–	–	0.02	–	–	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
230	–	0.04	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	0.14	0.02	–	0.03
234	–	–	–	–	–	0.14	0.01	0.01	–	0.18	–	0.63	0.36	0.58	–	0.05	–	0.09	–	–	0.19	–	–	–	–	–	–	0.18
238	0.25	0.32	0.33	–	–	0.40	0.15	0.45	0.02	–	–	–	0.03	–	0.02	0.35	0.36	0.04	–	–	–	–	–	0.25	0.04	0.08	0.03	0.05
242	0.20	0.24	–	0.39	0.02	0.21	0.05	–	0.13	0.32	0.37	0.15	0.24	–	–	0.05	0.09	0.50	0.73	0.86	0.35	0.10	0.23	0.08	0.11	–	–	0.11
246	0.12	0.19	0.42	0.33	0.57	0.14	0.47	0.43	0.59	–	0.43	0.04	0.05	–	–	0.30	0.27	0.22	–	–	–	0.60	0.53	0.08	0.02	0.27	0.47	0.13
250	–	0.02	–	0.06	0.02	0.08	0.21	0.10	0.13	0.05	0.02	–	–	–	–	0.15	–	0.07	0.27	0.04	–	–	0.03	0.38	0.05	0.19	0.03	0.08
254	–	–	–	–	–	0.04	0.11	–	–	0.05	0.02	0.02	0.16	0.08	0.15	0.05	–	0.04	–	–	0.10	–	–	0.02	0.02	0.31	–	0.05
258	–	–	–	–	–	–	–	–	–	0.24	–	0.17	0.12	0.33	0.17	–	–	–	–	–	0.26	–	–	–	–	–	0.06	–
262	–	–	–	–	0.03	–	–	–	0.06	–	0.02	–	–	0.01	0.06	–	–	–	–	–	–	0.04	0.05	–	0.02	–	–	0.03
266	–	–	–	–	–	–	–	–	–	0.05	–	–	0.01	–	0.09	–	–	–	–	–	0.10	–	–	0.02	–	–	0.03	0.08
270	–	–	–	–	0.36	–	–	–	0.07	–	0.15	–	–	–	0.02	–	–	–	–	–	–	0.23	0.18	–	–	–	–	–
274	–	–	–	–	–	–	–	0.01	–	0.03	–	–	–	–	0.04	0.05	–	–	–	–	0.01	–	–	–	0.04	–	–	–
278	0.25	0.13	0.17	0.11	–	–	–	–	–	–	–	–	–	–	–	–	0.18	–	–	–	–	–	–	–	–	–	–	–
282	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11
286	0.16	0.07	0.08	–	–	–	–	–	–	–	–	–	–	–	0.07	–	0.09	–	–	–	–	–	–	0.10	0.04	–	–	–
290	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
306	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
318	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
326	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.02	–	–	–
338	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
354	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–
398	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
Locus:	1051																											
N	38	27	8	9	29	39	38	38	26	19	30	27	38	37	28	10	11	28	15	25	36	25	20	30	28	24	16	19
220	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.32	–	–	–	–	–	–	–	–	–	0.05	–	–	–
224	0.30	0.46	0.13	0.72	0.02	0.19	0.24	–	–	0.05	–	0.11	0.21	–	–	–	0.50	0.50	0.77	0.82	0.03	–	–	–	–	–	–	–
228	–	–	–	–	–	–	–	–	–	–	–	0.06	0.13	0.05	–	–	–	–	–	–	0.24	–	–	–	0.05	–	–	–
232	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–	0.14	–	0.09	0.08
236	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.02	–	–	–	–	–	–	–	0.19	0.13	0.03
240	–	–	–	–	–	0.22	0.37	0.34	–	–	–	–	–	–	–	0.35	–	0.05	0.07	–	–	–	–	0.12	0.04	0.02	0.03	0.03
244	–	0.02	–	0.11	0.19	–	–	–	0.10	–	0.23	–	–	–	0.23	0.05	0.05	–	–	–	–	0.04	0.08	0.55	0.02	0.52	–	0.08
248	0.13	0.09	0.44	–	0.02	0.08	–	0.09	–	0.68	–	0.54	0.63	0.74	0.04	–	0.14	–	–	–	0.69	–	–	–	0.14	0.04	0.09	0.05
252	0.03	–	0.19	0.06	0.47	0.46	0.22	0.57	0.25	0.11	0.13	0.06	0.03	–	0.04	0.50	0.09	0.30	0.07	0.18	0.01	0.26	0.25	–	0.02	–	–	0.26
256	0.54	0.43	0.25	0.11	0.31	0.05	0.17	–	0.65	0.16	0.60	0.24	–	0.08	–	–	0.23	0.04	0.10	–	0.01	0.70	0.68	–	0.18	–	–	0.11
260	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–	0.32

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
264	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–	–	0.01	–	–	–	0.05	–	–	–
266	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
