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MICROSATELLITE ANALYSIS OF POPULATION STRUCTURE IN THE SANTA ANA SPECKLED DACE (*RHINICTHYS OSCULUS*)

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

by

Stacey Ann Nerkowski

June 2015

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Approved by:

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ABSTRACT

Rhinichthys osculus, the Speckled Dace, is one of the most ubiquitous fish in western North America. Within the Southern California region, the local taxon is known as the Santa Ana Speckled Dace. The purpose of this study was to characterize and identify polymorphic microsatellite markers for *R. osculus* in which twenty-three were identified through Illumina pair-end sequencing. Seven of these loci were then used to examine the patterns of genetic variation and population structure that occurred within and among the watersheds in the Southern California. The study also examined the regional relationships among Southern California, Central California and Owen's River Valley. Analysis of the microsatellite data revealed highly significant moderate levels of population structure exist within the Southern California region ($R_{ST}=0.160$, p=0.001). This structure is best explained by watershed as well as isolation by distance $(R^2=.2286, p=0.010)$. Highly significant geographic structure also exists among the geographic regions of Southern California, Central Coast, and Owen's River Valley regions (R_{ST=} 0.600, p-value=0.001) that are congruent with the regional differentiation elucidated by mtDNA sequence data. In both cases, the degree of population differentiation was correlated with isolation by distance. Utilizing this information we were able gain a better understanding of the evolutionary relationships among the Southern California populations of Santa Ana Speckled Dace. Within the Santa Ana Speckled Dace populations we examined four models to explain the geographic structure: watershed, mountain range,

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tributary, and isolation by distance. While all were significant, the tributary model exhibited the higher level of population structure (R_{ST=} 0.160, p-value=0.001) and a significant correlation was exhibited between geographic distance and population structure, suggesting isolation by distance may be playing a role. The results of the microsatellite analysis are congruent with an earlier broad scale analysis of mtDNA sequence data that suggests the Central California and the Owens Valley populations diverged from each other prior to the divergence of the Santa Ana Speckled Dace populations from the Colorado Basin populations, and that the Central Coast populations were not established as a result of a migration event from the Southern California populations, as was previously hypothesized. Primarily due to human activity, Santa Ana Speckled Dace habitat has become highly fragmented resulting in some populations becoming extirpated. We hope this study will guide the strategies for the conservation of the remaining populations of Santa Ana Speckled Dace and watershed management in Southern California.

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CHAPTER ONE

Overview

The degree of genetic differentiation and population substructure has been well examined in studies of vertebrate animal populations. The degrees of sequence divergence of particular genes have provided researchers with a portrait of the evolutionary relationships between species and their times since divergence from their common ancestors. The geographical distribution of a particular species can be associated with unique patterns of genetic differentiation among subpopulations. California encompasses some of the most geographically complicated patterns of genetic diversity on earth, and the California Floristic Province (including Southern California) is considered one of the world's 25 most biologically rich and endangered terrestrial ecoregions (Meyes, Mittermeier, Mittermeier, Fonseca, & Kent, 2000). Widespread species in the Southern California region have also shown pronounced fine-scale genetic differences among populations, possibly resulting from a highly varied and fragmented landscape ((Harrison S., 1999; Brodie III & Brodie Jr., 1990; Brown, Leebens-Mack, Thompson, Pellmyr, & Harrison, 1997; Maldonado, Davila, Stewart, Geffen, & Wayne, 1995; Metcalf, Nunney, & Hyman, 2001; Phillipsen & Metcalf, 2009; Vandergast, Bohonak, Weissman, & Fisher, 2007; Rodriguez-Robles, Denardo, & Staub, 1999).

Stream dwelling vertebrates, especially fish, provide an excellent model for examining such population substructure and genetic differentiation. Gene flow between tributaries and watersheds normally only occurs during times of flooding, allowing for these populations to be geographically isolated from one another for extended periods. Each population may be independently influenced by various evolutionary forces due to their isolation resulting in independent evolutionary lineages of genes to form.

Cyprinidae is one of the most diverse families of freshwater fish, and inhabits a variety of environments including lakes, ponds, creeks, tributaries and isolated springs across North America. Each of these environments contains different characteristics and histories. Due to these differences, the Cyprinidae family has proven to be a relevant model group to address the evolutionary effects of environmental and ecological factors (Simmons, Berendzen, & Mayden, 2003; Scott & Crossman, 1973)).

Rhinichthys osculus, the speckled dace, is one of the most ubiquitous freshwater fish in the western United States and occupies a variety of stream environments (Hubbs, Miller, & Hubbs, 1974). In the western United States, the speckled dace is the only native fish to be represented in the majority of the drainage systems (Miller R. R., 1958). Locally, the Santa Ana Speckled Dace is found in such creeks as Lytle Creek, Cajon Creek, Plunge Creek and Mill Creek (Figure 2). The speckled dace is a small, cyprinid fish that reaches a length of approximately 80mm (Moyle & Marchetti, 2006).

The local variation of the Speckled Dace is known as the Santa Ana Speckled Dace. The Santa Ana Speckled Dace's geographic range included the Santa Ana, San Gabriel and Los Angeles Watershed. Some populations have become extirpated, such as the Los Angeles River populations, as a result of anthropogenic effects such as the urbanization of the watershed and county controlled flood systems (Santa Ana River Watershed Project Authority, 2004). As the Santa Ana Speckled Dace's habitats become more and more fragmented, the populations are highly affected by climatic events such as fire and floods. As a result of their highly fragmented habitats and the extirpation of populations, the Santa Ana Speckled Dace was listed as a species of concern by the California Department of Fish and Wildlife (1995) and the United States Forest Service (1998). Due to the lack of peer reviewed descriptions of the Santa Ana Speckled Dace's taxonomic status including genetic data, they were never federally listed under the Endangered Species Act (Moyle & Marchetti, 2006).

Research is currently being performed in the lab of Dr. Anthony Metcalf at California State University, San Bernardino, on the *cytochrome b and d-loop* regions of mitochondrial DNA (mtDNA) of the Santa Ana Speckled Dace. In addition to the data acquired from the mtDNA sequences, this study proposes to analyze population level variation in this species using microsatellite loci. Microsatellite loci are 1-6 base tandem repeats found in nuclear DNA. They are useful for population genetic analysis because they are highly polymorphic, typically having 3 to 11 alleles with different numbers of repeats. The number of

times it repeats can vary between individuals, populations and even between species allowing it to be utilized as a fine scale marker (Hardy, Charbonnel, Fréville, & Heuertz, 2003).

We have isolated and characterized 23 polymorphic microsatellite loci for the Santa Ana Speckled Dace (Nunziata, Lance, Jones, Nerkowski, & Metcalf, 2013). The objective of this study is to utilize seven of the polymorphic microsatellite loci on samples of *R. osculus* representing the Southern California, Central Coast of California and Owens Valley populations. Utilizing the microsatellite data, we will examine gene flow and historic patterns of interbreeding among creeks to provide a genetic baseline for the tributaries within the Southern California region. In addition, comparing the Santa Ana Speckled Dace populations to those of other locations within California may provide insight into relationships and population structure among regions. With these data, we hope to gain a better understanding of the evolutionary histories and developments that have occurred among the local populations of *R. osculus*.

Phylogeography

Phylogeography is an interdisciplinary field of study that examines the processes leading to the geographic distribution and evolutionary relationships of populations and species, especially those of closely related species. Phylogeography expanded upon the concepts of historical biogeography, examining the geographical distributions of species utilizing climatic, geological, and other environmental forces. Phylogeography requires extensive input from a

variety of disciplines but especially those of molecular genetics/phylogenetics and population genetics. By analyzing the geographic distributions of current populations, Avise et al. (1987) stated that "one can begin to have a better understanding of the evolutionary footprints that may remain from ancestral populations that lead to the formation of the current population."

A species' geographic distribution typically results from geological and landscape barriers; these are barriers that result from physical topography changes such as the formation of mountain ranges, the creation of a river or lake, or the presence of a desert or elevation gradient. Each of these barriers can limit the migration and expansion of a population but can also cut off a population from its original source population. Over time, isolated populations may have been subjected to varying environmental stressors resulting in the population evolving independently from the ancestral population. This would result in a genetically distinct population which can be used in the mapping of the genetic and geographic distribution (Avise J. C., 2000).

How did the extant species of today arise from ancestral populations? What pressures or circumstances arose that caused a population to become geographically isolated, thereby creating the right conditions for divergence and eventually reproductive isolation? Natural selection plays a crucial role in the formation of new species but it can also contribute to population-level structure observed in geographical distributions (Avise, 1994; Avise, 2000).

Two competing schools of thought that account for the origins of spatially disjunct populations are vicariance and dispersal biogeography. Vicariance distribution results in emergence of new populations from the ancestral population due to environmental forces. Little to no gene flow exists between the ancestral population and the newly immerged populations, resulting in further divergence. A more varied historical relationship could occur through the dispersalist approach. This approach relies on the limited gene flow that would occur between emerging populations as a result of the modifications that have arisen since divergence from the ancestral population (Avise, 2000).

Early classification and description of species was typically based on a systematic approach where behavioral and morphological characteristics were used. Furthermore, subspecies classification was based in part on the geographical locations in which specimens were collected. Today, species and subspecies classification further describes the organisms by comparing and contrasting gene flow at the molecular level (Avise, 2000).

Migration (gene flow) contributes to the genetic variation of a population by allowing genes from one population to be brought into another population. It acts as a homogenizing force of evolution that tends to keep subpopulations of a species more similar genetically, resulting in little population substructure. Populations that are historically isolated, with little to no gene flow, typically have greater divergence in selectively neutral and non-neutral genes. Migration will tend to counteract the effects of genetic drift and selection. Migration can affect

genetic drift, but its effect is dependent on the relative strength of genetic drift (population size) and the relative strength of migration (migration rate). As heterozygosity increases within a population, genetic differentiation decreases, creating a more homogenized population (Hartl & Clark, 2006). When small populations colonize new areas, rare alleles are likely to be lost and heterozygosity is expected to decrease as generations progress. The new populations diverge genetically from the ancestral population and from each other.

When a new population is established by a few individuals colonizing an unoccupied area, initial genetic changes are likely to occur solely as a result of random processes. Rare alleles are likely to be lost in the founding event, and heterozygosity will be reduced in subsequent generations (Hedrick, 1998; Nei, 1987). The level of genetic differentiation in a population can provide researchers with an estimate of the level of migration and isolation. Populations that have little to no gene flow through migration should show high levels of genetic differentiation. As a result of genetic differentiation and migration limitations, a population's genetic structure can be established and analyzed.

Population Genetics

Population genetics examines genetic principles such as Mendelian genetics that affect the entire population of organisms. Population genetic studies examine population genetic parameters including genetic structure, gene flow and migration, population stability and demographic history, In addition they can be used to quantify the effects of habitat fragmentation to guide conservation efforts.

The allele frequencies within a population can be affected by five evolutionary forces; non-random mating, natural selection, mutation, genetic drift and migration. Each of these forces can place various pressures on a population but the effects on allele frequency are greatest when a population is isolated (Hartl & Clark, 2006). Freshwater vertebrate species are particularly characterized by geographic isolation where they tend to have fragmented habitats due to varying connectivity of drainage and watershed systems, mountain ranges and anthropogenic affects (Phillipsen & Metcalf, 2009). Through the analysis of molecular markers, one can analyze and describe the phylogeography and population genetic parameters of a species for both current and ancient isolation events.

Hardy-Weinberg Equilibrium Model

The Hardy-Weinberg Model examines the expected genotypic and allelic frequencies that occur in a population where random mating occurs. The Hardy-Weinberg Model is a reference model that allows for no evolutionary forces including no migration, no genetic drift with an infinite population size, no mutation, no selection and where mating is random. Heterozygosity can maintain the frequency of rare recessive alleles within a population. Heterozygosity can range in value from 0 to almost 1, where increasing values

represent an increase in heterozygosity frequency within a population. Higher than expected levels of heterozygosity, typically indicate high levels of immigration and gene flow (Hartl & Clark, 2006).

<u>Genetic Structure: Fixation Index (F_{ST})</u>

To examine and quantify the inbreeding effect of population substructure, Sewall Wright defined the fixation index. The fixation index, F_{ST} , allows the user to examine genetic differentiation of subpopulations within a total population. The F_{ST} , can range from 0 to 1, where an increasing value represents greater genetic structure (Wright, 1931). Analogs of F_{ST} have been developed to further analyze population structure with use of various molecular markers such a ϕ_{ST} (Excoffier, Smouse, & Quattro, 1992) and R_{ST} (Balloux & Goudet, 2002).

Gene Flow and Migration

As discussed above, gene flow contributes to the genetic variation of a population by allowing genes from one population to be brought into another population. Migration is the exchange/movement of genes (individuals) from one population to another. In populations that receive no immigration, the presence of a small number of individuals able to reproduce will limit the genetic variation that is exhibited within the population over generations. Wright (1931) introduced effective population size as the ideal population size that would undergo genetic drift at the same rate as exhibited by the actual population. In the case of mtDNA and N_e, genetic drift will have a four-fold greater effect on mtDNA than that of nuclear DNA. N_e can be calculated by NF/2 where NF is equal to the number of

breeding females in the population. Since mtDNA is haploid, it contains a single set of genes from the mother instead of the two paternal and two maternal gene copies received in nuclear DNA (Hartl & Clark, 2006). The effective population size that can be estimated from mtDNA is ¼ of that from nuclear autosomal sequences; this could result in an increased lineage assorting rate and allele extinction rate (Hartl & Clark, 2006).

Mitochondrial DNA

During the 1970's, population genetics began analyzing evolutionary relationships with the use of mitochondrial DNA (mtDNA) markers. Animal mtDNA is maternally inherited and has a non-recombining mode of inheritance (Dawid & Blackler, 1972), a rapid rate of evolution (an average rate of 0.02 substitutions per base pair per million years) (Brown, Matthew George, & Wilson, 1979) and extensive intraspecific polymorphism (Dawid & Blackler, 1972; Moritz, Dowling, & Brown, 1987). mtDNA quickly became the molecular marker of choice when analyzing phylogenetic relationships and in developing phylogeography.

In addition, mtDNA was useful in evaluating gene flow and geographic isolation because the effective population size (Ne) of mtDNA is ¼ of that of nuclear autosomal sequences, resulting in more rapid divergence of populations due to genetic drift (Hartl & Clark, 2006; Avise, 1994). Researchers could utilize this information to examine the degree of genetic drift and geographic variation

that lead to the divergence of the organism from its ancestral predecessors. The mitochondrion is capable of self-replication due to the possession of its own genome (Dawid & Blackler, 1972).

The mitochondrial genome is highly compact such that no introns or long intergenic stretches of non-coding sequences exist, compared to what is typically found in the nuclear genome. Animal mtDNA is uniform in size across most taxa, being approximately 16kb in length and having a very stable gene arrangement in vertebrates (Brown, Matthew George, & Wilson, 1979; Dawid & Blackler, 1972). Due to the small size of the mitochondrial genome, replication occurs at a much faster rate than would normally occur in the nuclear genome (Brown, Matthew George, & Wilson, 1979). Since mitochondrial DNA is maternally transmitted, the recombination of genomic haplotypes is almost non-existent (Brown, Matthew George, & Wilson, 1979; Dawid & Blackler, 1972; Moritz, Dowling, & Brown, 1987). Nuclear DNA would usually be subjected to the variation and shuffling that would occur from bi-parental genetic material during meiosis, whereas mtDNA lacks this occurrence. As a result, the variation that occurs within the genome is generally the result of a mutation (Brown, Matthew George, & Wilson, 1979; Dawid & Blackler, 1972; Moritz, Dowling, & Brown, 1987).

Over the past 30 years, mtDNA sequences have been used on numerous vertebrate taxa including mammals, reptiles and amphibians, and numerous invertebrate species. Typically, in most phylogeographic studies, mtDNA

haplotypes that are similar or identical will be spatially proximal to one another. Greater sequence divergence occurs in a genealogical split that typically results in the differentiation and isolation of a population in separate refugia (Avise, 2009). Usually, this isolation can align with historical terrain changes in the geological record and are often concordant with landscape level variation.