268	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	0.30	0.02	–	0.09	–
272	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–	–	–	–	–	–	–	0.13	0.02	–	–
276	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	0.04	0.17	–	–
280	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	0.02	0.03	–
288	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
292	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	0.02	0.38	–
296	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
308	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	0.02	–	–	–	–
312	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
316	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	–
320	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
324	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–	–
328	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
332	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
340	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–

Locus:	249																											
N	38	27	8	9	29	39	38	38	26	19	31	28	38	39	28	10	11	30	15	28	37	27	20	30	28	24	17	19
131	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03
139	0.26	0.17	–	–	–	0.01	0.01	–	–	–	–	–	–	–	–	–	0.18	–	–	0.11	0.07	–	–	–	0.23	–	–	0.03
143	–	0.04	0.06	–	–	–	–	–	–	0.08	–	–	0.07	–	–	–	–	–	0.03	–	0.05	–	–	–	0.05	0.10	–	–
147	0.28	0.30	0.31	0.50	0.02	0.45	0.21	0.49	0.12	–	0.08	–	–	–	0.02	0.45	0.23	0.35	0.60	0.38	0.07	0.04	–	0.18	0.05	0.19	–	0.05
151	0.11	0.19	0.31	0.28	–	0.47	0.68	0.51	–	0.42	–	0.43	0.32	0.68	0.16	0.50	0.27	0.52	0.20	0.48	0.54	–	–	0.07	–	0.38	0.27	0.03
155	0.30	0.19	0.06	0.06	0.02	0.06	0.09	–	0.15	0.05	0.24	–	–	–	–	–	0.23	–	–	–	0.07	0.15	0.03	0.28	0.11	0.17	–	0.05
159	0.04	0.07	0.13	–	–	–	–	–	0.04	0.13	–	0.39	0.20	0.22	0.23	–	–	–	–	–	0.07	–	–	0.15	0.23	–	–	0.05
163	–	–	–	–	–	–	–	–	–	0.26	–	0.18	0.33	0.10	0.14	–	–	0.03	0.13	0.04	0.14	–	–	0.08	0.14	–	0.15	0.24
167	0.01	0.02	0.13	0.06	0.40	–	–	–	0.35	–	0.27	–	–	–	–	–	0.09	0.03	0.03	–	–	0.11	0.15	–	0.13	–	–	0.11
171	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	0.07	–	–	–	–	–	–	–	–	0.03	0.21
175	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	0.05	0.02	0.15	0.08
179	–	0.02	–	0.11	0.47	–	–	–	0.14	–	0.21	–	–	–	0.34	–	–	–	–	–	–	0.52	0.43	–	–	–	–	–
183	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	0.02	–	–	–	–
187	–	–	–	–	0.10	–	–	–	0.21	0.05	0.19	–	0.09	–	0.02	–	–	–	–	–	–	0.19	0.40	0.07	–	0.06	0.35	0.05
191	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.03	0.08
199	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.15	–	0.04	0.03	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
Locus:	1088																											
N	38	27	6	9	26	38	38	34	28	19	31	24	38	39	26	10	12	26	13	22	37	26	20	30	28	23	18	19
107	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
111	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	0.05
115	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	0.32	0.02	0.02	0.11	0.34
119	–	–	–	–	0.02	–	–	–	–	0.08	–	0.19	0.04	–	0.12	–	–	–	0.23	0.02	0.04	–	–	0.17	–	0.22	0.08	0.03
123	–	0.02	–	0.11	0.94	–	–	–	0.89	0.74	0.97	0.67	0.87	0.68	0.04	–	–	0.08	–	–	0.88	0.94	0.95	0.12	0.11	0.11	–	0.05
127	–	–	–	–	–	0.30	0.20	0.59	–	0.08	–	–	0.01	0.05	0.15	0.55	–	0.29	0.08	–	–	–	–	–	0.32	–	–	0.13
131	–	–	–	–	–	0.11	0.07	0.25	–	–	–	–	–	–	0.02	–	–	0.04	–	–	–	–	–	0.13	0.27	0.41	0.19	0.13
135	0.32	0.33	0.42	0.11	0.04	0.15	0.12	–	–	–	–	–	–	–	0.06	–	0.21	0.08	–	–	–	–	–	–	–	0.02	0.06	–
139	0.17	0.15	0.08	0.17	–	0.11	0.03	–	–	0.03	–	–	–	–	–	–	0.04	0.04	0.27	0.57	0.01	–	–	–	0.02	–	0.03	0.03
143	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	0.21	–	0.08	0.02	–	–	0.03	–	–	0.05	0.13	0.04	–	0.05
147	–	0.06	0.33	–	–	–	–	–	–	0.03	–	–	0.03	–	–	0.15	0.04	–	–	–	–	–	–	0.20	0.02	0.07	–	0.08
151	0.42	0.35	0.17	0.06	–	0.16	0.25	0.13	0.11	–	0.03	–	–	–	0.10	0.30	0.50	0.08	–	–	–	0.06	0.05	0.02	0.04	0.07	0.08	–
155	–	0.02	–	0.11	–	0.09	0.32	0.03	–	–	–	–	–	–	0.06	–	0.08	0.14	0.12	–	–	–	–	–	0.04	–	0.11	0.05
159	0.09	0.07	–	0.44	–	0.09	0.03	–	–	–	–	–	–	–	0.02	–	0.04	0.12	0.23	0.34	–	–	–	–	–	0.02	0.33	–
163	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	–	–	0.04	0.08	0.07	–	–	–	–	–	0.02	–	–
167	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–
171	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–
175	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
195	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–	–	–	–	–	–	–	–	–
217	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
221	–	–	–	–	–	–	–	–	–	–	–	0.13	0.05	0.26	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–
Locus:	1011																											
N	38	27	7	9	26	38	38	36	28	19	31	27	38	39	26	10	12	30	16	29	37	26	20	30	28	24	17	19
128	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	0.03
136	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	–	0.03
152	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–
156	–	–	–	–	–	0.04	–	0.10	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	0.05	–	–	0.08
160	–	0.02	–	–	–	0.37	0.41	0.53	–	–	–	–	–	–	0.02	0.40	–	0.17	–	–	–	–	–	0.32	–	0.08	–	0.16
164	–	0.02	–	–	–	0.32	0.25	0.38	0.14	0.21	0.08	0.24	0.18	0.21	0.04	0.40	–	0.17	0.22	–	0.12	0.19	0.08	0.27	0.09	0.54	0.24	0.03
168	–	–	–	–	–	–	–	–	–	0.11	–	–	0.13	–	–	–	–	–	–	–	0.30	–	–	–	0.16	–	0.71	0.24
172	0.21	0.17	0.50	0.67	0.29	0.21	0.20	–	0.13	0.13	0.27	–	0.12	–	0.06	0.05	0.25	0.50	0.75	1.00	–	0.27	0.10	–	0.07	–	–	–
176	0.32	0.28	0.36	0.17	0.58	0.04	–	–	0.38	0.29	0.26	0.76	0.41	0.74	0.19	0.15	0.13	0.05	–	–	0.35	0.44	0.43	–	0.04	–	–	–
180	0.01	–	–	–	–	–	–	–	–	0.05	–	–	0.07	–	0.04	–	–	0.02	–	–	0.04	–	–	0.05	0.09	0.25	0.03	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
184	0.37	0.48	0.14	0.17	0.02	0.03	0.15	–	–	–	–	–	–	–	0.02	–	0.63	0.02	–	–	–	–	–	0.12	0.04	0.04	0.03	0.26
188	0.09	0.04	–	–	0.12	–	–	–	0.34	0.05	0.21	–	0.03	–	0.39	–	–	0.08	0.03	–	–	0.04	0.20	0.23	0.27	–	–	0.03
192	–	–	–	–	–	–	–	–	0.02	0.16	0.18	–	0.07	–	0.08	–	–	–	–	–	0.18	0.06	0.20	–	0.05	–	–	0.03
196	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	0.02	0.07	0.02	–	0.03
200	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.02	–	–	0.08
220	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03
248	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–
Locus:	1058																											
N	37	27	6	8	25	38	38	35	27	19	31	24	38	38	24	10	10	29	14	18	37	26	20	30	22	20	14	11
177	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18	0.16	0.75	0.46	0.09