As advances were made in molecular methods, the investigation of geographic distribution and variation could be analyzed at the molecular level, shedding light on intraspecific phylogeographic structures. Typically, phylogeography has been used to analyze the evolutionary distinct population segments of a species, but it has also been used as a tool in analyzing historical gene flow between populations (Avise, Ball, & Arnold, 1988), estimating population sizes throughout a species evolutionary lineage (Avise, Neigel, & Arnold, 1984), as well as defining the evolutionary trajectory for a species (Sites Jr. & Crandall, 1997). In recent studies, mtDNA was used to analyze the phylogeography and population history of various mammals (Vila, et al., 1999; Paetkau, Calvert, Stiriling, & Strobeck, 1995), birds (Mila, Girman, Kimura, & Smith, 2000; Zink, 1996), amphibians (Phillipsen & Metcalf, 2009; Tan & Wake, 1995) and insects (Vandergast, Bohonak, Weissman, & Fisher, 2007).

Southern California Population Studies

Vicariant events throughout the geological history of California have played a crucial role in shaping the diversification and distributions of California's extant species. Cismontane California is divided by the Transverse Range and

the Peninsular Range. The Transverse Range is a group of mountains that exist in Southern California. This mountain range gets its name from its East-West orientation rather than the North-South orientation typically exhibited by mountain ranges throughout North America. The Peninsular Range runs predominantly north-south, and includes the Santa Ana Mountains, the San Jacinto Mountains and the Laguna Mountains. It has been proposed that the transverse range has played an important role in the speciation and genetic distribution of species across Southern California. "Transverse Range Break " has been attributed to distinct north/south and/or east/west lineage breakages of numerous species.

The use of mtDNA was utilized to examine lineage breaks within the Transverse Mountain range. Chatzimanolis & Caterino (2007) examined the phylogeographic structure and localized lineage breaks of the rove beetle, *Sepedophilus castaneus*, through the amplification of the *Cytochrome Oxidase I* (*CO1*) gene in mtDNA. Their results revealed significant genetic and geographic structure among the *S. castaneus* populations of central and Southern California. Haplotypes fell into four distinct clades which represented Southwestern Sierra Nevada, Saint Lucias and two across the transverse range. Upon further analysis of their data, researchers believe an ancient isolation existed between the Sierra Nevadan population and that of the Eastern Transverse Range (Chatzimanolis & Caterino, 2007).

Research utilizing mtDNA has also revealed the genetic differentiation between populations and regions for the Jerusalem cricket in Southern

California. Vandergast, Bohonak, Weissman, & Fisher(2007) examined the phylogeography and genetic differentiation among current and prehistoric populations of *Stenopelmatus mahogany*. mtDNA's cytochrome oxidase gene was sequenced and analyzed for each sample. High elevation haplotype surrounding the Los Angeles basin represented a single clade, and other regional populations represented corresponding clades. One haplotype was represented in both the San Bernardino and Tequesquite sampling sites, which provided evidence into the implication that a single long distance dispersal event led to the creation of the Tequesquite population (Vandergast, Bohonak, Weissman, & Fisher, 2007).

The Transverse Range has been known to can act as a geographic and isolating barrier for one species but not affect the distribution of a similar species. Burns et al. (2006) examined the phylogeography of the wrentit, *Chamaea fasciata*, across the California Floristic Province. Within mtDNA, they amplified and sequenced protein-coding portions of *ATPase6*, *ATPase8*, *COII*, *COIII* and *cytochrome b*. Researchers were able to identify 6 distinct clades that were restricted geographically. Upon further investigation, they suggested that the wrentit was isolated during the Pleistocene into the Southern refugia, and only recently has the wrentit undergone a range expansion. Researchers compared their data to similar data for the California Thrasher, which reflected a distinct lineage break between the Transverse Ranges, unlike that of the wrentit (Burns & Barhoum, 2006).

Most researchers examine the phylogeography and genetic distribution of terrestrial animals, yet limited studies have been performed on the phylogeography of freshwater vertebrates of the Southern California region. Southern California is composed of numerous drainage and watersheds which could have been influenced by the landscape and geological history of the area. Gene flow between watersheds and drainages is based on periods of connectivity typically resulting from pluvial events.

Phillipsen & Metcalf (2009) examined the patterns of genetic variation that existed among putative landscape barriers across the range of the California Tree Frog, *Pseudacris cadaverina*. Using mtDNA cytochrome b sequences, they found that high levels of genetic differentiation existed in the Southern California populations as revealed by an AMOVA. Gene flow was limited between regional populations due to watersheds, mountain ranges and allopatric fragmentation between coastal and desert populations. Strong support for a Transverse Range Break among *P. cadaverina* was exhibited by their data (Phillipsen & Metcalf, 2009).

The majority of phylogeography studies conducted on the inhabitants of the Southern California region have utilized mtDNA as the molecular marker, and very little has been done using only nuclear DNA (nDNA) or a combination of nDNA and mtDNA. Spinks, Thomson, & Shaffer (2010) examined the phylogeography of the western pond turtle, *Emys marmorata*. Researchers utilized the ND4 site of mtDNA and five nuclear loci to map the phylogeography

within the California region. They utilized five nuclear loci that have relatively low intraspecific variation among populations, and compared them to the ND4 phylogeography trees. Their data suggested a north/south split among populations with an integration of the two Central Coast populations; this was further supported by the evidence of gene flow from the northern populations and the San Joaquin populations into the Central Coast region (Spinks, Thomson, & Shaffer, 2010). This study revealed the applications of nDNA in phylogeographic studies and enrichment as an applicable molecular marker. Appropriate, population level nDNA markers were found to be difficult to acquire in the early years of phylogeography, resulting in most of the published research utilizing mtDNA.

Mitochondrial DNA Disadvantages

Mitochondrial DNA continues to be essential in the field of phylogeography, yet it has many disadvantages that have lead researchers to the identification and development of nuclear DNA markers (Avise, et al., 1987; Avise, 1991; Harrison R., 1989; Moritz, Dowling, & Brown, 1987; Simon, 1991). In animals, mtDNA pseudogenes, have been observed in the nuclear genome, which is highly undesirable in population genetic studies. Pseudogenes are sequences of DNA that exhibit gene-like qualities but are non-functional due to their lack of the ability to code for proteins. Corrections have been established to treat samples that may have mtDNA pseudogenes but these treatments have

shown limited effectiveness (Bensasson, Zhang, Hart, & Hewitt, 2001; Zhang & Hewitt, 1996).

An additional shortcoming of mitochondrial DNA is that it can be highly conserved between individuals of the same species and even those of the same genus. As a result, its uses in population genetic studies can be greatly limited among some animal taxa, such as those belonging to the grasshopper and locust order (Bensasson, Zhang, & Hewitt, 2000). Population structure would be difficult to establish when the mitochondrial genome is nearly identical in every individual of the population or species. The lack of recombination in the mitochondrial genome provides a limited and biased view of the evolutionary lineage of an organism, which could be very different when compared to the overall population or species lineage. In addition, due to the maternally biased evolutionary perspective, population structure could easily be affected by sexbiased dispersal (Bensasson D., Zhang, Hart, & Hewitt, 2001; Zhang & Hewitt, 1996). The maternal inheritance only enables researchers to trace the maternal lineage through phylogenies and evolutionary relationships with other species/taxa (Forbes & Boyd, 1996; Schlötterer & Harr, 2000).

Microsatellites

nDNA is comprised of multiple genetic structures known as chromosomes. nDNA is bi-parentally inherited and in vertebrates encodes for the vast majority of genome when compared to mtDNA. It can be utilized as a molecular marker

when examining population genetics and phylogeography through direct assessment of the sequence variation. Numerous nDNA markers have been identified including restriction fragment length polymorphisms (RFLPs), variable number tandem repeats (VNTRs), and multi-locus and single-locus microsatellites. The rate of synonymous substitution for nDNA is 10⁻⁸ to 10⁻⁹ mutations per generation, making them effectively neutral in the presence of natural selection (Kumar & Subramanian, 2001).

The 1980's gave rise to the molecular revolution which provided researchers new powerful techniques and technologies based on the perception that polymorphisms could be used a molecular markers. A variety of molecular markers were identified on both mitochondrial DNA (mtDNA) as well on nuclear DNA (nDNA) which were utilized in population genetics and conservation biology. Due to the maternal bias in mtDNA, a new fine-scale molecular marker needed to be identified for use in phylogenetic relationships within species as well as interspecies relationships. Microsatellites became an important source of polymorphic genetic markers for the construction of linkage maps, parentage testing, genetic population structure and assessing evolutionary relationships.

Microsatellites are tandem repeats of 1 to 6 base pairs in nDNA scattered across chromosomes. In fish species, microsatellite arise approximately once every 10kbp (O'Connell & Wright, 1997). The lengths of the sequences are often di-, tri-, or tetra- nucleotide repeats which can be repeated up to approximately 100 times at a locus (Epifanio, Johnson, & Kassler, 2003). The repeat number

found at each locus can vary between an individual's alleles but also within the population and/or between species. Since nDNA is bi-parentally inherited (one copy is maternally inherited while the other is paternally inherited), an individual can vary in the number of repeat sequences for each allele, thus can either be homozygous or heterozygous at a specific locus. On this basis, we can use a variety of microsatellite loci to specify fingerprints which are used to identify specific individuals, as well as to employ population genetic analysis directly via heterozygosity (AI-Rabab'ah & Williams, 2002; Garcia-Moreno, Matocq, Roy, Geffen, & Wayne, 1996; Schlötterer & Harr, 2000).

Microsatellite mutation rates in repeat number range from 10⁻⁶ to 10⁻² mutations per generation and are significantly higher than nuclear base substitution rates (O'Connell & Wright, 1997). The proposed mutation mechanism has been called "DNA (replication) slippage". It is assumed that during DNA replication the nascent and template strand realign out of normal alignment. If DNA synthesis continues on this molecule, the repeat number of the microsatellite is altered (Schlötterer & Harr, 2000).

Microsatellite markers are ideal for conservation biology due to their ability to be used to detect bi-parentally inherited polymorphisms as well as recent and past species admixture. Genetic admixture occurs when individuals from two or more previously geographically isolated populations begin interbreeding. Admixture results in the introduction of new genetic lineages into a population. The mutation rates and variability within microsatellite loci play important roles in

determining what type of analysis and research can be done with certain loci (Estoup & Angers, 1998; O'Connell & Wright, 1997)

Microsatellite loci are typically conserved in related taxa, but the repeat range will vary. Researchers can typically use microsatellites identified in a species and use them on related taxa in which microsatellites have not been characterized. Turner, Dowling, Broughton, & Gold (2004) used microsatellite loci that were isolated from one cyprinid fish, the common shiner (Luxilus cornutus) and attempted cross-species amplification in four other cyprinid species. Of the eight loci used in the experiment, five of the microsatellite loci produced "well-resolved, polymorphic and scorable products (Turner, Dowling, Broughton, & Gold, 2004)." Girard & Angers (2006) performed a similar experiment among several *Leuciscinae* species where they attempted crossspecies amplification of 11 microsatellite loci isolated from *Rhinichthys* cataractae (Longnose Dace). Between four to ten loci were successfully amplified in five of the closely related species. Both of these cases provided microsatellite loci amplification across species that did not previously have microsatellite loci identified (Girard & Angers, 2006).

The advantages to the use of microsatellites have led to the gradual replacement of allozyme and mtDNA markers in population level and phylogeographic studies. Microsatellites are easy to amplify, due to their short fragment lengths, and relatively easy to isolate. In addition, very small amounts of tissue are required for the isolation of microsatellites. This allows for non-

lethal sample acquisition which extremely advantageous in populations that are endangered or have small population sizes (McConnell, O'Reilly, Hamilton, Wright, & Bentzen, 1995)

Two models that are appropriate for analyzing microsatellite data are the stepwise mutation model (SMM) and the two-phase model (TPM). The SMM suggests that in the evolution of microsatellites, they can only gain or lose a single repeat. This suggests that those individuals that differ by a single repeat are more closely related to one another than those that differ in larger repeat numbers (Jarne & Lagoda, 1996). The TPM incorporates the mutational processes of the SMM but allows for multiple mutations to occur instead of a single repeat number mutation (Di Rienzo, et al., 1994).

Genetic survey data can be collected and analyzed to infer the demography of a population (Avise, 1994). Population expansion and decline can be evaluated with microsatellite data by examining the level of heterozygosity (repeat numbers) that exists at each locus within a population. Based on the assumptions of mutation models, populations that are in decline reduce allelic diversity faster than heterozygosity or gene diversity (Nei, Maruyama, & Chakraborty, 1975). When the observed diversity in repeat number is greater than the expected diversity, a population may be declining in size. On the other hand, when allelic diversity is in deficit compared to the expected diversity, the population may currently be in a time of expansion (Shi, Kerdelhue, & Ye, 2012). Spatial Analysis of Molecular Variance (SAMOVA) can

then be utilized define groups of populations that are geographically homogeneous and maximally differentiated from each other, and the results can identify genetic barriers between these groups (Dupanloup, Schneider, & Excoffier, 2002).

Microsatellites can also have disadvantages, with the greatest being null alleles. Null alleles are non-amplifying alleles usually attributed to point mutations occurring at a primer annealing site. Microsatellite loci have been found to sometimes include a null allele. If a null allele is present and not accounted for it will create a false homozygote reading when scoring the genotypes. This mis-scoring will lead to a deficiency in the level of heterozygosity observed in the population. Null alleles can also cause erroneous results in the elimination of putative parents or the degree of relatedness between individuals in a kinship analysis (Ardren, Borer, Thrower, Joyce, & Kapuscinski, 1999; McConnell, O'Reilly, Hamilton, Wright, & Bentzen, 1995; O'Reilly & Wright, 1995). An additional disadvantage of microsatellites arises through stutter or shadow bands. During the amplification of a microsatellite loci via PCR, a ladder of bands 1-2 bps apart can be generated on the polyacrylamide gel; these are known as stutter or shadow bands. The bands typically result from slipped-strand mis-pairing that could occur during PCR. These stutter bands can result in mis-scoring of the alleles, yet O'Reilly & Wright (1995) observed that little to no stuttering occurs in tri- and tetra nucleotide repeat sequences.

Microsatellite Studies in Marine and Freshwater Vertebrates

Due to the high mutation rates exhibited by microsatellites, mtDNA is also needed to fully examine the phylogeography of many vertebrate species. mtDNA analysis contributes to the detection of older lineages within a species and can identify the relationships between species through a common ancestor. Microsatellites can provide insight into the population structure and gene flow that currently exists in a population, or changes that have occurred more recently in evolutionary time. Utilizing both of these molecular markers, researchers can shed much light on the evolution of particular species.

In fisheries science and conservation, allozyme and mitochondrial DNA genetic tags were used instead of co-dominant nuclear marker to differentiate and monitor fish populations. However, the problem that arose with these methods were that many required lethal sampling, and low genetic variability was often observed. Researchers in fisheries science have turned to microsatellite markers to examine fish populations. Borer, Miller, & Kapuscinski (1999) used microsatellite loci to examine variability among 186 individuals of walleye (*Stizostedion vitreum*) sampled from northern Minnesota. Based on their data, they were able to determine that there was very little relatedness among the individuals within and between the sampled populations and that within populations, high levels of genetic variation existed (Borer, Miller, & Kapuscinski, 1999).

Ward, Bowers, Hensley, Mobley, & Belouski (2007) examined microsatellite loci in the spotted sea trout (*Cynoscion nebulosus*) that were collected from nine different bays around Texas. Statistically significant differences were observed among the bays in which the upper Texas coast bays clustered together and the lower Texas coast bays clustered together. These findings disagreed with previous allozyme and mtDNA studies. Since allozyme and mtDNA are not fine-scale markers, they may not have been able to detect the different selection pressures currently in place against the populations (Ward, Bowers, Hensley, Mobley, & Belouski, 2007).

Microsatellites have also been used to examine the levels of genetic diversity that exist among populations in order to implement conservation policies. Turner, Dowling, Broughton, & Gold (2004) identified microsatellite loci from the common shiner, *Luxilus cornutus*, which were successfully amplified across four species among the subfamily *Leusicinae*. The microsatellite loci in this study revealed its utility in population and conservation genetics applications for numerous cyprinid fish species (Turner, Dowling, Broughton, & Gold, 2004).