181	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	0.02	0.02	–	–	0.05
185	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.07	–
189	0.16	0.20	0.25	0.44	–	0.25	0.05	–	–	–	–	–	–	–	0.38	–	0.15	0.48	0.79	0.94	–	–	–	0.02	0.02	0.10	0.07	0.18
193	0.08	0.07	–	–	0.02	0.72	0.55	0.99	–	0.37	–	0.77	0.62	0.55	0.06	0.95	0.10	0.35	0.07	–	0.39	–	–	0.08	0.18	–	–	0.18
197	–	–	0.08	–	–	–	0.01	–	0.13	–	0.39	–	–	–	0.06	–	–	–	0.07	–	–	0.10	0.18	0.02	0.27	–	–	–
201	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	0.05	–	–	–	–	–	0.02	0.02	–	0.39	0.05
205	0.18	0.07	0.08	–	–	–	–	–	–	–	–	–	–	–	0.17	–	0.05	0.05	–	–	–	–	–	0.23	0.05	0.08	–	0.09
209	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	0.03	0.02	0.03	–	0.09
213	–	–	–	–	–	–	–	–	–	0.40	–	0.21	0.29	0.43	0.23	–	–	0.03	–	–	0.45	–	–	–	0.05	–	–	–
217	0.24	0.15	0.25	0.25	0.34	–	0.18	0.01	0.37	0.08	0.18	0.02	0.09	–	0.04	0.05	0.25	–	–	–	0.15	0.17	0.30	–	–	–	–	–
221	0.34	0.46	0.33	0.31	0.64	0.03	0.20	–	0.50	0.08	0.44	–	–	–	–	–	0.40	0.03	0.07	0.06	0.01	0.73	0.53	–	0.02	–	–	–
225	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.38	0.02	0.05	–	–
229	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–
233	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
237	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–
241	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.09
245	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09
249	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	0.09
253	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
Locus:	3.00																											
N	38	26	4	6	27	39	38	38	27	19	31	28	38	39	23	10	9	30	13	24	37	22	20	29	28	24	18	19
137	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–	0.05	–	0.02	0.16	0.50	0.36	–
145	0.58	0.31	0.25	0.67	1.00	0.90	0.74	0.86	1.00	0.34	1.00	0.57	0.61	0.45	0.98	1.00	0.33	0.97	0.54	0.48	0.42	0.96	1.00	0.14	0.64	0.42	0.44	0.95
149	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
169	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	–	–	0.03
173	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	–	–	0.17	–
177	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	–	–	–	–	–	–	0.29	–	–	–	0.03
181	0.42	0.67	0.75	0.33	–	0.10	0.26	0.15	–	0.61	–	0.43	0.40	0.55	–	–	0.61	0.03	0.46	0.52	0.58	–	–	0.05	0.04	0.06	–	–
189	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.41	0.02	0.02	0.03	–
195	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Locus:	1083																											
N	38	27	8	9	29	39	38	38	29	19	32	28	38	39	26	10	12	29	16	29	37	25	20	30	28	24	18	17
124	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–
132	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06
136	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.31	–
140	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
144	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.20	–	–	–
148	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.25	–	–	0.03	–	–	–	–	–	–	0.18	–	–	0.03
156	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.05	0.13	0.08	0.09
160	0.01	–	–	0.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	–	–	–	–	0.02	–	–	–
164	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	0.02	–	0.03
168	–	–	–	–	–	0.13	0.01	0.01	–	0.18	–	0.57	0.40	0.59	–	–	–	0.09	–	–	0.19	–	–	–	–	–	–	0.18
172	0.25	0.32	0.25	–	–	0.41	0.15	0.42	0.02	–	–	–	0.01	–	0.02	0.40	0.42	0.07	–	–	–	–	–	0.25	0.04	0.06	0.03	0.06
176	0.20	0.24	0.06	0.39	0.02	0.19	0.07	–	0.14	0.24	0.36	0.16	0.25	–	–	–	0.08	0.48	0.75	0.85	0.35	0.10	0.23	0.08	0.11	–	–	0.06
180	0.12	0.19	0.38	0.33	0.57	0.14	0.46	0.45	0.57	0.05	0.48	0.09	0.05	0.01	–	0.35	0.21	0.21	–	–	–	0.64	0.53	0.08	0.04	0.27	0.44	0.09
184	–	0.02	–	0.06	0.02	0.09	0.21	0.11	0.12	0.03	–	–	–	–	–	0.15	–	0.09	0.25	0.03	–	–	0.03	0.38	0.07	0.19	0.03	0.09
188	–	–	–	–	–	0.04	0.11	–	–	0.11	–	0.02	0.15	0.06	0.14	0.05	–	0.03	–	–	0.10	–	–	0.02	0.02	0.33	–	0.06
192	–	–	–	–	–	–	–	–	–	0.24	–	0.16	0.13	0.32	0.19	–	–	–	–	–	0.26	–	–	–	–	–	0.06	–
196	–	–	–	–	0.03	–	–	–	0.07	–	0.03	–	–	0.01	0.08	–	–	–	–	–	–	0.04	0.05	–	0.02	–	–	0.03
200	–	–	–	–	–	–	–	–	–	0.05	–	–	0.01	–	0.10	–	–	–	–	–	0.10	–	–	0.02	–	–	0.03	0.09
204	–	–	–	–	0.36	–	–	–	0.09	–	0.13	–	–	–	0.04	–	–	–	–	–	–	0.22	0.18	–	–	–	–	–
208	–	–	–	–	–	–	–	0.01	–	0.03	–	–	–	–	0.04	0.05	–	–	–	–	0.01	–	–	–	0.04	–	–	–
212	0.25	0.13	0.19	0.11	–	–	–	–	–	–	–	–	–	–	–	–	0.17	–	–	–	–	–	–	–	–	–	–	–
216	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	0.15

Appendix 3.1, continued

	DDMA	DDMB	DDMC	SSC95b	WMC95	CLK	CLN	FC	BCB	LWC	MWMC	RC	SC	SL	UCC	UFC	UM	UMT	USSCA	USSCB	UWC	UWMC	WMC	HCS	NFNR	MSS	MWS	NFAR
220	0.16	0.07	0.13	–	–	–	–	–	–	–	–	–	–	–	0.08	–	0.08	–	–	–	–	–	–	0.10	0.02	–	–	–
224	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
244	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
252	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
268	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
272	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
288	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
Locus:	1081																											
N	38	27	8	6	26	39	38	37	28	19	32	28	37	39	26	10	12	27	16	25	37	25	20	29	28	23	17	18
154	0.45	0.44	0.06	0.17	0.42	0.08	–	–	0.16	0.05	0.14	0.13	0.16	–	0.02	–	0.46	0.15	0.06	0.10	0.11	0.30	0.25	0.02	0.20	–	0.15	0.11
158	0.30	0.30	0.50	0.58	0.15	0.39	0.43	0.73	0.30	0.90	0.31	0.82	0.81	0.99	0.15	0.50	0.33	0.67	0.72	0.78	0.78	0.48	0.35	0.10	–	0.02	0.21	0.06
162	–	–	–	–	–	–	–	–	–	0.05	–	–	0.03	–	0.02	0.10	–	–	0.06	–	0.05	–	–	0.21	0.16	–	–	0.03
166	0.13	0.17	0.25	–	–	0.14	0.29	–	–	–	–	0.05	–	–	0.35	–	0.13	0.09	0.03	0.12	0.05	–	–	–	0.05	0.07	0.06	0.06
170	0.05	0.02	0.13	0.25	0.42	0.01	–	–	0.54	–	0.55	–	–	–	0.29	–	0.04	–	–	–	–	0.22	0.40	0.02	0.14	–	–	0.31
174	0.05	0.06	–	–	–	0.35	0.28	0.27	–	–	–	–	–	–	–	0.40	0.04	0.09	0.13	–	–	–	–	–	0.11	0.22	0.27	0.08
178	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.04	0.07	0.09	0.19
182	0.01	0.02	0.06	–	–	–	–	–	–	–	–	–	–	0.01	0.12	–	–	–	–	–	–	–	–	–	0.02	–	–	–
186	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	–	–	–	–	–	–	–	–	0.53	0.18	0.54	–	–
190	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	0.08
194	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.06
198	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03
202	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.24	–
206	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–
218	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–
226	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–