Species diversity and geographic distribution were examined by Fernandes-Matioli, Matioli, & Almeida-Toledo (2000) using microsatellites in the genus *Gymnotus*, a monophyletic group of electrogenic freshwater fish in the Amazon basin. Researchers used microsatellites as flanking primer pairs to see what sequences were amplified in four different *Gymnotus* species. One of their primers amplified species-specific products in which researchers were able to
determine geographic distribution of *G. sylvius*, which was used to further describe the species (Fernandes-Matioli, Matioli, & Almeida-Toledo, 2000).

The California Floristic Province is a region with great biodiversity among terrestrial and aquatic plants and animals. Aguilar & Jones (2009) examined two native California minnows, *Lavina symmetricusl* (roach) and *Lavina exilicauda* (hitch), for nuclear (8 microsatellites) and mitochondrial (control region and ND2) diversification. mtDNA analysis revealed two distinct, highly divergent clades for *L. symmetricus*, representing the Gualala and Pit Rivers. It was estimated that these two clades diverged from an ancestral population of *Lavina* approximately 3-6 million years ago. The problem that arose was that there were no distinct haplotypes that could distinguish the two different species within the Sacramento and San Joaquin region. Upon analysis of the microsatellite data, the subspecies previously described for each species were identified as genetically distinct units and conservation management could then be implemented to manage them as such units (Aguilar & Jones, 2009).

Further investigation needs to be performed on freshwater vertebrate phylogeography for the Southern California region. For this reason, *Rhinichthys osculus*, the speckled dace, would prove to be a valuable species in further examining the divergence patterns that exist among the watershed populations of Southern California using both mtDNA and nDNA. In addition, gene flow can be examined for the drainage systems that currently house populations of *R. osculus*. With this information we can provide further insight into the

phylogeography and geographic distribution of our local populations for conservation and reintroduction use.

Phylogeography of the Speckled Dace

Rhinichthys osculus, the speckled dace, is considered to be one of the most ubiquitous freshwater fish in the Western United States and occupies a variety of environments (Hubbs, Miller, & Hubbs, 1974) from the Columbia Basin in Canada to the Sonora Basin of Mexico (Lee, et al., 1980). In the Western United States, the speckled dace is the only native fish to be represented in the majority of the major drainage systems (Miller R. R., 1958). *Rhinichthys osculus* belongs to the Cyprinidae, one of the most diverse families of freshwater fish. The speckled dace ranges in size from 5-9 cm in length. They have morphological adaptations such as streamlined bodies and large falcate fins that allow them to swim in swift currents but also maintain their position when needed (Smith & Dowling, 2008). The current geographic distribution of the Speckled Dace in California is represented in Figure 1.

The species belonging to the Cyprinidae inhabit a variety of environments including lakes, ponds, creeks, tributaries and even isolated springs across North America. Species of the Cyprinidae family have proven to be relevant models to address environmental and ecological changes from an evolutionary perspective (Girard & Angers, 2006; Scott & Crossman, 1973; Simmons, Berendzen, & Mayden, 2003).

R. osculus was first identified as the western genus *Apocope* which researchers thought contained at least 12 different species. Upon further taxonomic analysis, *Apocope* became a sub-genus and the species are now considered a single-wide ranging species known as *Rhinichthys osculus* (Girard) (Jordan, Evermann, & Clark, 1930; Miller & Miller, 1948). Each geographical population adapted morphological characteristics that were better suited for that environment, giving rise to the vast morphological differentiation that exists between regional populations. Oakey, Douglas, & Douglas (2004) examined the molecular phylogeny of *R. osculus* and compared it to the hydrographic evolution of Western North America. Researchers mapped 112 restriction sites in mtDNA among 59 sampled populations. Upon construction of their phylogenetic trees, two large clades, representing the Colorado River clade and the Snake River clade, were predominant in their results. Within the major clades were smaller sub-basin clades that were created due to aridity, tectonics and elevation (Oakey, Douglas, & Douglas, 2004).

Smith & Dowling (2008) were able to determine that the estimated time of divergence for *R. osculus* from an ancestral fish species occurred approximately 6.3 million years ago (mya) in the Colorado River Basin. Based on mtDNA sequences of the cytochrome b region and ND4 site, they projected that *R. osculus* diverged from the Colorado River basin and into connecting watersheds (including the Los Angeles Basin) approximately 1.9 mya. Within the Colorado River Basin, divergence occurred 1.9-1.3 mya between the upper and lower

basin populations. Once these populations diverged, morphological adaptations were arose for each region based on the current gradient that existed in each habitat (Smith & Dowling, 2008).

R. osculus is the most frequently occurring freshwater fish in the state of Oregon. Pfrender, Hicks, & Lynch (2004) examined a 670 bp segment of cytochrome b in mtDNA which revealed deep divergence and genetic variation among basins. When researchers applied Kimura's molecular clock hypothesis to their data, they were able to attribute the divergence in populations to vicariant events that occurred during the late Miocene and early Pliocene. Two additional mtDNA lineages were discovered to co-occur in the Klamath River Basin which may reflect the isolation and then re-connectivity of the populations within the basin, and provides some insight into the population substructure that exists in some of the Northern Californian populations (Pfrender, Hicks, & Lynch, 2004).

Recently, Hoekzema & Sidlauskas (2014) identified cryptic species of Speckle Dace in the Oregon Great Basin region utilizing both phylogenetics and population genetics. They utilized mtDNA's ND2 region and nuclear DNA's S7 sequence data to further analyze the phylogenetic relationships of the various Speckle Dace populations within the Oregon Great Basin region. Eight microsatellites were used to examine the population genetics of each population. They were able to identify 3 distinct clades within the region and high levels of population structure occurring in an isolated spring (Hoekzema & Sidlauskas, 2014).

One of the largest river basins in the Southern California region is the Santa Ana River watershed, covering 6,900 km² in four different California counties including San Bernardino, Riverside, and Los Angeles (Santa Ana River Watershed Project Authority, 2004; Santa Ana Watershed Association, 2009). The watershed's topography is highly variable with high elevation peaks in the north and east and more arid, semi-desert conditions found to the west along the coastal plains (Santa Ana Watershed Association, 2009).

The Santa Ana Watershed can be divided into the Upper and Lower Watershed. The Upper Watershed is located between San Gorgonio Peak (San Bernardino Mountains) and the Prado Basin, whereas the Lower Basin consists of the regions below Prado Basin to the Pacific Ocean. Within each watershed are tributaries which are habitat for *R. osculus*. Santiago Creek is the only major tributary within the Lower Watershed that previously contained populations of *R. osculus* but those populations are extirpated (Santa Ana River Watershed Project Authority, 2004; Santa Ana Watershed Association, 2009). Indian Creek is also part of the Santa Ana Watershed and is located in the San Jacinto Mountains of the Peninsular Range.

R. osculus carringtonii is the local subspecies that was identified within the Santa Ana River Watershed by Cornelius (1969) based on morphological measurements. He concluded that *R. osculus carringtonii* was more closely related to the populations of *R. osculus* yarrow from Arizona's Virgin River and the Colorado Basin, rather than to populations of *R. osculus carringtonii* found in

Northern California. This was further supported by the research of Oakey, Douglas, & Douglas (2004) suggesting that that Colorado Basin population diverged approximately 1.9 mya and gave rise to the Los Angeles Basin populations.

The Santa Ana River Watershed has gone through many geological and locational changes throughout history. During the end of the last glacial period, the Wisconsinian Glaciation event, many of the rivers within Southern California flooded due to the melting of glacial ice (Colburn, 2006). It was during this time that the Santa Ana River began eroding away the granite formations of the surrounding mountains, including the Peninsular Ranges. As a result of this flooding, many of the tributaries, which would normally be isolated, came into connection with one another. It was during these kinds of events that researchers (Pfrender, Hicks, & Lynch, 2004; Smith & Dowling, 2008) believe connectivity between populations occurred. Once isolation retuned, the populations eventually became genetically structured. *R. osculus* would prove to be a valuable tool for examining population structure among and within tributaries.

The Santa Ana Speckled Dace, one of the rarest native freshwater fish in Southern California, occupies only remnants of its native range (Figure 2) as a result of anthropogenic effects such as urbanization developments and county controlled flood measures. The population within the Los Angeles River system were extirpated in the early 1990's (Santa Ana Watershed Association, 2009;

Santa Ana River Watershed Project Authority, 2004). The populations within the Santa Ana and San Gabriel watersheds are in imminent danger of extirpation. These populations have a very limited range and can be greatly affected by fires and floods (Moyle, Yoshiyama, Williams, & Wikramanayake, 1995).

Government agencies have listed the Santa Ana Speckle Dace as a species of concern in both 1995 (California Department of Fish and Wildlife) and 1998 (United States Forest Service). The Santa Ana Speckled Dace never received federal protection under the Endangered Species Act due to the lack of peer reviewed descriptions of their taxonomic status (Moyle & Marchetti, 2006).

The fires and floods that occurred within the Santa Ana Watershed during 2003-2004 impacted the geographic distribution of *R. osculus*. Prior to the fires and floods, the five northernmost drainages that drain into the Santa Ana River had recorded population of *R. osculus*. These drainages included Lytle Creek, Cajon Creek, Twin/Strawberry Creek, City Creek and Plunge Creek. After the fire and floods occurred, the populations in Twin/Strawberry Creek and City Creek were extirpated. If agencies such as California Department of Fish and Game or the United States Forest Service decide to reintroduce populations back into areas in which *R. osculus* was extirpated or to introduce them into new suitable habitats, an analysis of the genetic variation that occurs between drainages as well as within drainage needs to be performed. This will allow for the stock population to contain a suitable amount of genetic variation to sustain a viable population.

The primary focus of this study is to identify and characterize microsatellite loci that can be used to characterize the genetic variation present within and among the populations of *R. osculus* inhabiting the Santa Ana and San Gabriel River watersheds of Southern California. Utilizing the microsatellite data, the degree of gene flow will be examined to determine if the current lack of connectivity between the drainages has caused each tributary to show patterns of genetic differentiation and population structure. In addition, the Santa Ana Speckled Dace populations will be compared to populations located in the Owens Valley region and those found on the Central Coast of California to provide further insight into the patterns of genetic differentiation of the Santa Ana Speckled Dace. I hypothesize that there is a high level of genetic structure that occurs in the speckled dace. Current mtDNA in Tony Metcalf's lab has shown a 6.33-6.78% genetic difference between the Central Coast and Owens Valley regions to that of Southern California. This suggests independent evolutionary trajectories. Microsatellite loci will be used to further examine this regional structure. I hypothesize that a high level of genetic structure will occur among the three regions due to the lack of gene flow and isolation by distance between each of the three regions.

In this study, specimens of *R. osculus* were obtained from various sample locations throughout the Santa Ana and San Gabriel River Watershed to examine population substructure among and within tributaries in the Southern California region. Previous work has been done our lab to examine the extent of

genetic variation that occurs among mtDNA's *cytochrome b* and *d-loop* region, but this provides only a maternal perspective of the evolutionary lineage for *R*. *osculus* in the Southern California region. Genetic differentiation results from the isolation of a population and the forces that act upon that population resulting in unique genetic structures. Using microsatellite loci genetic diversity and population structure were examined for the Santa Ana Speckled Dace populations providing a complete genetic description of *R. osculus* at the population and individual level, for this region.

The Southern California region has gone through extensive geological and climatal changes throughout its history. This has altered the landscape and topography of the region, during pluvial and arid intervals (Colburn, 2006). Each of these events could alter the evolutionary history of a species by influencing the levels of dispersal as well as gene flow that can exist between populations. Freshwater tributaries within the Santa Ana River Watershed have also undergone such events that which can lead to the divergence of aquatic populations. *R. osculus* was once able to freely inhabit the free flowing and perennial rivers within the Santa Ana River Watershed, but due to changes in climate and geology and more recent anthropogenic causes in the last century, their habitat became discontinuous and fragmented. Currently, due to the discontinuous and fragmented habitats of *R. osculus*, the degree of gene flow between population is very limited, if it exists at all. (Cornelius, 1969; Oakey, Douglas, & Douglas, 2004) I therefore expect to see evidence of historical

admixture when connectivity between the tributaries was possible, but as a result of current anthropogenic effects leading to fragmented habitats, the populations should exhibit a moderate degree of structure as a result of their isolation.

Through my analysis I hope to gain a better understanding of the molecular evolution, phylogeography, population genetics and conservation of the local populations of the Santa Ana Speckled Dace.



Figure 1. Distribution of the Speckled Dace within California. Areas bordered in dark are known to contain Rhinichthys osculus within river drainages of that region. Watershed sampling locations are denoted in red numbered circles. 1=Santa Ana River, 2=San Gabriel River; 3=Santa Maria River and San Luis Obispo River; 4=Owens River. (Map adapted from Nico, L., & Fuller, P. (2015). *Rhinichthys osculus*. Retrieved from USGS Nonindigenous Aquatic Species Database: http://nas.er.usgs.gov/)



Figure 2. Current and Historical Distribution of the Santa Ana Speckled Dace (R. osculus) Map taken from (Santos, N. R., Katz, J. V., Moyle, P. B., & Viers, J. H. (2014). *RHINICHTHYS OSCULUS SUBSPECIES*. Retrieved from UC DAVIS PISCES: http://pisces.ucdavis.edu/content/rhinichthys-osculus-subspecies-2).

CHAPTER TWO MATERIALS AND METHODS

Specimen Collection

In order to evaluate patterns of genetic variation that exist between the various tributaries within the population inhabiting Southern California, the Central Coast of California and the Owen's River Valley, samples of *Rhinichthys* osculus were collected from various sampling sites as illustrated by Figure 1. Samples were acquired in conjunction with the United States Forest Service and California Department of Fish and Wildlife. Additional samples were collected using proper electroshocking techniques in accordance with permits issued to the Metcalf Lab by the United States Forest Service at various sampling locations. For each specimen that was collected by the Metcalf Lab, GPS coordinates were taken from the sampling location on the designated tributary. A minimum of seven *R. osculus* individuals were collected from each tributary. *R. osculus* sampling sites within the Santa Ana Watershed (Southern California population) included Plunge Creek, City Creek, Twin/Strawberry Creek, Cajon Creek, Lytle Creek, Mill Creek, and Indian Creek (Figure 2). The San Gabriel River Watershed (Southern California population) included sampling sites within Cattle Canyon Creek, Fish Creek, North Fork, West Fork, and East Fork of the San Gabriel River (Figure 2). In addition, two samples were obtained from the Hain's River located within the Los Angeles River Watershed (Southern California population) (Figure 2). *R. osculus* specimens representing the Central Coast

population were collected in two different watersheds, the San Luis Obispo Watershed and the Santa Maria Watershed. The San Luis Obispo watershed included specimens collected from San Luis Obispo Creek, Stenner Creek, and Brizziolari Creek (Figure 1). Tributaries within the Santa Maria Watershed, in which R. osculus specimens were collected, was composed of the Cuyama River, Manzana Creek, and Davy Brown Creek (Figure 1). *R. osculus* samples representing the Owen's River Watershed were obtained from Marvin's Marsh and Pine Creek (Figure 1). A list of all samples (n=146) and locations are represented in Table 1.