Appendix 3.2. Descriptive statistics for SNP loci, including frequency of designated predominately "rainbow" allele (nucleotide given as adenine [A], guanine [G], cytosine [C], and thymine [T]) ":"= 1 base pair deletion; alternate SNP allele given parenthetically), number of individuals amplified for each assay (n). Observed (Ho) and unbiased expected [He(nb)] heterozygosities and F_{IS} values also shown. Codes as given in Table 3.1.

		B9_164				CHIT 80				CRB2677 117			
	Code	N	A(:)	He(nb)	Ho	N	C(G)	He(nb)	Ho	N	T(G)	He(nb)	Ho
1	DDM95	38	0.24	0.37	0.37	38	0.00	0.00	0.00	38	0.00	0.00	0.00
2	DDM96	27	0.30	0.42	0.44	27	0.02	0.04	0.04	27	0.00	0.00	0.00
3	DDM97	11	0.14	0.25	0.27	11	0.00	0.00	0.00	9	0.00	0.00	0.00
4	SSC95	10	0.30	0.44	0.40	10	0.00	0.00	0.00	10	0.00	0.00	0.00
5	WMC95	28	0.29	0.42	0.50	29	0.00	0.00	0.00	29	0.00	0.00	0.00
6	BCB	32	0.55	0.50	0.41	33	0.00	0.00	0.00	31	0.00	0.00	0.00
7	UWMC	40	0.28	0.40	0.35	40	0.00	0.00	0.00	35	0.00	0.00	0.00
8	MWMC	32	0.31	0.44	0.44	33	0.00	0.00	0.00	30	0.00	0.00	0.00
9	WMC	20	0.25	0.38	0.40	20	0.00	0.00	0.00	19	0.00	0.00	0.00
10	SL	36	0.57	0.50	0.58	36	0.00	0.00	0.00	36	0.00	0.00	0.00
11	SHT	33	0.80	0.32	0.33	34	0.00	0.00	0.00	33	0.00	0.00	0.00
12	PST	16	0.41	0.50	0.44	16	0.00	0.00	0.00	16	0.00	0.00	0.00
13	LKRI	27	0.39	0.48	0.41	29	0.00	0.00	0.00	28	0.00	0.00	0.00
14	RFC06	35	0.80	0.32	0.40	35	0.00	0.00	0.00	35	0.00	0.00	0.00
15	RC	30	0.40	0.49	0.47	30	0.00	0.00	0.00	30	0.00	0.00	0.00
16	TAM	30	0.93	0.13	0.13	30	0.00	0.00	0.00	30	0.00	0.00	0.00
17	UWC	37	0.89	0.20	0.16	39	0.00	0.00	0.00	38	0.00	0.00	0.00
18	LWC	19	0.87	0.23	0.16	19	0.00	0.00	0.00	19	0.00	0.00	0.00
19	SC	38	0.78	0.35	0.34	39	0.00	0.00	0.00	39	0.00	0.00	0.00
20	LIO	40	0.63	0.47	0.55	40	0.00	0.00	0.00	40	0.00	0.00	0.00
21	USSC02	39	0.00	0.00	0.00	40	0.00	0.00	0.00	40	0.00	0.00	0.00
22	USSC01	20	0.00	0.00	0.00	28	0.02	0.04	0.04	22	0.00	0.00	0.00
23	SSCPB	36	0.46	0.50	0.53	40	0.00	0.00	0.00	36	0.01	0.03	0.03
24	DDM06	30	0.45	0.50	0.37	30	0.00	0.00	0.00	30	0.00	0.00	0.00
25	DDM	35	0.33	0.45	0.43	39	0.01	0.03	0.03	35	0.00	0.00	0.00
26	UM	13	0.31	0.44	0.46	14	0.00	0.00	0.00	13	0.00	0.00	0.00
27	ALP	40	0.06	0.12	0.13	40	0.00	0.00	0.00	38	0.00	0.00	0.00
28	UMT	29	0.12	0.22	0.24	30	0.07	0.13	0.13	30	0.00	0.00	0.00
29	SMC06	40	0.51	0.51	0.58	40	0.13	0.22	0.25	40	0.06	0.12	0.13
30	SMC	18	0.39	0.49	0.56	21	0.05	0.09	0.10	20	0.00	0.00	0.00
31	LMC	35	0.27	0.40	0.54	37	0.00	0.00	0.00	36	0.00	0.00	0.00
32	JAC06	39	0.42	0.49	0.59	40	0.00	0.00	0.00	40	0.20	0.32	0.20
33	CLN	39	0.08	0.14	0.10	39	0.00	0.00	0.00	38	0.00	0.00	0.00
34	CLK	39	0.04	0.07	0.08	39	0.00	0.00	0.00	37	0.03	0.05	0.00
35	LKBRN06	8	0.75	0.40	0.50	8	0.25	0.40	0.25	8	0.06	0.13	0.13
36	LKRBRN	29	0.22	0.35	0.24	36	0.15	0.26	0.25	36	0.14	0.24	0.22
37	UFC	11	0.00	0.00	0.00	11	0.00	0.00	0.00	7	0.14	0.26	0.00
38	FC	36	0.18	0.30	0.19	38	0.00	0.00	0.00	36	0.00	0.00	0.00
39	TMC	17	0.29	0.43	0.47	19	0.00	0.00	0.00	16	0.00	0.00	0.00
40	UCC	29	0.93	0.13	0.14	30	0.53	0.51	0.27	29	0.00	0.00	0.00
41	LCC	40	0.74	0.39	0.43	40	0.00	0.00	0.00	40	0.00	0.00	0.00
42	NFAR	20	1.00	0.00	0.00	20	0.63	0.48	0.55	19	0.29	0.42	0.16
43	NFNR	31	0.92	0.15	0.16	30	0.33	0.45	0.00	31	0.02	0.03	0.03
44	HCS	30	1.00	0.00	0.00	30	0.73	0.40	0.53	28	0.79	0.34	0.29