Molecular Methods

DNA was isolated using phenol-chloroform extraction methods utilizing phase lock gels (PLG) as set forth in Eppendorf's Phase Lock Gel Manual (Mouse Tail Genomic DNA Isolation Protocol) or Qiagen DNeasy kits. For phenol-chloroform extractions using Phase Lock Gel tubes, a 10-50 mg tissue sample was extracted from each specimen and then washed three times to remove ethanol. To further extract ethanol from the tissue, samples were placed under vacuum conditions and left for 90 minutes. Each sample was then placed in a 1.5 milliliter centrifuge tube and digested with a lysis Buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) and proteinase-K in 55°C water bath for at least 12 hours. Genomic DNA was then extracted from the solution using 2 phenol-chloroform-isoamyl (25:24:1) and 1 chloroform-isoamyl (24:1) extractions in Phase Lock Gel (Heavy) tubes. Further treatments of the solution

with sodium acetate and 95% ethanol were utilized. The tubes were then centrifuged to create a genomic DNA pellet in which the remaining solution was then decanted. Samples were air dried for at least 45 minutes and then each DNA extraction was suspended in 150µL of TE Buffer (1mM Tris-HCL, 1mM EDTA, pH 8.0). Genomic DNA extractions were then visualized using agarose gel electrophoresis. Using the Qiagen DNeasy kits, a 10-25 mg piece of tissue was removed from each whole sample specimen. The tissue was then rinsed three times and placed under vacuum conditions for at least 90 minutes in order to fully extract the ethanol from the tissue sample. The sample was then transferred to a sterile 1.5 milliliter microcentrifuge tube where Qiagen's Buffer ATL and proteinase-K were added to the tube in order to digest the tissue sample. The tube was then placed in a 55°C water bath for at least 12 hours. Qiagen's Buffer AL and 95% ethanol were then added to the tube and centrifuged. The solution was then transferred to a Qiagen spin column and collection tube, which was further treated with additional rounds of centrifugation with Qiagen Buffer AW1 and Buffer AW2. Spin columns were transferred to new collection tubes after each round of centrifugation. The spin column was then transferred to a 1.5 milliliter microcentrifuge tube where Qiagen's Buffer AE was added and then the tube was centrifuged; resulting in the extraction of the specimen's genomic DNA. DNA concentrations were analyzed using spectrophotometry (A260/A280 and A234/A260) to ensure the purity and yield of the samples.

Microsatellite loci identification and isolation as well as primer development was performed in conjunction with the Savannah River Ecology Lab. 24 *R. osculus* specimens were used to create an Illumina pair-end shotgun library in which five million successful reads were used to identify 4635 nuclear base pair repeats. Primers were created for these microsatellite loci and they were screened to identify sequences that would occur only once; identifying 48 loci. Eight *R. osculus* samples were used to examine the polymorphic characteristics of the loci in which 23 microsatellite loci appeared as prospective candidates. To further access the reliability and polymorphism of the microsatellite loci, the 23 microsatellite loci were tested across all 24 specimens encompassing various watersheds within California (Nunziata, Lance, Jones, Nerkowski, & Metcalf, 2013). Table 2 summarizes the 23 microsatellite loci that were identified for *R. osculus*.

Once the microsatellite loci had been characterized and identified, each microsatellite locus underwent preliminary screening to determine which loci were to be used in this study. Following a protocol for visualizing microsatellites using unlabeled primer for the long nose dace on 2% metaphor gels (Girard & Angers, 2006), in which a subset of 40 of the 135 *R. osculus* samples were used for amplification of each of the 23 loci. Each 25µL PCR reaction consisted of 1.5µL of 1.5mM of MgCl2, 1µL of each 10µM dNTP solution, 0.5µL of Taq polymerase, 2.5µL of 10x Taq polymerase buffer, 2µL of each primer (5mM), 14.5µL dH2O, and 75ng of genomic DNA. The PCR program, utilizing BioRad

C1000 Thermal Cycler, consisted of an initial denaturing temperature of 92°C for 30s, then 45 cycles of the following profile: 92°C for 30 seconds for denaturation, 15 seconds at the annealing temperature 65°C, and 5 seconds at 68°C. The final phase of the cycle is a 2 minute extension at 68°C. PCR amplicons were then visualized utilizing a 2% METAPHOR© gel and analyzed as to the successfulness of the loci on the 40 samples with the current DNA extractions.

Each of the 146 samples of *R. osculus* underwent PCR amplification for seven of the 23 polymorphic microsatellite loci; for this study Rhos 5, 8, 9, 14, 23, 25 and 29 were utilized based on reliability and repeatability. PCR conditions were optimized and carried out in 10µL volumes: 1.0 µL template DNA (approximately 20-40 ng/µL), Rhos 23, 25, and 29 used 0.6µL of each primer (1mM) (*Rhos8* and 14 used 0.5µL, *Rhos9* used 0.4µL, and *Rhos5* used 0.4µL), 0.2µL Taq polymerase, 1.0µL dNTP's (2mM), 5.0µL buffer (10x buffer with 15mM) MgCl2), and sterile water (molecular grade) brought up each reaction to 10µL. The PCR profile for all reactions, across all seven loci was: 95°C for 5 minutes (initial denaturation), 36 cycles of denaturation at 95°C for 20 seconds, annealing at 65°C for 20 seconds, and extension at 72°C for 30 seconds; and a final extension of 72°C for 3 minutes. 3µL of LI-COR, Inc. Blue Stop Solution was added to each sample and then denatured using the PCR machine at 95°C for 3 minutes. Samples were then immediately placed on ice and then run on a 6.5% polyacrylamide gel using a LI-COR 4300 automated sequencer. Each sample was visualized on three different gels to ensure the accuracy of the microsatellite

scoring. Microsatellite images were visualized, analyzed and scored using SAGA GT software (LI-COR, INC.).

Population Genetics

The goal of this study was to characterize the genetic variation and population structure that occurs among and within the populations of speckled dace within the Southern California region and compare it to those speckled dace populations residing in the Owens River and Central Coast ranges. Within the Southern California populations, genetic variation and population structure were analyzed using four different models: each tributary, between watersheds (Santa Ana and San Gabriel), between mountain ranges, and isolation by distance. The analysis will provide insight into the current and historical gene flow occurring in the Southern California region. In addition, analyzing the microsatellite data for each region within California may provide further insight into the regional population level structure and provide a more complete genetic characterization of the Santa Ana Speckled Dace.

Genetic diversity indices were calculated for each region, watershed, mountain range, and tributary using GENALEX version 6.501 (Peakall & Smouse, 2006;Peakall & Smouse, 2012). This included number of alleles per locus (N_A), observed (H_O) and expected heterozygosity (H_E), allelic richness (AR), percent polymorphic loci, and the number of private alleles.

Genetic differentiation can be examined through an F-statistic created by Wright (1931) known as the F_{ST} . F_{ST} values can range from 0 to 1, where

increasing values represent higher degrees of genetic differentiation. The F_{ST} value compares the level of genetic variation that occurs within the subpopulations and compares it to the level in the overall population (Hartl & Clark, 2006; Wright, 1931). Analogs of F-statistics such as R_{ST} values (Balloux & Goudet, 2002) have been developed to analyze population structure utilizing microsatellite loci; this model accounts for step-wise mutations which are characteristic of such loci. R_{ST} values were examined in GENALEX version 6.501 (Peakall & Smouse, 2006;Peakall & Smouse, 2012). Pairwise F_{ST} values were calculated in GENALEX following Weir & Cockerham (1984).

AMOVA, analysis of molecular variance, is a statistical model that examines patterns of molecular variation within and among population using Fstatistics or its analogs (Excoffier, Smouse, & Quattro, 1992; Excoffier & Slatkin, 1995; Excoffier, Laval, & Schneider, 2005). GENALEX version 6.501 was used to run AMOVAs. Four different AMOVA's were implemented; the first three examined the patterns of genetic variation that occurred within the Southern California region (by tributary, watershed, and mountain range) and the fourth examined the patterns of genetic variation and differentiation between the regions within California. Individuals were assigned to populations in the first AMOVA by the tributary in which they were collected; where each tributary within the Santa Ana and San Gabriel River Watersheds were considered independent populations. Individuals, for the second AMOVA, were assigned to populations based on the watershed in which the tributary was located (Santa Ana vs. San

Gabriel River Watershed in which Hain Creek was considered part of the San Gabriel River due to the small sample size). Individuals for the third AMOVA were assigned to populations based on the mountain region in which the headwaters of the tributary was located (San Jacinto, San Bernardino or San Gabriel Mountains). Finally, the fourth AMOVA assigned the individuals to populations based on the region of collection (Southern California, Central Coast, and Owen's River populations). Significance testing of F-statistic values and variance components were based on 999 permutations and evaluated at the 0.05 probability level.

A Mantel test was performed in GENALEX version 6.501 to examine the correlation between the pairwise F_{ST} and R_{ST} values from the microsatellite data, and geographic distance (km) between populations or regions. Pairwise geographic distances (km) were determined using centroid calculations. Significant results from the Mantel test indicates isolation by distance among the populations sampled.

Patterns of genetic variability and inferred populations (K) were identified using STRUCTURE (version 2.3.4) (Falush, Stephens, & Pritchard, 2003; Falush, Stephens, & Pritchard, 2007, Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard, Stephens, & Donnelly, 2000) K will indicate the number of populations that are best represented based on the genetic variation and similarities in the haplotypes of the microsatellite data. In addition, STRUCTURE will be used to determine the proportion of each individual's genome that arose

from the inferred populations. A parameter was set in which the admixture model was used, with correlated allele frequencies, and LOCOPRIOR (prior location of capture). Under this parameter, 15 independent runs of K = 1-10 (where K represents the number of putative clusters) with an initial burn-in period of 100,000 iterations and a run length of 1,000,000 Markov chain Monte Carlo iterations was performed. STRUCTURE HARVESTER 6.92 (Earl & vonHoldt, 2012) was used to assess the most probable number of clusters represented by the data using the second order rate of change, ΔK , and LnP(D) under the Evanno, Regnaut, & Goudet (2005) method (Appendix C). Utilizing the 15 iterations of the most probable K, data was inputted into CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) using a full-search algorithm. Graphical representation of the STRUCTURE results was created using DISTRUCT (Rosenberg, 2004)

To further examine clusters of genetically similar individuals, a discriminate analysis of principal components (DAPC) was performed in R (version 3.2.0) package *adegenet* (Jombart & Ahmed, 2011). A DAPC does not assume a particular underlying model of gene flow as is used in a principal component analysis (PCoA)

Table 1. *Rhinichthys osculus* Sampling Locations. (n) represents sample size at each location; Mountain Range codes are as follows: San Bernardino (SB), San Gabriel (SG), San Jacinto (SJ), Coastal Ranges (CR), and Eastern Sierra Nevada (SN). Regions are coded as Southern California (SOCAL), Central California (CENTRAL), and Owen's Basin (OWENS).

		MOUNTAIN		
POPULATION	n	WATERSHED	RANGE	REGION
CAJON CREEK	21	SANTA ANA	SB	SOCAL
CITY CREEK	14	SANTA ANA	SG	SOCAL
LYTLE CREEK	28	SANTA ANA	SG	SOCAL
INDIAN CREEK	7	SANTA ANA	SJ	SOCAL
MILL CREEK	5	SANTA ANA	SB	SOCAL
PLUNGE CREEK	21	SANTA ANA	SB	SOCAL
TWIN CREEK	12	SANTA ANA	SB	SOCAL
FISH CREEK	5	SAN GABRIEL	SG	SOCAL
CATTLE CANYON	3	SAN GABRIEL	SG	SOCAL
NORTH FORK SAN GABRIEL RIVER	2	SAN GABRIEL	SG	SOCAL
EAST FORK SAN GABRIEL RIVER	2	SAN GABRIEL	SG	SOCAL
WEST FORK SAN GABRIEL RIVER	1	SAN GABRIEL	SG	SOCAL
HAIN CREEK	2	LOS ANGELES	SG	SOCAL
PINE CREEK	3	OWENS	SN	OWENS
MARVIN'S MARSH	3	OWENS	SN	OWENS
BRIZZOLARI CREEK	3	SAN LUIS OBISPO	CR	CENTRAL
STENNER CREEK	3	SAN LUIS OBISPO	CR	CENTRAL
SAN LUIS OBISPO CREEK	3	SAN LUIS OBISPO	CR	CENTRAL

			MOUNTAIN	
POPULATION	n	WATERSHED	RANGE	REGION
DAVY BROWN CREEK	2	SANTA MARIA	CR	CENTRAL
CUYAMA RIVER	2	SANTA MARIA	CR	CENTRAL
MANZANA CREEK	4	SANTA MARIA	CR	CENTRAL

Table 2: Microsatellite Loci and Primer Information Developed in Conjunction with the Savannah River Ecology Lab. The size (bp) indicates the range of observed alleles in base pairs and includes the length of the CAG tag; TA refers to the annealing temperature where TD65 indicates a touchdown protocol and number of individuals genotyped is N. * indicates CAG tag (5'- CAGTCGGGCGTCATCA-3') label. Loci bolded were used in genotyping the 146 speckled dace samples (Modified from Nunziata, S. O., Lance, S. L., Jones, K. L., Nerkowski, S. A., & Metcalf, A. E. (2013). Developement and characterization of 23 microsatellite markers for Rhinichthys osculus using paired-end Illumina shotgun sequencing. *Conservation Biology Notes*.)

Locus	Primer Sequence 5'> 3'	Repeat motif	Size (bp)	ТА
Rhos1	F: *TTAAATTGTGCCAAATGATGC R: AAGACACATTTGTTTGGAAGGC	AATG	228-236	65
Rhos3	F:*TCAGCTAACCAAATATTGCATGG R:ACAAACGGGAAGGAGCAGG	ATCT	268-436	65
Rhos5	F:*TGGCATTGAGCGAGGTCC R:ACGATATTTAGCTGTCATCATCCG	ATCT	237-313	65
Rhos8	F: *TCGCAAAGATTCACAAACGG R: ATCAGCTCACAATGATCCGC	AAAG	173-281	65
Rhos9	F: CGAGAATGACTCAACATTAACC R: ATGTTGGCACGTGAAAGCC	ATCT	145-185	65
Rhos10	F:*TTGGACAGCTGTATGAATTGGG R:ATTGCAGGACCACCAACACC	ATCT	305-377	65

Locus	Primer Sequence 5'> 3'	Repeat motif	Size (bp)	ТА
Rhos14	F: *GGTGCAGCTTTGAGAGGG R: CCTATATTAAACTCTATGAGCCATAAATCC	ATCT	262-322	65
Rhos16	F:* TGTTACTAATAATCATGTCCTGAAGAGG R: CGCTACTCTGGGTTTGAATGC	AATG	168-312	65
Rhos18	F: *AACTATAACCAGGTGTTACAGTGGG R: CGTAGTACAATGTTACACAATAATAGGC	ATCT	252-360	TD65
Rhos20	F:*GAGGACTGTTTCTATCCCGGC R:CTGGAAATCACAAACCAGGG	ATCT	246-366	65
Rhos21	F: *CCTACAATGTTTGTTTGTTTACACG R: CTTTGAGGAGATTAACTCTTTCCC	AAAG	162-246	65
Rhos22	F: *CAATGTTTCCAATTCTATAACAAAGG R: GAAGACATCAGACATCCAATTTCC	ATCT	365-413	65
Rhos23	F:*TGTGTAAACCAGTAGACTTTCTAATATACC R:GACAATGAAACAATGATTACTTACAGC	ATCT	218-358	65
Rhos25	F: *TGTCAGTATCAGTCTTTCCTTCGC R: GAGCCATATGCATGGAGAGC	AAAG	126-214	65
Rhos26	F: *TGGAATATATCCAGTACCAACTTCC R: GATGGACATAGAACAATGGATGG	ATGG	103-163	65
Rhos27	F:*AAACTGTAACCTAAATGGGTATTAAAGAGG R: GCATGAAACGATGTGAAATAGCC	ATCT	274-330	65

Locus	Primer Sequence 5'> 3'	Repeat motif	Size (bp)	ТА
Rhos29	F: *TCTCAAGCTCTTATTCTGATGATCC R: GCTTACTGTGCTGTCTGTGGC	ATCT	204-264	65
Rhos31	F: *TCATACTGCCGTCTAGTGGTGG R: GTCATCGGGTCAGCAGAGG	АТСТ	173-305	65
Rhos33	F:*AGGTGATGCCACACATGACG R:TCAAAGAATCTGAGCGTCGG	ATCT	324-372	65
Rhos35	F:*TGTTCAACAGGCCTCAAACC R:TCATTACCTTATTAAAGGGACAGTGC	ATCT	302-354	65
Rhos36	F: *TGTTCAACAGGCCTCAAACC R: TCCCTTTATACTTTCAGCTGCTCC	AAAG	144-204	TD65
Rhos42	F: *AAATGAGCAAGTGAGCCAGC R: TGATCATTAGGAAGGATACACTGC	AAAC	140-191	TD65
Rhos43	F: *AGTGGAACATCAGTCACTGCG R: TGATGATATGTGCATCAAGCG	AAC	256-274	TD65

CHAPTER THREE

RESULTS

Genetic Diversity

Population genetic analysis was used to assess the levels of genetic differentiation within and among the Speckled Dace populations of California. Genetic indices including the number of alleles examined (N), alleles for each locus (N_A), observed (H_o) and expected (H_E) heterozygosity and the Fixation Index ($F_{IS} = (He - Ho) / He = 1 - (Ho / He)$) for each of the seven microsatellite loci examined are presented in Table 1. *Rhos5* had a range of 220-308 bp in which 21 alleles were identified among the 146 *R. osculus* samples. *Rhos8* exhibited a range between 160-298 bp, 26 different alleles were identified; *Rhos9* exhibited a range of 117-225 bp, 22 alleles were identified; *Rhos14's* exhibited a range of 117-225 bp, 22 alleles were identified; *Rhos23* exhibited a range of 196-340 bp, 24 alleles identified; *Rhos25* exhibited a range of 108-220 bp, 26 alleles identified; and *Rhos29* exhibited a range of 175-291 bp, 20 alleles were identified.

Mean heterozygosities across the seven loci ranged from 0.333 (Brizzolari) to 1.000 (East Fork and North Fork San Gabriel River). Significant deviations from Hardy-Weinberg expectations occurred in five populations, with two or three of the microsatellite loci analyzed, within all the sampled regions.

Loci deviating from Hardy-Weinberg proportions occurred within the Santa Ana River Watershed only (Table 3).

Table 5 summarizes each tributary's genetic indices including allelic richness (AR), observed (H_o) and expected (H_E) heterozygosity, and the number of private alleles. Private alleles were identified in all of the tributaries within the Southern California populations except for Mill (n=5), Cattle Canyon (n=3) and West Fork of the San Gabriel River (n=1). This may simply be due to the sample size obtained from these populations. The greatest number of private alleles was observed in Plunge Creek

Table 3.	Speckled Dace Populations Deviating from Expected Hardy-Weinberg
Proportio	ons

POPULATION	LOCUS	p-VALUE
INDIAN CREEK	Rhos25	0.021
PLUNGE CREEK	Rhos14	0.009
PLUNGE CREEK	Rhos23	<0.001
TWIN CREEK	Rhos25	0.003
TWIN CREEK	Rhos29	0.019
CITY CREEK	Rhos5	0.008
CITY CREEK	Rhos23	<0.001
CAJON CREEK	Rhos9	0.03
CAJON CREEK	Rhos25	0.012
LYTLE CREEK	Rhos8	0.007
LYTLE CREEK	Rhos9	0.007
LYTLE CREEK	Rhos25	<0.001
Statistics are based on Chi-	-Square Tests for Hardy-We	inberg Equilibrium. Only

loci listed are display significant deviations from expected proportions. All tributaries deviating from Hardy-Weinberg proportions are located in the Southern California region.

POP		Rhos 5	Rhos 9	Rhos 25	Rhos 23	Rhos 14	Rhos 8	Rhos 29
	Ν	123	123	123	123	123	123	123
	Na	19	13	24	17	16	20	18
SOCAL	Ne	11.540	5.227	11.295	8.832	7.610	10.212	7.378
OCOAL	Ho	0.805	0.618	0.626	0.520	0.650	0.724	0.724
	H _e	0.93	0.809	0.911	0.887	0.869	0.902	0.864
	F	0.119	0.236	0.313	0.413	0.251	0.198	0.163
	Ν	6	6	6	6	6	6	6
OWENS	Na	8	8	2	9	7	9	8
VALLEY	Ne	6.000	5.538	1.385	8.000	6.000	7.200	7.200
	Ho	0.833	0.667	0.333	1.000	0.500	1.000	0.333
OWENS	H_{e}	0.833	0.819	0.278	0.875	0.833	0.861	0.861
VALLEY	F	0.000	0.186	-0.200	-0.143	0.400	-0.161	0.613
	Ν	17	17	17	17	17	17	17
	Na	13	10	3	15	4	1	12
CENTRAL	Ne	8.892	5.838	1.966	7.811	1.357	1.000	6.964
COAST	Ho	0.765	0.529	0.353	0.647	0.118	0.000	0.412
	H_{e}	0.888	0.829	0.491	0.872	0.263	0.000	0.856
	F	0.138	0.361	0.282	0.258	0.553	#N/A	0.519
Genetic diver	sity include	s number of s	amples for a	ach region (N) number of	allalas prosor	t for each lo	ci(NI)

Table 4. Genetic Diversity for the Southern California, Owens Valley, and Central Coast Populations of Speckled Dace.

Genetic diversity includes number of samples for each region (N), number of alleles present for each loci (N_a), number of effective alleles (N_e), observed (H_o) and expected (H_e) heterozygosities for each region, and fixation index (F).

Table 5. Santa Ana Speckled Dace Genetic Diversity Summaries For Each of the Tributaries in the Southern California Region. Genetic diversity summaries for each of the 12 tributaries sampled in the Southern California region where the number of samples (n), average allelic richness across all seven loci (AR), observed (Ho) and expected (H_E) heterozygosities, and private alleles (PA) are identified.

POPULATION	n	AR	H _o	H _E	PA
CAJON CREEK	21	9.714	0.782	0.827	2
CITY CREEK	14	8.429	0.673	0.780	2
INDIAN CREEK	7	4.143	0.490	0.536	1
LYTLE CREEK	28	5.429	0.571	0.626	2
MILL CREEK	5	4.286	0.743	0.634	0
PLUNGE CREEK	21	8.714	0.673	0.825	6
TWIN CREEK	12	6.857	0.595	0.711	1
CATTLE CANYON	3	4.857	0.905	0.754	0
FISH CREEK	5	4.714	0.600	0.651	1
NF SAN GABRIEL	2	3.143	1.000	0.643	1
EF SAN GABRIEL	2	3.143	1.000	0.643	2
WF SAN GABRIEL	1	1.714	0.714	0.357	0
HAIN RIVER	2	2.286	0.643	0.500	1

Population Genetic Structure

Geographic structuring among the three regions sampled was evaluated in STRUCTURE HARVESTER utilizing the STRUCTURE runs for K=1-10. The most probable K was identified as K=3 when examining the cluster arrangement between the three regions sampled in California. All STRUCTURE runs of K=3 produced similar membership coefficients (q scores). Geographic subdivision revealed three groups, Central Coast, Southern California and Owens Valley (Figure 8a). The results of the DAPC corroborated the results of STRUCTURE where K=3 (Figure 7) was inferred in which the Southern California, Central Coast and Owens River populations clustered independently of one another, supporting the genetic differentiation observed between the three regions. Each sample was properly assigned to the region it was collected based on the microsatellite data analysis.

The greatest amount of genetic differentiation was observed when comparing the three regions within California (R_{ST} =0.600, p<0.05). 60% of the variation observed in the microsatellite data occurred among the three regions whereas differences within populations accounted for only 40% of the variance (Table 6 and Table 7b). Pairwise F_{ST} values from the AMOVA analysis are summarized in Tables 7a and 8a. A Mantel test found a significant correlation (F_{ST} : R^2 =.1808, p=0.010) between population pairwise F_{ST} values and geographic distance (km), suggesting isolation by distance between the three regions of California (Figure 3). In addition, when accounting for the stepwise

mutation model exhibited by microsatellites, a Mantel test found a significant moderate correlation between pairwise R_{ST} values and geographic distance (R^2 =0.361, p=0.010; Figure 4).

COMPARISON	R _{ST} VALUES	p-VALUES	N _M	
Among all tributaries within Southern California	0.160	0.001	1.373	
Between Santa Ana and San Gabriel River Watersheds	0.151	0.001	1.447	
Between San Jacinto, San Bernardino and San Gabriel Mountain Ranges	0.077	0.001	3.134	
Between Southern California, Central Coast and Owens Valley	0.600	0.001	0.168	
$P_{\rm excluse}$ and their eignificance levels for an everall $\Delta MO V/A$ and then $\Delta MO V/A$'s				

Table 6. R_{ST} and N_M values for Analysis of Molecular Variance Comparisons

R_{ST} values and their significance levels for an overall AMOVA and then AMOVA's analyzing different models that would explain population structure within and among the Santa Ana Speckled Dace populations.

Further geographic structuring was evaluated for the Southern California

populations. STRUCTURE runs of K=1-10 were analyzed in STRUCTURE

HARVESTER where the most probable K was identified as K=2; in which the

Lytle Creek population was appeared to represent a single inferred population

genotype and the remaining populations in Southern California represented an

admixture of both of the inferred populations (Figure 8b). The greatest amount of

admixture with the Lytle population was observed in the most proximal tributary, Cajon Creek, and decreased with geographic distance. The Mantel test revealed a moderate correlation between geographic distance and genetic structure, suggesting isolation by distance is a contributing factor to differentiation among the Southern California populations (F_{ST} : R^2 =0.1808, p=0.010 (Figure 5); R_{ST} : R^2 =0.361, p=0.010(Figure 6)).

Results from the AMOVA analysis indicated significant levels of genetic differentiation occurred in all scenarios examined. The lowest R_{ST} values occurred when analyzing tributaries within Southern California were assigned to populations based on the mountain range in which they reside (R_{ST} =0.070; p<0.05). The majority of the variation, 92.3%, is occurred within the populations and not among them. When analyzing each tributary as its own population, moderate levels of population structure was observed (R_{ST} =0.160; p<0.05). When comparing the tributaries based on watersheds provided similar R_{ST} values (R_{ST} =0.151, p<0.05). In each analysis, 15-16% of the total variation occurred among tributaries or watersheds (Table 6). The remaining 84-85% of the variation could be accounted for within the tributary or watershed, suggesting moderate levels of population genetic differentiation.

Gene flow between speckled dace populations can be examined through the migration rate (N_M). Table 6 illustrates the number of migrants per generation for each of the comparisons utilized in the AMOVA scenarios. Under assumptions of equilibrium, the greatest number of migrants is observed among

mountain range model (N_M =3.134) and the weakest migration rate occurs between the three regions sampled within California (N_M =0.168), suggesting that these regions had little to no gene flow occurring historically. Table 7. (a) AMOVA Pairwise R_{ST} Values Among the Southern California, Owens Valley and Central Coast Regions and (b) AMOVA Pie Graph Representing the Percentage of Molecular Variation Among and Within Sampled Regions.

(a)		SOCAL	OWENS	CENTRAL
	SOCAL	0.000	0.001	0.001
	OWENS	0.406	0.000	0.001
	CENTRAL	0.659	0.412	0.000

Pairwise RST values are displayed below the diagonal and bolded p-values represent significant values based on 999 permutations above the diagonal.

(b)

PERCENTAGES OF MOLECULAR VARIANCE



Table 8 (a) AMOVA Pairwise F_{ST} Values Among the Southern California, Owens Valley and Central Coast Regions and (b) AMOVA Pie Graph Representing the Variation Among and Within Sampled Regions.

(a)		SOCAL	OWENS	CENTRAL
	SOCAL	0.000	0.001	0.001
	OWENS	0.090	0.000	0.001
	CENTRAL	0.197	0.260	0.000

Pairwise FST values are displayed below the diagonal and bolded p-values represent significant values based on 999 permutations above the diagonal.

(b)

PERCENTAGES OF MOLECULAR VARIANCE




Figure 3. Cumulative Pairwise F_{ST} Values Between Each Tributary and Geographic Location For All Sampling Sites Within California. Performing a mantel test showed a moderate level of correlation between genetic and geographic distance (km). (R^2 =0.1808, p<0.001)







GEOGRAPHIC DISTANCE vs F_{st}

Figure 5. Cumulative Pairwise F_{ST} Values Between Each Tributary and Geographic Location For All Sampling Sites Within the Southern California Region. Performing a mantel test showed a moderate level of correlation between genetic and geographic distance (km).



Figure 6. Cumulative Pairwise R_{ST} Values Between Each Tributary and Geographic Location For All Sampling Sites Within California. Performing a mantel test showed a moderate level of correlation between genetic and geographic distance (km) (R^2 =0.2286, p=0.010)



Figure 7. Discriminate Analysis of Principle Components (DAPC) For the First Two Axes Which Identifies K=3. Where 1 represents the southern California populations, 2 represents the Owens River populations; and 3 represents the Central Coast populations.



Figure 8. STRUCTURE Results for (a) Three Genetic Structures (K=3) for the Three Regions Sampled in California; Southern California, Owens Valley and Central Coast. Vertical Bars Represent 146 Speckled Dace Samples, While Color Represents the Proportion of Ancestry From Each Population. (b) Two Genetic Clusters (K=2) That Were Identified From the 123 individuals of the Southern California Santa Ana Speckled Dace Populations.

CHAPTER FOUR DISCUSSION

Microsatellite Analysis

The characterization and identification of the 23 polymorphic microsatellite loci for the Speckled Dace that we established have shown to be successful markers when examining the populations within California. The seven loci that I chose to utilize in this study were able to identify population structure and patterns of gene flow occurring within the Santa Ana Specked Dace but also within the speckled dace populations found in the Central Coast of California and Owens Valley. The numbers of alleles identified per locus (19-26) were comparable to the alleles identified in other microsatellites studies done with the speckled dace (Hoekzema & Sidlauskas, 2014; Kinziger, Nakamoto, Anderson, & Harvey, 2011). In addition, all loci utilized amplified in 100% of the individuals sampled.

Population Structure among the Three Regions

My study revealed a lack of connectivity between the three regions sampled in California. This is some of the first genetic evidence supporting a hypothesis that the Santa Ana Specked Dace populations (Southern California populations) are discontinuous from the other two regions sampled. Current mtDNA studies being performed in Tony Metcalf's lab with *cytochrome b* and *dloop* (Jay VanMeter, unpublished data) suggest a 6-8% genetic difference

between the Southern California populations and those of the Central Coast and Owens Valley, inferring independent evolutionary trajectories. The microsatellite data corroborates the mtDNA evidence, providing a clear picture of the genetic structure among these regional populations.

Smith & Dowling (2008) suggested that the Los Angeles Basin populations diverged from the Colorado Basin ~1.9 mya. They further suggest that the Owen's Valley populations would have colonized that region sometime between the divergence from a sister species of *R. osculus* ~6.3 mya and the colonization event that occurred in the Colorado Basin, ~3.6 mya. The Owen's Valley dace and the Southern California populations should show more similarity to one another than to those on the Central Coast. Microsatellite genotypes suggest that the Speckled Dace populations differentiate into three genetic populations as is supported with the DAPC (Figure 7). Based on this hypothesis, we would expect that the Owen's Valley dace and the Santa Ana Speckled dace to be more similar to one another, in that they diverged from a more recent common ancestor than the common ancestral populations that would be associated the Central Coast populations. Following the same model, we would expect the Owens Valley dace to show less genetic structure with the Central Coast dace due to less divergence events than those events leading to the Southern California populations (Figure 9). This is clearly supported when examining pairwise R_{ST} values which account for the stepwise mutation model exhibited by microsatellites, rather than the infinite allele model observed with F_{ST} values.

Significant, high levels of differentiation between the regional populations is exhibited (Table 7a, $R_{ST} = 0.406-0.659$) but greatest between Santa Ana Speckled Dace and those of the Central Coast ($R_{ST}=0.659$). The AMOVA analysis indicated that 60.0% of the genetic variance observed in the microsatellite loci is due to the difference between each of the regions rather than within each region. Additionally, isolation by distance may be contributing to the structure observed among the sampled regions. This suggests that the gene flow between each of the regions is absent and has been for some time.