45	MSS	30	1.00	0.00	0.00	30	0.72	0.41	0.37	28	0.66	0.46	0.11
46	MWS	30	1.00	0.00	0.00	26	0.98	0.04	0.04	30	0.10	0.18	0.20

Appendix 3.2, continued

		F5 306				Omy_f1 259-260				LDH 156			
	Code	N	G(T)	He(nb)	Ho	N	::(AA)	He(nb)	Ho	N	T(C)	He(nb)	Ho
1	DDM95	38	0.68	0.44	0.47	32	0.03	0.06	0.06	38	0.00	0.00	0.00
2	DDM96	27	0.70	0.42	0.37	23	0.07	0.12	0.13	27	0.02	0.04	0.04
3	DDM97	11	0.45	0.52	0.55	11	0.23	0.37	0.45	7	0.00	0.00	0.00
4	SSC95	10	0.35	0.48	0.50	7	0.21	0.36	0.14	10	0.00	0.00	0.00
5	WMC95	29	0.71	0.42	0.38	14	0.50	0.52	0.29	29	0.00	0.00	0.00
6	BCB	33	0.65	0.46	0.33	31	0.27	0.40	0.16	32	0.00	0.00	0.00
7	UWMC	40	0.48	0.51	0.65	24	0.13	0.22	0.08	33	0.00	0.00	0.00
8	MWMC	33	0.39	0.48	0.55	29	0.40	0.49	0.17	33	0.00	0.00	0.00
9	WMC	20	0.43	0.50	0.55	12	0.71	0.43	0.25	20	0.00	0.00	0.00
10	SL	36	0.00	0.00	0.00	36	0.00	0.00	0.00	35	0.00	0.00	0.00
11	SHT	34	0.31	0.43	0.56	8	0.00	0.00	0.00	34	0.18	0.30	0.35
12	PST	16	0.00	0.00	0.00	6	0.00	0.00	0.00	16	0.00	0.00	0.00
13	LKRI	26	0.04	0.08	0.08	27	0.17	0.28	0.19	29	0.00	0.00	0.00
14	RFC06	35	0.00	0.00	0.00	28	0.00	0.00	0.00	34	0.00	0.00	0.00
15	RC	30	0.03	0.07	0.07	30	0.15	0.26	0.17	29	0.00	0.00	0.00
16	TAM	30	0.00	0.00	0.00	29	0.31	0.44	0.41	29	0.00	0.00	0.00
17	UWC	40	0.00	0.00	0.00	39	0.23	0.36	0.26	37	0.00	0.00	0.00
18	LWC	19	0.05	0.10	0.11	19	0.13	0.23	0.26	19	0.00	0.00	0.00
19	SC	39	0.00	0.00	0.00	38	0.25	0.38	0.29	39	0.00	0.00	0.00
20	LIO	40	0.00	0.00	0.00	40	0.20	0.32	0.30	40	0.00	0.00	0.00
21	USSC02	40	0.31	0.44	0.48	39	0.45	0.50	0.44	32	0.00	0.00	0.00
22	USSC01	28	0.20	0.32	0.18	24	0.25	0.38	0.25	24	0.00	0.00	0.00
23	SSCPB	40	0.41	0.49	0.58	37	0.15	0.26	0.19	40	0.00	0.00	0.00
24	DDM06	30	0.33	0.45	0.47	24	0.02	0.04	0.04	30	0.00	0.00	0.00
25	DDM	38	0.32	0.44	0.26	31	0.10	0.18	0.06	35	0.00	0.00	0.00
26	UM	14	0.68	0.45	0.50	9	0.00	0.00	0.00	13	0.00	0.00	0.00
27	ALP	40	0.90	0.18	0.15	39	0.17	0.28	0.03	39	0.14	0.25	0.28
28	UMT	30	0.08	0.16	0.17	30	0.22	0.35	0.37	30	0.12	0.21	0.10
29	SMC06	40	0.66	0.45	0.38	39	0.49	0.51	0.15	40	0.00	0.00	0.00
30	SMC	21	0.71	0.42	0.38	16	0.28	0.42	0.19	20	0.00	0.00	0.00
31	LMC	33	0.73	0.40	0.48	36	0.03	0.05	0.00	36	0.00	0.00	0.00
32	JAC06	40	0.46	0.50	0.63	40	0.06	0.12	0.03	40	0.00	0.00	0.00
33	CLN	39	0.15	0.26	0.26	39	0.00	0.00	0.00	39	0.00	0.00	0.00
34	CLK	39	0.08	0.14	0.10	39	0.09	0.17	0.18	39	0.00	0.00	0.00
35	LKBRN06	8	0.56	0.53	0.63	8	0.19	0.33	0.13	8	0.25	0.40	0.25
36	LKRBRN	35	0.23	0.36	0.40	35	0.16	0.27	0.31	33	0.08	0.14	0.15
37	UFC	11	0.18	0.31	0.36	11	0.00	0.00	0.00	11	0.05	0.09	0.09
38	FC	38	0.25	0.38	0.45	37	0.00	0.00	0.00	39	0.00	0.00	0.00
39	TMC	19	0.08	0.15	0.16	17	0.00	0.00	0.00	16	0.00	0.00	0.00
40	UCC	29	0.34	0.46	0.14	30	0.00	0.00	0.00	28	0.00	0.00	0.00
41	LCC	40	0.08	0.14	0.15	27	0.00	0.00	0.00	40	0.00	0.00	0.00
42	NFAR	20	0.75	0.38	0.40	20	0.85	0.26	0.20	18	0.14	0.25	0.28
43	NFNR	31	0.98	0.03	0.03	31	0.11	0.20	0.16	30	0.33	0.45	0.40
44	HCS	30	1.00	0.00	0.00	30	0.57	0.50	0.53	30	0.97	0.07	0.00
45	MSS	30	1.00	0.00	0.00	31	0.50	0.51	0.48	30	0.88	0.21	0.23
46	MWS	30	1.00	0.00	0.00	30	0.82	0.30	0.23	30	0.48	0.51	0.50

Appendix 3.2, continued

		OMY 180				RAPD 132				ID1c 77-83				RTDL_695	
	Code	N	G(C)	He(nb)	Ho	N	A(T)	He(nb)	Ho	N	AGTTAAT(7bp:)	He(nb)	Ho	N	T(C)