It was also hypothesized that the Central Coast populations were established from the migration of the Southern California populations into the region (Cornelius, 1969). The microsatellite data does not support this hypothesis. A high level of population differentiation occurs between the Southern California populations and that of the Central Coast populations (Table 8a: F_{ST} =0.143; Table 7a: R_{ST} =0.659). This would suggest that the Central Coast populations were established from a colonization event that most likely occurred from the population that diverged independently from those that colonized Owens Valley, Colorado Basin, and the Southern California populations. In addition, when examining the membership coefficient of each sampled individual, three inferred populations were identified. The Central Coast populations show minimal admixture with the other two inferred populations further supporting the idea that the central coast populations diverged independently of the populations that colonized the other two regions. Furthermore, the admixture of the two

inferred populations that are observed in the Owens samples supports the conclusion that the Owens Valley populations and the Southern California populations diverged from the same ancestral lineage and the lack of connectivity among watersheds.

The lack of gene flow (Table 6, N_M =0.168) between each of the regions is partially due to geographic distance (Mantel test, R²=0.361, p=0.010) but also landscape barriers such as mountain ranges. The Central coast populations are west of the Sierra Nevada Mountains and north of the Transverse Range; the Owen's populations lie east of the Sierra Nevada Mountains and north of the Transverse Range; whereas the Southern California populations lie south of the Sierra Nevada Mountains and south of the Transverse Mountain range. Historical gene flow would have been restricted by the elevation gradients and landscape barriers presented by these mountain ranges.

Current gene flow is further restricted within each region due to the fragmented habitats created through anthropogenic effects. Due to the limited sample sizes for both Central Coast and Owens Valley populations, further sampling may provide a better insight into the population structure occurring within each of the regions. Since the geographic distance between each of the sampled regions is great, sampling of intermediate zones may provide further insight into the divergence events that led to the genetic diversity and population

structure exhibited among each of the regions.



Figure 9. Diagram Depicting Phylogenic Divergence Events Leading to the Colonization of the Santa Ana Speckled Dace Populations of Southern California. Diagram adapted from Smith, G. R., & Dowling, T. E. (2008). Correlating hydrographic events and divergence times of speckled dace (Rhinichthys: Teleostei: Cyprinidae) in the Colorado River drainage. *The Geological Society of America - Special Paper*, 301-315.

Southern California Populations

Overall, my study has revealed that the Santa Ana Speckled Dace

populations in Southern California exhibit moderate levels of genetic structure.

There is a significant genetic differentiation of the microsatellite genotypes and a

moderate level of population structure correlating to isolation by distance within the Southern California region (Mantel test (F_{ST}/R_{ST})); R^2 =.281/ R^2 =.2286,p=0.01).

This moderate level of population structure was consistent with our expectations due to the highly fragmented habitats that have resulted from both past Pleistocene climate change and more recent anthropogenic effects. During pluvial periods at the end of the Pleistocene, connectivity among the tributaries in the Los Angeles Basin would have been at its peak due to the melting of the Wisconsinian glacier (Colburn, 2006). Up to around a century ago, connectivity still may have been achieved during pluvial events, but then anthropogenic advances led to the restricted flow of most of the tributaries in the region. This would have restricted gene flow between the populations of the Santa Ana Speckled Dace. STRUCTURE analysis indicated that Santa Ana Speckled Dace consists of two ancestral populations (K=2), suggesting that the Southern California populations are not a single panmictic group but rather genetic structure has occurred between the populations.

Four models have been proposed to examine populations structure and gene flow among the Southern California populations of the Santa Ana Speckled Dace; (1) the watershed in which they inhabit (Santa Ana vs San Gabriel River watersheds), (2) the mountain ranges in which the headwaters for each tributary reside (San Jacinto, San Bernardino or San Gabriel Mountains), (3) each tributary is considered its own distinct population, and (4) isolation by distance.

Watershed Model

Watersheds contain the habitats and environmental structures of the tributaries (Frissell, Liss, Warren, & Hurley, 1986). This can isolate organisms to their perspective watersheds and restrict connectivity. Connectivity between the Santa Ana and San Gabriel River watersheds may have occurred historically, but in the last 100 years, flood control has altered many of the original paths in which the tributaries and main rivers flowed (Santa Ana River Watershed Project Authority, 2004). Connectivity between these two watersheds is currently discontinuous due to the fragmented habitats, providing a perfect model to examine current gene flow and population structure. Upon analysis of the microsatellite data utilizing an AMOVA, 15.1% of the genetic variation occurred as a result of differences among the watershed rather than within the watershed, suggesting a moderate level of population structure is occurring between the two watersheds (N_M =1.447).

The San Gabriel watershed's tributaries exhibit very little population structure, providing evidence that the tributaries sampled from this watershed represent a panmictic population (Appendix B, R_{ST} =0.00-0.193). Many of the samples were obtained from locations near the confluences of these tributaries to the San Gabriel River, allowing for possible gene flow between each of the sampled tributaries. Due to the limited sample size obtained from the Hain River (n=2) (Los Angeles River Watershed) and the proximate location to the San Gabriel River, Hain was considered part of the San River Watershed for this



Figure 10. Map Representing the Santa Ana River Watershed Where Colored Stars Represent Confluences of Interest. The red star represents the northeastern most tributaries (Mill, City, Plunge and Twin Creek) confluence; the yellow star represents the Cajon/Lytle confluence; and the green star represents Indian Creek's confluence. Map adapted from (Shannon1. (2015, March 27). *Map of the Santa Ana River basin.* Retrieved from Newkis: http://www.newikis.com/en/commons/File:Santa-ana-river-new.jpg)

study. Very little genetic structure occurred among Hain and the San Gabriel

tributaries (R_{ST}=0.00-0.291), indicating that gene flow would have occurred

among the Los Angeles and San Gabriel River watershed. The pairwise R_{ST}

values for within the San Gabriel tributaries are comparable to those of Hain and

the San Gabriel tributaries further supporting this gene flow model.

Mountain Range Model

The Southern California region is comprised of various mountain ranges that could account for different genetic breaks in organisms. For Santa Ana Speckled Dace populations, three mountain ranges contain the headwaters for the tributaries in question. The San Gabriel Mountains provide the headwaters to Mill, Plunge, Twin, and City Creek. The San Jacinto Mountains are the location for the headwaters to Indian Creek. The San Gabriel Mountains contain the headwaters to all forks of the San Gabriel River, including Fish Creek and Cattle Canyon, as well as Lytle and Cajon Creek of the Santa Ana River watershed. Numerous freshwater organism studies have identified the Transverse Range Break (separating the San Gabriel and San Bernardino Mountains) as a distinct phylogenetic lineage break between populations (Phillipsen & Metcalf, 2009; Spinks, Thomson, & Shaffer, 2010; Chatzimanolis & Caterino, 2007). This lineage break is not well supported by the microsatellite data. The AMOVA indicated that very little to no population structure was observed between mountain ranges. 7.7% of the genetic variance was among mountain ranges and the migration rate was highest (N_M =3.134) in this model. This would suggest that the mountains in which the headwaters for each tributary are located do not act as genetic barriers for Santa Ana Speckled Dace. Cajon and Lytle Creek are part of the Santa Ana watershed and may have had historical connectivity with the other tributaries within the Santa Ana watershed. Therefore, the mountain

range model does not accurately reflect the current geographic structure or the patterns of gene flow.

Tributary Model

Historic connectivity between adjacent streams would have been common prior to the establishment of county-controlled flood measures. As a result of these flood control measures and an increase in human population density within the Southern California region, tributaries have become highly fragmented and isolated from each other. Most of the Santa Ana Speckled Dace populations reside in the highlands of each tributary, preventing further connectivity among tributaries. As a result of their increased isolation from each other, tributary populations are rarely sharing migrants. The greatest levels of significant population structure occur under the tributary model, where each tributary is considered its own population (R_{ST} =0.160). The values most likely indicate a degree of historical population structure, but given the current flood control measures instituted by local county and state agencies, population structure is expected to increase.

Isolation by Distance

Analysis of the microsatellite data has revealed a significant correlation between population structure and geographic distance among the tributaries within the Southern California region (R^2 =0.2286, p-value=0.010; Figure 6), suggesting isolation by distance. The microsatellite data suggests that the populations found within the tributaries that occupy the northeast region of the

Santa Ana watershed (Mill, Plunge, City and Twin) would have had the greatest opportunity for gene flow during pluvial events. In addition, the confluences for each of the tributaries with the Santa Ana River are very close to one another (Figure 10), allowing further opportunities for gene flow ($R_{ST}=0.00-0.222$). Lytle and Cajon Creek are located in the northwestern region of the Santa Ana watershed and are proximate to one another. Greater amounts of gene flow would be expected between these two tributaries as is supported by the microsatellite data (Appendix, R_{ST} =.018). The confluence for Lytle/Cajon is found south of the confluence for the northeastern tributaries (Figure 10), resulting in possible gene flow in the most proximate tributaries to the confluence, but should decline with geographic distance from the confluence (Appendix B). Indian Creek's confluence is the southernmost confluence (Figure 10) within the sample for the Santa Ana watershed. Minimal gene flow would be expected between the northernmost populations and Indian Creek, as is evident with the microsatellite data (Appendix B, R_{ST}=0.146-0.335). This east to west pattern is supported by STRUCTURE (Figure 8b) where the contributions of the two ancestral populations stretch proportionally from Indian Creek in east to Lytle Creek in the west.

Conservation Implications

The Santa Ana Speckled Dace has been listed as a Species of Concern by the California Department of Fish and Wildlife and the United State Forest Service. Due to the effects of fires and floods in the area, some of the populations have more recently become extirpated. The population genetic data provided by this study has important implications in the conservation management strategies for the Santa Ana Speckled Dace. Habitat loss and fragmentation has placed the Santa Ana Speckled Dace on the pathway to possible extinction within the Southern California region. Introduction and reintroduction of the Santa Ana Speckled Dace populations has been attempted by the United States Forest Service within Lytle Creek, but with no recorded success. Since our lab began work on the Santa Ana Speckled Dace, three of the seven tributary populations within the Santa Ana Watershed have become extirpated as a result of fire and floods and may be reintroduced in the future. This study has revealed that moderate levels of population structure occur between each of the tributaries within the Southern California region due to restricted gene flow. Therefore, it is important to consider this when deciding upon a stock population to be utilized for such purposes.

It has been shown that geographic distance and genetic differentiation are moderately correlated with each other. This is important to consider when reestablishing an extirpated population. By choosing a tributary that is in proximal location to the extirpated population, a better representation of the original ancestral alleles that were present in that tributary may be re-established. In addition, the microsatellite data has provided a baseline for the most recent genetic composition of each of the tributaries within the Southern California region. Utilizing this information, we can monitor the changes in the genetic

composition of the populations to further evaluate the effects of habitat fragmentation and isolation on the Santa Ana Speckled Dace populations.

The Santa Ana Speckled Dace populations have shown to be genetically distinct from other regions within California. With further evaluation of the mitochondrial and nuclear genome, being performed by our lab, the Santa Ana Speckled Dace may show to be reciprocally monophyletic for all markers, suggesting that the Southern California population be a distinct taxa, or species.

Conclusions

The speckled dace of the Central Coast, Owens Valley and Southern California have shown to be discontinuous, highly differentiated populations, with no current gene flow occurring between the regions. The data presented in this study supports the findings of Smith & Dowling (2008) suggesting that divergence events led to the Owens Valley, Colorado Basin and Southern California populations. The microsatellite data revealed that the Southern California populations do not share ancestry with the Central Coast populations as was previously hypothesized. Further analysis needs to be performed examining the intermediate ranges between the three geographic regions sampled to provide further insight into the divergent events that led to the colonization of the extant populations.

The Santa Ana Speckled Dace populations within Southern California exhibit moderate levels of population structure and genetic variation. Habitat loss and fragmentation resulting from anthropogenic effects has restricted gene flow

resulting in the greatest levels of genetic differentiation occurring among each tributary within the Southern California region. Current levels of genetic diversity and gene flow have been established for the Santa Ana Speckle Dace. This study shows that the Santa Ana Speckled Dace is minimally a conservation management unit, and has established a genetic profile for each of the tributaries that can be utilized in conservation management strategies to maintain the local biodiversity of the Santa Ana Speckled Dace. APPENDIX A

MICROSATELLITE DATA

RHOS5 ALLELE FREQUENCIES (SOUTHERN CALIFORNIA POPULATIONS)														
Locus	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 5	N	7	5	21	12	14	21	28	3	2	2	5	1	2
	220	0.000	0.000	0.000	0.000	0.000	0.048	0.321	0.000	0.000	0.000	0.100	0.000	0.000
	224	0.000	0.000	0.000	0.208	0.107	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	228	0.000	0.100	0.071	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	232	0.714	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	236	0.071	0.100	0.095	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	240	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.250	0.250	0.000	0.000	0.000
	244	0.000	0.000	0.000	0.000	0.357	0.000	0.000	0.167	0.500	0.000	0.000	0.000	0.000
	248	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	252	0.000	0.200	0.262	0.125	0.036	0.119	0.089	0.333	0.250	0.000	0.100	0.500	0.000
	256	0.214	0.100	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	260	0.000	0.000	0.000	0.083	0.000	0.024	0.054	0.000	0.000	0.000	0.400	0.500	0.750
	264	0.000	0.200	0.095	0.167	0.000	0.167	0.161	0.167	0.000	0.500	0.100	0.000	0.000
	268	0.000	0.000	0.071	0.042	0.000	0.071	0.054	0.000	0.000	0.000	0.000	0.000	0.250
	272	0.000	0.100	0.000	0.042	0.071	0.071	0.000	0.000	0.000	0.250	0.000	0.000	0.000
	276	0.000	0.200	0.238	0.000	0.143	0.143	0.304	0.000	0.000	0.000	0.100	0.000	0.000
	280	0.000	0.000	0.167	0.042	0.000	0.095	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	284	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.167	0.000	0.000	0.100	0.000	0.000
	288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000
	292	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	300	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
	308	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

Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 5	Ν	3	3	3	3	3	2	2	4
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	244	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	248	0.000	0.167	0.167	0.000	0.000	0.000	0.000	0.250
	252	0.000	0.000	0.000	0.000	0.000	0.750	0.250	0.125
	256	0.000	0.167	0.000	0.000	0.000	0.250	0.250	0.375
	260	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
	264	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
	268	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	272	0.333	0.167	0.000	0.000	0.333	0.000	0.250	0.000
	276	0.167	0.000	0.000	0.500	0.000	0.000	0.250	0.000
	280	0.500	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	284	0.000	0.167	0.000	0.167	0.167	0.000	0.000	0.000
	288	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	292	0.000	0.000	0.500	0.000	0.167	0.000	0.000	0.250
	300	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	308	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000

RHOS5 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)



Allele Frequency for RHOS 5

RHOS9	ALLELE I	FREQUI	ENCIES	(SOUTI	HERN C	ALIFOI	RNIA PO	PULAT	TIONS)					
Locus	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 9	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	117	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250
	121	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	125	0.000	0.000	0.000	0.000	0.036	0.048	0.000	0.500	0.000	0.000	0.000	0.000	0.000
	129	0.000	0.000	0.000	0.000	0.071	0.024	0.000	0.000	0.000	0.250	0.100	0.000	0.000
	133	0.000	0.100	0.095	0.583	0.036	0.214	0.018	0.000	0.000	0.250	0.800	0.000	0.000
	137	0.500	0.700	0.452	0.208	0.179	0.071	0.089	0.167	0.500	0.000	0.100	0.500	0.000
	141	0.000	0.200	0.167	0.042	0.143	0.333	0.804	0.167	0.250	0.250	0.000	0.000	0.000
	145	0.071	0.000	0.071	0.042	0.143	0.214	0.089	0.000	0.250	0.250	0.000	0.000	0.750
	149	0.214	0.000	0.000	0.000	0.179	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	153	0.000	0.000	0.000	0.083	0.071	0.024	0.000	0.000	0.000	0.000	0.000	0.500	0.000
	157	0.214	0.000	0.143	0.000	0.036	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	161	0.000	0.000	0.071	0.000	0.071	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	165	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	169	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
	173	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
	177	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
	181	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
	185	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
	189	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
	193	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
	197	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
	225	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

Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 9	Ν	3	3	3	3	3	2	2	4
		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	117	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	121	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	125	0.167	0.500	0.000	0.000	0.000	0.000	0.000	0.000
	129	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	133	0.167	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	141	0.000	0.000	0.000	0.167	0.167	0.000	0.000	0.000
	145	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.125
	149	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	153	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	157	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	161	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000
	165	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	169	0.000	0.000	0.000	0.167	0.000	0.250	0.000	0.375
	173	0.000	0.000	1.000	0.500	0.167	0.000	0.000	0.125
	177	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
	181	0.000	0.000	0.000	0.000	0.000	0.250	0.500	0.125
	185	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.250
	189	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	193	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	197	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000

RHOS9 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)



Allele Frequency for RHOS 9

Locus	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 25	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	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
	112	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
	116	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	128	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	132	0.000	0.000	0.000	0.000	0.036	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.250
	136	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	140	0.000	0.200	0.238	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	144	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	148	0.643	0.000	0.024	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.500
	152	0.000	0.000	0.119	0.083	0.107	0.000	0.054	0.167	0.000	0.250	0.600	0.000	0.250
	156	0.000	0.100	0.000	0.250	0.143	0.071	0.554	0.167	0.000	0.250	0.000	0.000	0.000
	160	0.000	0.600	0.024	0.167	0.036	0.000	0.000	0.333	0.500	0.000	0.000	0.500	0.000
	164	0.000	0.000	0.000	0.000	0.214	0.071	0.000	0.167	0.500	0.000	0.000	0.500	0.000
	168	0.000	0.000	0.167	0.125	0.214	0.143	0.000	0.000	0.000	0.000	0.100	0.000	0.000
	172	0.000	0.100	0.095	0.000	0.107	0.167	0.054	0.167	0.000	0.500	0.200	0.000	0.000
	176	0.000	0.000	0.000	0.000	0.000	0.238	0.161	0.000	0.000	0.000	0.100	0.000	0.000
	180	0.071	0.000	0.095	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	184	0.071	0.000	0.071	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	188	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.000	0.000	0.071	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	196	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	200	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	208	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.024	0.036	0.000	0.000	0.000	0.000	0.000	0.000
	216	0.071	0.000	0.000	0.000	0.000	0.071	0.125	0.000	0.000	0.000	0.000	0.000	0.000
	220	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000

RHOS25 ALLELE FREQUENCIES (SOUTHERN CALIFORNIA POPULATIONS)

Locus	Allele/n	ele/n PINE MARVIN		BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA	
RHOS 25	Ν	3	3	3	3	3	2	2	4	
	108	0.000	0.000	0.667	0.500	1.000	0.500	0.250	0.750	
	112	0.000	0.000	0.333	0.167	0.000	0.500	0.750	0.250	
	116	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000	
	128	0.667	1.000	0.000	0.000	0.000	0.000	0.000	0.000	
	132	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	144	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	148	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	176	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

RHOS25 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)



Allele Frequency for RHOS 25

Locus	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 23	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	196	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
	200	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
	204	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
	208	0.000	0.000	0.000	0.000	0.071	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	216	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
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.200	0.000	0.000
	224	0.000	0.000	0.024	0.000	0.000	0.048	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	228	0.000	0.100	0.167	0.042	0.143	0.262	0.464	0.000	0.000	0.000	0.000	0.000	0.000
	232	0.000	0.400	0.119	0.042	0.000	0.214	0.268	0.167	0.000	0.250	0.000	0.000	0.500
	236	0.929	0.000	0.000	0.042	0.036	0.095	0.214	0.000	0.000	0.000	0.000	0.000	0.000
	240	0.000	0.000	0.000	0.000	0.036	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	244	0.071	0.000	0.143	0.000	0.393	0.167	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	248	0.000	0.000	0.214	0.000	0.036	0.000	0.000	0.167	0.000	0.000	0.500	0.000	0.000
	252	0.000	0.500	0.048	0.125	0.000	0.024	0.000	0.167	0.000	0.250	0.100	0.000	0.000
	256	0.000	0.000	0.286	0.083	0.179	0.024	0.000	0.167	0.000	0.250	0.000	0.000	0.000
	260	0.000	0.000	0.000	0.083	0.107	0.000	0.000	0.000	0.250	0.000	0.000	1.000	0.000
	264	0.000	0.000	0.000	0.458	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
	268	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.167	0.000	0.000	0.200	0.000	0.000
	276	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.500
	280	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
	296	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
	300	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
	304	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
	340	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000

PHOS22 ALLELE EDECLIENCIES (SOLITHEDN CALEODNIA DODUL ATIONS)

Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 23	Ν	3	3	3	3	3	2	2	4
	196	0.000	0.000	0.500	0.167	0.000	0.000	0.000	0.250
	200	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000
	204	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000
	208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	216	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	220	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
	224	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	228	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.250
	236	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.500
	240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	244	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	248	0.167	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	256	0.000	0.333	0.333	0.500	0.500	0.000	0.000	0.000
	260	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	264	0.000	0.167	0.000	0.167	0.000	0.000	0.000	0.000
	268	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	276	0.167	0.000	0.000	0.000	0.000	0.250	0.250	0.000
	280	0.333	0.000	0.000	0.000	0.000	0.250	0.000	0.000
	296	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	300	0.000	0.167	0.000	0.000	0.167	0.000	0.000	0.000
	304	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	340	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

RHOS23 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)

Allele Frequency for RHOS 23



Locus

LOCUS	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 14	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	230	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
	238	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	242	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
	246	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	250	0.071	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	254	0.000	0.000	0.071	0.042	0.036	0.262	0.607	0.000	0.000	0.000	0.000	0.000	0.000
	258	0.000	0.000	0.048	0.000	0.036	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	262	0.071	0.000	0.000	0.292	0.000	0.000	0.000	0.167	0.000	0.000	0.300	0.000	0.000
	266	0.071	0.000	0.190	0.083	0.000	0.143	0.000	0.000	0.250	0.500	0.300	0.000	0.000
	270	0.571	0.000	0.000	0.125	0.107	0.286	0.179	0.167	0.000	0.000	0.300	0.000	0.500
	274	0.143	0.300	0.119	0.125	0.464	0.143	0.196	0.167	0.000	0.000	0.100	0.000	0.500
	278	0.071	0.000	0.071	0.083	0.179	0.048	0.000	0.333	0.250	0.000	0.000	1.000	0.000
	282	0.000	0.700	0.095	0.083	0.071	0.024	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	286	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	290	0.000	0.000	0.095	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	294	0.000	0.000	0.000	0.000	0.107	0.024	0.000	0.000	0.000	0.500	0.000	0.000	0.000
	298	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000
	302	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	314	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

RHOS14 ALLELE FREQUENCIES (SOUTHERN CALIFORNIA POPULATIONS)

Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 14	Ν	3	3	3	3	3	2	2	4
	230	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	238	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	242	0.000	0.000	0.167	1.000	1.000	1.000	1.000	1.000
	246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	254	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	258	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000
	262	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	266	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	270	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	274	0.000	0.333	0.500	0.000	0.000	0.000	0.000	0.000
	278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	282	0.000	0.167	0.167	0.000	0.000	0.000	0.000	0.000
	286	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	294	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	298	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	302	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	314	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000

RHOS14 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)



Allele Frequency for RHOS 14
LOCUS	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 8	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	160	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
	168	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
	172	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
	176	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
	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
	184	0.000	0.000	0.000	0.125	0.179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	188	0.000	0.000	0.000	0.000	0.214	0.119	0.339	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.500	0.200	0.167	0.000	0.036	0.024	0.000	0.000	0.000	0.000	0.000	0.500	0.000
	196	0.000	0.000	0.119	0.083	0.179	0.357	0.268	0.000	0.000	0.500	0.000	0.000	0.000
	200	0.000	0.300	0.190	0.708	0.107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	204	0.357	0.000	0.286	0.000	0.071	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.250
	208	0.000	0.000	0.000	0.000	0.071	0.071	0.036	0.000	0.000	0.250	0.100	0.000	0.000
	212	0.143	0.000	0.000	0.083	0.036	0.238	0.161	0.167	0.000	0.000	0.200	0.000	0.000
	216	0.000	0.300	0.000	0.000	0.000	0.071	0.000	0.167	0.000	0.000	0.000	0.000	0.250
	220	0.000	0.100	0.119	0.000	0.107	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000
	224	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.167	0.250	0.000	0.200	0.000	0.000
	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.250	0.000	0.100	0.000	0.000
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.250	0.000	0.000	0.000
	236	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.500
	240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.500	0.000
	244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.000
	256	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	260	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
	264	0.000	0.100	0.000	0.000	0.000	0.024	0.143	0.000	0.000	0.000	0.000	0.000	0.000
	268	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	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

RHOS8 ALLELE FREQUENCIES (SOUTHERN CALIFORNIA POPULATIONS)

кпозо	ALLELE F	KEQUEIN	LIES (CENTI	AL AND OW	ENS FOFUI	LATIONS)			
Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 8	Ν	3	3	3	3	3	2	2	4
	160	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	168	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	172	0.167	0.333	0.000	0.000	0.000	0.000	0.000	0.000
	176	0.167	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	180	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	184	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	200	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	208	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	228	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	256	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	260	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	264	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	268	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	298	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000

RHOS8 ALLELE FREQUENCIES (CENTRAL AND OWENS POPULATIONS)



Allele Frequency for RHOS 8

RHOS29 AI	LELE FR	EQUEN	VCIES (S	SOUTH	ERN CA	ALIFOR	NIA PO	PULAT	TONS)					
LOCUS	Allele/n		IND	PLNG	TWIN	CITY	CAJON	LYTLE	CATTLE	EFSGR	NFSGR	FISH	WFSGR	HAIN
RHOS 29	Ν	7	5	21	12	14	21	28	3	2	2	5	1	2
	175	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
	191	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	195	0.071	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	199	0.357	0.100	0.024	0.000	0.036	0.167	0.000	0.000	0.250	0.000	0.000	0.000	0.000
	203	0.071	0.200	0.238	0.042	0.000	0.024	0.000	0.167	0.000	0.000	0.500	0.000	0.500
	207	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
	211	0.000	0.000	0.000	0.500	0.000	0.048	0.250	0.000	0.000	0.000	0.000	0.000	0.000
	215	0.000	0.300	0.143	0.042	0.000	0.071	0.000	0.000	0.000	0.000	0.100	0.000	0.000
	219	0.286	0.100	0.119	0.167	0.036	0.000	0.000	0.167	0.000	0.000	0.100	0.000	0.500
	223	0.000	0.000	0.095	0.083	0.000	0.024	0.000	0.000	0.000	0.000	0.100	0.500	0.000
	227	0.000	0.100	0.214	0.167	0.607	0.310	0.482	0.000	0.250	0.000	0.000	0.000	0.000
	231	0.000	0.000	0.024	0.000	0.071	0.071	0.000	0.333	0.250	0.000	0.200	0.000	0.000
	233	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
	235	0.214	0.000	0.000	0.000	0.214	0.048	0.000	0.000	0.000	0.500	0.000	0.500	0.000
	239	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.167	0.250	0.000	0.000	0.000	0.000
	247	0.000	0.000	0.024	0.000	0.036	0.119	0.250	0.000	0.000	0.000	0.000	0.000	0.000
	255	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
	277	0.000	0.000	0.024	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	291	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

RHOS29	ALLELE	FREQUEN	CIES (CENT	RAL AND OW	ENS POPU	LATIONS)			
Locus	Allele/n	PINE	MARVIN	BRIZZOLARI	STENNER	SAN LUIS OBI	DAVY BROWN	CUYAMA	MANZANA
RHOS 29	Ν	3	3	3	3	3	2	2	4
	175	0.167	0.000	0.000	0.000	0.000	0.250	0.000	0.000
	187	0.000	0.333	0.000	0.000	0.000	0.000	0.250	0.000
	191	0.000	0.167	0.167	0.167	0.167	0.000	0.000	0.000
	195	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
	199	0.000	0.333	0.000	0.333	0.333	0.000	0.000	0.000
	203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	207	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	215	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	219	0.000	0.000	0.000	0.000	0.000	0.000	0.500	1.000
	223	0.000	0.000	0.333	0.000	0.000	0.250	0.000	0.000
	227	0.333	0.000	0.000	0.333	0.333	0.000	0.000	0.000
	231	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000
	233	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000
	235	0.000	0.000	0.167	0.000	0.000	0.000	0.250	0.000
	239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	247	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	255	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	277	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
	291	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000



Allele Frequency for RHOS 29

APPENDIX B

PAIRWISE CHARTS

GEOGRAPHIC DISTANCE – 21 POPULATIONS

IND	MILL	PLNG	TWIN	CITY	CAJO N	LYTLE	CATTL	EFSG R	NFSG R	FISH	WFSG R	HAIN	PINE	MAR V	BRIZ	STEN	SLO	DB	CUY	MZN A	
0.000																					IND
40.35																					мш
9	0.000																				IVILL
45.76																					PLUN
4 57.00	5.856	0.000																			GE
57.90	18.79	12.93	0.000																		TWIN
70.67	30 52	24 92	13 59																		
2	2	1	3	0.000																	CITY
72.45	34.73	28.92	16.11	12.95																	CAJO
3	6	3	3	5	0.000																N
75.14	38.28	32.54	19.94	16.94																	INTLE
4	8	0	3	6	4.285	0.000															
97.54	64.76	59.33	47.50	43.99	32.32	28.06															CATTL
5	5	2	0	4	9	9	0.000														
125.2	94.53	89.20	77.51	/3.3/	62.23	58.01	30.01	0.000													EFSGR
20 125 2	94 53	4 89 20	2 77 51	73 37	62.23	58.01	30.01	0.000													NESG
28	4.55	4	2	, 5.57	9	1	50.01	0.000	0.000												R
106.2	76.75	71.65	60.65	58.60	46.40	42.12	15.11	19.08	19.08												
39	7	9	4	2	7	6	5	3	3	0.000											FISH
125.2	94.53	89.20	77.51	73.37	62.23	58.01	30.01			19.08											WFSG
28	5	4	2	6	9	1	6	0.000	0.000	3	0.000										R
139.2	110.2	104.0	93.62	89.89	78.58	74.33	46.26	16.56	16.56	35.59	16.56										HAIN
80	40	40	8	5	6	0	5	9	9	5	9	0.000									
399.1	359.7	355.1	345.2	331.6	335.4	334.9	330.0	324.4	324.4	353.3	324.4	326.4	0.000								PINE
85 121 C	54 205 1	200.4	200 5	21	270.6	270.1	264 7	2502	250.2	260.6	2502	250.7	0.000								
434.0	62	90.4	380.5	200.9 42	15	06	14	52	530.2	05	52	68	1	0 000							MAR
385.7	352.7	346.8	334.1	325.8	318.0	314.3	288.7	260.5	260.5	279.6	260.5	247.3	297.8	313.1	0.00						
78	59	59	59	34	47	21	04	87	87	36	87	12	91	13	0						BRIZ
387.6	354.3	348.6	335.9	327.5	319.8	316.0	290.5	262.4	262.4	281.4	262.4	249.1	297.7	312.7	2.01	0.00					STEN
09	54	31	24	66	11	91	10	27	27	79	27	82	20	31	8	0					STEN
382.9	349.8	344.0	331.3	323.0	315.2	311.5	285.9	257.7	257.7	276.8	257.7	244.4	297.5	313.0	2.89	4.88	0.00				SLO
55	01	84	91	94	79	47	01	59	59	05	59	62	36	61	2	3	0				010
302.7	272.0	266.5	254.2	247.5	238.2	234.2	207.3	178.0	178.0	196.7	178.0	163.4	306.5	330.4	88.1	90.1	85.3	0.00			DB
202.1	43	264.2	251.6	08 242 F	58 225 5	78	26	177.0	177.0	26	177.0	76	94	202.4	90	91	70.9	28.0	0.00		
3U3.1 /13	270.0	204.3 15	251.0 56	243.5 80	235.5 //0	231./ 85	206.0	111.9	1//.9	18/10	111.9 Z	104.9 51	279.U 80	302.4 12	82.0 56	84.4 72	/9.8 /0	28.0	0.00		CUYA
302.0	271 3	265.8	253 5	246.7	237 5	233 5	206.6	177 २	177 २	40 196 0	ده ۲77 ۹	162.8	306.7	330 3	88.7	90.7	85.9	0.67	27 9	0.00	
80	64	32	34	31	79	99	48	30	30	48	30	01	97	52	92	92	09	9	77	0.00	MZNA