1	DDM95	38	0.17	0.29	0.08	38	0.00	0.00	0.00	38	0.20	0.32	0.39	38	0.00
2	DDM96	27	0.28	0.41	0.26	27	0.00	0.00	0.00	27	0.35	0.46	0.56	27	0.04
3	DDM97	9	0.11	0.21	0.22	11	0.00	0.00	0.00	10	0.15	0.27	0.10	11	0.00
4	SSC95	10	0.20	0.34	0.20	10	0.00	0.00	0.00	10	0.30	0.44	0.40	10	0.00
5	WMC95	29	0.03	0.07	0.00	29	0.00	0.00	0.00	29	0.97	0.07	0.00	29	0.00
6	BCB	33	0.11	0.19	0.03	33	0.00	0.00	0.00	32	0.73	0.40	0.34	33	0.00
7	UWMC	40	0.00	0.00	0.00	39	0.00	0.00	0.00	39	0.90	0.19	0.21	40	0.00
8	MWMC	27	0.00	0.00	0.00	33	0.00	0.00	0.00	33	0.88	0.22	0.12	33	0.00
9	WMC	20	0.00	0.00	0.00	20	0.00	0.00	0.00	20	0.95	0.10	0.10	20	0.00
10	SL	35	0.26	0.39	0.23	36	0.00	0.00	0.00	35	0.94	0.11	0.06	34	0.47
11	SHT	34	0.10	0.19	0.21	34	0.00	0.00	0.00	34	0.88	0.21	0.24	34	0.35
12	PST	16	0.16	0.27	0.31	16	0.00	0.00	0.00	16	0.91	0.18	0.19	16	0.25
13	LKRI	26	0.04	0.08	0.08	29	0.00	0.00	0.00	29	0.90	0.19	0.21	29	0.14
14	RFC06	35	0.47	0.51	0.66	35	0.00	0.00	0.00	35	1.00	0.00	0.00	35	1.00
15	RC	29	0.03	0.07	0.07	30	0.00	0.00	0.00	30	0.90	0.18	0.20	30	0.13
16	TAM	30	0.25	0.38	0.43	30	0.00	0.00	0.00	30	0.70	0.43	0.53	30	0.73
17	UWC	40	0.29	0.41	0.28	38	0.00	0.00	0.00	38	0.55	0.50	0.42	39	0.67
18	LWC	19	0.16	0.27	0.21	19	0.00	0.00	0.00	19	0.63	0.48	0.53	19	0.84
19	SC	39	0.41	0.49	0.36	39	0.00	0.00	0.00	39	0.69	0.43	0.36	39	0.79
20	LIO	40	0.46	0.50	0.48	40	0.00	0.00	0.00	40	0.89	0.20	0.18	40	0.83
21	USSC02	39	0.00	0.00	0.00	40	0.00	0.00	0.00	37	0.12	0.22	0.19	40	0.00
22	USSC01	28	0.18	0.30	0.29	26	0.00	0.00	0.00	19	0.61	0.49	0.37	28	0.00
23	SSCPB	40	0.19	0.31	0.18	40	0.00	0.00	0.00	40	0.43	0.49	0.50	40	0.20
24	DDM06	28	0.43	0.50	0.21	30	0.00	0.00	0.00	29	0.16	0.27	0.31	30	0.00
25	DDM	36	0.28	0.41	0.28	38	0.00	0.00	0.00	40	0.14	0.24	0.28	40	0.00
26	UM	14	0.21	0.35	0.14	13	0.00	0.00	0.00	14	0.29	0.42	0.57	14	0.00
27	ALP	40	0.44	0.50	0.53	40	0.00	0.00	0.00	40	0.79	0.34	0.43	40	0.00
28	UMT	30	0.22	0.35	0.23	30	0.03	0.07	0.07	30	0.68	0.44	0.50	30	0.13
29	SMC06	39	0.40	0.49	0.23	40	0.00	0.00	0.00	39	0.26	0.39	0.36	40	0.00
30	SMC	18	0.39	0.49	0.33	18	0.00	0.00	0.00	21	0.29	0.42	0.57	21	0.00
31	LMC	23	0.43	0.50	0.17	36	0.00	0.00	0.00	37	0.34	0.45	0.57	36	0.00
32	JAC06	39	0.36	0.47	0.31	40	0.00	0.00	0.00	40	0.53	0.51	0.60	37	0.14
33	CLN	39	0.17	0.28	0.23	39	0.00	0.00	0.00	39	0.74	0.39	0.46	39	0.00
34	CLK	39	0.18	0.30	0.31	39	0.01	0.03	0.03	39	0.86	0.25	0.28	39	0.00
35	LKBRN06	8	0.44	0.53	0.88	8	0.06	0.13	0.13	8	0.69	0.46	0.63	8	0.25
36	LKRBRN	34	0.24	0.37	0.24	35	0.04	0.08	0.09	36	0.85	0.26	0.19	35	0.00
37	UFC	11	0.09	0.17	0.18	11	0.00	0.00	0.00	11	1.00	0.00	0.00	11	0.09
38	FC	38	0.14	0.25	0.24	39	0.00	0.00	0.00	39	1.00	0.00	0.00	40	0.00
39	TMC	19	0.18	0.31	0.37	19	0.00	0.00	0.00	18	0.97	0.06	0.06	19	0.00
40	UCC	29	0.16	0.27	0.24	28	0.02	0.04	0.04	29	0.24	0.37	0.41	29	0.21
41	LCC	40	0.24	0.37	0.43	40	0.00	0.00	0.00	40	0.94	0.12	0.13	40	0.65
42	NFAR	20	0.68	0.45	0.45	20	0.05	0.10	0.00	20	1.00	0.00	0.00	18	1.00
43	NFNR	31	0.60	0.49	0.48	31	0.00	0.00	0.00	29	1.00	0.00	0.00	31	1.00
44	HCS	30	0.75	0.38	0.37	30	0.95	0.10	0.10	30	1.00	0.00	0.00	30	1.00
45	MSS	29	0.72	0.41	0.28	30	0.92	0.16	0.10	28	0.98	0.04	0.04	30	1.00
46	MWS	30	0.58	0.49	0.50	30	0.08	0.16	0.17	29	1.00	0.00	0.00	30	1.00

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