PAIRWISE R_{ST} VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR 21 POPULATIONS

IND	MILL	PLN	TWN	CITY	CAJON	LYTLE	CATTL	EFSG	NFSG	FISH	WFSG	HAIN	PINE	MARV	BRIZ	STEN	SLO	DB	CUY	MZNA	
0.000	0.002	0.002	0.001	0.005	0.001	0.004	0.004	0.001	0.019	0.001	0.003	0.002	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001	IND
0.222	0.000	0.397	0.002	0.002	0.007	0.011	0.175	0.003	0.397	0.076	0.084	0.275	0.001	0.004	0.001	0.001	0.001	0.002	0.001	0.001	MILL
0.146	0.000	0.000	0.002	0.001	0.001	0.001	0.320	0.002	0.417	0.046	0.270	0.298	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	PLNG
0.335	0.208	0.098	0.000	0.001	0.001	0.001	0.012	0.001	0.319	0.001	0.074	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	TWIN
0.149	0.222	0.130	0.215	0.000	0.002	0.003	0.001	0.001	0.218	0.001	0.053	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	CITY
0.199	0.154	0.080	0.198	0.148	0.000	0.102	0.015	0.001	0.144	0.001	0.016	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	CAJON
0.158	0.158	0.112	0.232	0.124	0.018	0.000	0.078	0.001	0.161	0.002	0.053	0.017	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	LYTLE
0.315	0.067	0.015	0.205	0.250	0.161	0.092	0.000	0.034	0.466	0.358	0.457	0.460	0.001	0.013	0.002	0.001	0.002	0.001	0.012	0.001	CATTL
0.598	0.483	0.380	0.425	0.540	0.548	0.467	0.305	0.000	0.137	0.001	0.269	0.068	0.007	0.083	0.006	0.003	0.004	0.026	0.016	0.004	EFSGR
0.273	0.000	0.000	0.027	0.048	0.088	0.069	0.000	0.211	0.000	0.149	0.346	0.290	0.180	0.096	0.006	0.006	0.004	0.032	0.027	0.003	NFSGR
0.422	0.126	0.079	0.313	0.416	0.235	0.183	0.000	0.441	0.126	0.000	0.057	0.375	0.001	0.002	0.002	0.001	0.001	0.003	0.001	0.001	FISH
0.386	0.174	0.031	0.143	0.179	0.222	0.149	0.000	0.125	0.000	0.193	0.000	0.265	0.080	0.114	0.008	0.001	0.012	0.015	0.035	0.001	WFSGR
0.382	0.052	0.027	0.308	0.396	0.308	0.237	0.000	0.291	0.035	0.000	0.108	0.000	0.181	0.298	0.005	0.008	0.006	0.022	0.039	0.004	HAIN
0.415	0.292	0.278	0.397	0.413	0.472	0.430	0.279	0.287	0.088	0.382	0.131	0.097	0.000	0.456	0.006	0.024	0.041	0.040	0.082	0.002	PINE
0.414	0.319	0.349	0.418	0.521	0.549	0.518	0.283	0.193	0.195	0.362	0.190	0.051	0.000	0.000	0.003	0.004	0.001	0.016	0.024	0.001	MARV
0.727	0.728	0.637	0.771	0.744	0.712	0.654	0.746	0.772	0.650	0.770	0.720	0.679	0.267	0.439	0.000	0.101	0.109	0.102	0.263	0.058	BRIZ
0.736	0.766	0.627	0.768	0.759	0.714	0.653	0.787	0.805	0.718	0.794	0.786	0.731	0.309	0.354	0.148	0.000	0.457	0.293	0.441	0.150	STENNER
0.719	0.718	0.618	0.737	0.740	0.709	0.651	0.716	0.732	0.623	0.743	0.690	0.633	0.197	0.310	0.150	0.010	0.000	0.169	0.487	0.021	SLO
0.705	0.753	0.640	0.768	0.742	0.722	0.653	0.750	0.739	0.657	0.783	0.723	0.682	0.273	0.302	0.212	0.000	0.147	0.000	0.410	0.274	DB
0.683	0.727	0.596	0.746	0.721	0.683	0.611	0.738	0.754	0.637	0.763	0.726	0.665	0.226	0.299	0.070	0.000	0.000	0.000	0.000	0.412	CUY
0.762	0.809	0.664	0.807	0.773	0.730	0.657	0.834	0.860	0.785	0.835	0.842	0.808	0.454	0.531	0.170	0.112	0.276	0.067	0.000	0.000	MZNA

PAIRWISE F_{ST} VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR 21 POPULATIONS

IND	MILL	PLNG	TWIN	CITY	CAJON	LYTLE	CATTL	EFSGR	NFSGR	FISH	WFSGR	HAIN	PINE	MARV	BRIZ	STEN	SLO	В	CUY	MZNA	
0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	IND
0.304	0.000	0.001	0.001	0.001	0.001	0.001	0.003	0.002	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.001	MILL
0.202	0.088	0.000	0.001	0.001	0.001	0.001	0.004	0.005	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	PLNG
0.296	0.173	0.128	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	TWIN
0.237	0.165	0.081	0.160	0.000	0.001	0.001	0.004	0.025	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	CITY
0.208	0.147	0.073	0.138	0.066	0.000	0.001	0.001	0.001	0.063	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	CAJON
0.346	0.266	0.189	0.252	0.175	0.090	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	LYTLE
0.254	0.097	0.059	0.136	0.078	0.069	0.219	0.000	0.468	0.067	0.002	0.007	0.006	0.007	0.002	0.003	0.001	0.003	0.003	0.004	0.001	CATTL
0.286	0.133	0.080	0.178	0.072	0.093	0.254	0.000	0.000	0.061	0.001	0.056	0.020	0.018	0.010	0.006	0.003	0.005	0.037	0.019	0.005	EFSGR
0.312	0.177	0.089	0.144	0.102	0.044	0.217	0.061	0.094	0.000	0.002	0.025	0.032	0.008	0.008	0.006	0.003	0.005	0.024	0.031	0.001	NFSGR
0.319	0.237	0.127	0.160	0.192	0.138	0.301	0.097	0.193	0.134	0.000	0.005	0.005	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	FISH
0.396	0.294	0.208	0.286	0.224	0.240	0.393	0.186	0.170	0.309	0.328	0.000	0.025	0.007	0.006	0.004	0.006	0.005	0.017	0.033	0.005	WFSGR
0.291	0.248	0.157	0.242	0.185	0.140	0.309	0.139	0.187	0.195	0.188	0.396	0.000	0.004	0.002	0.004	0.008	0.003	0.017	0.034	0.003	HAIN
0.306	0.194	0.094	0.162	0.118	0.109	0.275	0.105	0.122	0.122	0.170	0.314	0.223	0.000	0.003	0.001	0.005	0.002	0.001	0.004	0.001	PINE
0.327	0.240	0.166	0.231	0.168	0.165	0.318	0.137	0.182	0.151	0.251	0.365	0.263	0.095	0.000	0.001	0.004	0.005	0.002	0.009	0.001	MARV
0.430	0.344	0.235	0.314	0.252	0.244	0.384	0.251	0.320	0.311	0.336	0.472	0.388	0.308	0.322	0.000	0.027	0.001	0.005	0.013	0.001	BRIZ
0.413	0.333	0.209	0.296	0.235	0.216	0.349	0.247	0.284	0.275	0.324	0.462	0.365	0.280	0.302	0.171	0.000	0.301	0.018	0.016	0.001	STEN
0.432	0.361	0.239	0.324	0.258	0.244	0.384	0.278	0.316	0.320	0.365	0.501	0.417	0.309	0.332	0.231	0.024	0.000	0.003	0.007	0.001	SLO
0.427	0.333	0.218	0.303	0.266	0.228	0.370	0.223	0.265	0.298	0.331	0.449	0.382	0.275	0.331	0.281	0.151	0.186	0.000	0.376	0.003	DB
0.371	0.315	0.217	0.293	0.242	0.219	0.360	0.217	0.266	0.252	0.322	0.457	0.333	0.265	0.309	0.284	0.135	0.199	0.036	0.000	0.048	CUY
0.425	0.414	0.295	0.360	0.334	0.296	0.419	0.345	0.424	0.421	0.416	0.567	0.450	0.404	0.431	0.330	0.238	0.248	0.205	0.101	0.000	MZNA

PAIRWISE RST VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR SOUTHERN CALIFORNIA WATERSHEDS

SANTA ANA SAN GABRIEL

0.000	0.001	SANTA ANA
0.147	0.000	SAN GABRIEL

Rst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.

PAIRWISE FST VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR SOUTHERN

CALIFORNIA WATERSHEDS

SANTA ANA	SAN GABRIEL	

0.000	0.001	SANTA ANA
0.053	0.000	SAN GABRIEL

107

Fst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.

PAIRWISE R_{ST} VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR SOUTHERN

CALIFORNIA MOUNTAIN RANGES

SAN JACINTO	SAN BERNARDINO	SAN GABR	IEL
0.000	0.002	0.001	SAN JACINTO
0.135	0.000	0.001	SAN BERNARDINO
0.141	0.060	0.000	SAN GABRIEL

Rst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.

PAIRWISE R_{ST} VALUE BELOW DIAGNOL AND p-VALUE ABOVE DIAGANOL FOR SOUTHERN

CALIFORNIA MOUNTAIN RANGES

SAN JACINTO	SAN BERNARDINO	SAN GABRIEL	
0.000	0.001	0.001	SAN JACINTO
0.183	0.000	0.001	SAN BERNARDINO
0.215	0.059	0.000	SAN GABRIEL

Fst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.

APPENDIX C

INPUT FILES

GENALEX MICROSATELLITE DATA FILE – 21 POPULATIONS

7	147	21	7	5	21	12	14	21	28	3	2	2	5	2	2	3	3	3	3	3	2	2	4	1	14 7
			INDI AN CK	MI LL	PLU NG E	TWI N	CIT Y CK	CAJ ON	LY TL E	CATT LE CAN	EF SAN GAB	NF SAN GAB	FI S H	WF SAN GAB	H Al N	PI N E	MAR VIN	BRIZZ OLARI	STE NNE R	SAN LUIS OBI	DAVY BROW N	CUY AM A	MAN ZAN A		CA LIF O
Sam ple	Рор	RH OS 5		RH OS 9		RH OS 25		RH OS 23		RHOS 14		RHOS 8		RHOS 29			х	Y							
INDC K-01	INDIA N CK	23 2	232	13 7	137	144	180	236	23 6	270	274	204	2 1 2	203	21 9		33.7 6196 4	- 116.8 83911							
INDC K-02	INDIA N CK	23 2	236	13 7	157	148	148	236	23 6	270	278	192	2 1 2	199	19 9		33.7 6196 4	- 116.8 83911							
INDC K-03	INDIA N CK	23 2	256	14 9	157	148	148	236	23 6	250	270	204	2 0 4	219	23 5		33.7 6196 4	- 116.8 83911							
INDC K-04	INDIA N CK	23 2	232	14 5	149	148	148	236	23 6	270	274	192	2 0 4	235	23 5		33.7 6196 4	- 116.8 83911							
INDC K-05	INDIA N CK	23 2	232	13 7	137	128	148	236	24 4	262	266	192	2 0 4	199	21 9		33.7 6196 4	- 116.8 83911							
INDC K-06	INDIA N CK	23 2	232	14 9	157	148	148	236	23 6	270	270	192	1 9 2	195	19 9		33.7 6196 4	- 116.8 83911							
INDC K-07	INDIA N CK	25 6	256	13 7	137	184	216	236	23 6	270	270	192	1 9 2	199	21 9		33.7 6196 4	- 116.8 83911							
MILL -1	MILL	25 2	256	13 7	141	156	160	232	25 2	282	282	192	2 0 0	219	22 7		34.0 7778 9	- 117.0 9945							
MILL -2	MILL	25 2	264	13 7	141	160	160	232	25 2	274	282	216	2 1 6	203	20 7		34.0 7778 9	- 117.0 9945							
MILL -3	MILL	26 4	276	13 7	137	160	160	228	25 2	282	282	192	2 6 4	207	21 5		34.0 7778 9	- 117.0 9945							
MILL -4	MILL	23 6	276	13 7	137	140	172	232	23 2	274	282	200	2 2 0	199	21 5		34.0 7778 9	- 117.0 9945							
MILL -5	MILL	22 8	272	13 3	137	140	160	252	25 2	274	282	200	2 1 6	203	21 5		34.0 7778 9	- 117.0 9945							
PC- 01	PLUNG E	25 2	252	13 3	137	140	188	228	22 8	238	238	192	1 9 6	223	22 7		34.1 0891 7	- 117.1 5075							

PC- 02	PLUNG E	23 6	280	13 7	137	116	116	256	25 6	238	238	192	1 9 6	223	22 7	34.1 0891 7	- 117.1 5075	
PC- 03	PLUNG E	22 8	264	13 7	141	180	192	248	24 8	238	238	204	2 2 0	227	22 7	34.1 0891 7	- 117.1 5075	
РС- 04	PLUNG E	25 2	264	13 7	157	140	152	224	24 8	302	302	200	2 0 4	203	21 5	34.1 0891 7	- 117.1 5075	
PC- 05	PLUNG E	25 2	276	14 1	157	140	160	256	25 6	278	286	204	2 2 4	223	22 3	34.1 0891 7	- 117.1 5075	
PC- 06	PLUNG E	25 2	280	13 7	157	140	180	256	25 6	254	282	204	2 6 8	195	21 9	34.1 0891 7	- 117.1 5075	
РС- 07	PLUNG E	26 4	280	13 7	137	140	180	232	23 2	274	258	220	2 6 8	203	22 7	34.1 0891 7	- 117.1 5075	
PC- 08	PLUNG E	25 2	280	13 3	145	152	152	228	25 6	286	286	192	2 0 4	231	20 3	34.1 0891 7	- 117.1 5075	
PC- 09	PLUNG E	25 2	280	13 7	137	152	192	232	23 2	254	274	196	2 0 4	203	22 7	34.1 0891 7	- 117.1 5075	
РС- 10	PLUNG E	27 6	276	13 3	137	140	140	228	22 8	254	266	192	2 2 0	203	24 7	34.1 0891 7	- 117.1 5075	
PC- 11	PLUNG E	22 8	268	13 7	141	168	168	244	24 4	278	282	200	2 0 4	227	27 7	34.1 0891 7	- 117.1 5075	
PC- 12	PLUNG E	22 8	268	13 3	137	168	192	248	24 8	266	278	192	2 0	195	20 3	34.1 0891 7	- 117.1 5075	
PC- 13	PLUNG E	23 6	280	13 7	145	136	184	248	24 8	246	274	200	2 0 0	215	22 7	34.1 0891 7	- 117.1 5075	
РС- 14	PLUNG E	25 2	280	13 7	137	168	180	252	25 6	274	274	196	2 0 4 2	215	21 5	34.1 0891 7 24.1	- 117.1 5075	
РС- 15	PLUNG E	25 2	276	14 1	141	168	168	228	25 6	290	290	200	2 0 0 2	175	17 5	0891 7 24.1	- 117.1 5075	
РС- 16	PLUNG E	23 6	252	14 1	161	148	168	232	25 2	282	282	192	2 0 0 2	199	20 3	0891 7 24.1	- 117.1 5075	
PLN G-1	PLUNG E	23 6 25	276	13 7	141	140	184	248	24 8 25	266	290	200	2 0 4 2	203	20 3 21	0891 7	117.1 5075	
G-2	E	25	268	7	157	140	172	228	6	258	290	204	2 6	215	9	0891	- 117.1	

													8			7	5075
PLN G-3	PLUNG E	27 6	276	13 7	145	140	184	244	24 4	266	286	192	2 0 4	219	22 7	34.1 0891 7	- 117.1 5075
PLN G-4	PLUNG E	26 4	276	13 7	161	172	172	244	24 4	266	266	204	2 2 0	215	21 9	34.1 0891 7	- 117.1 5075
PLU N-1	PLUNG E	27 6	276	15 7	161	152	172	256	25 6	266	266	196	2 2 4	203	21 9	34.1 0891 7	- 117.1 5075
TWI N-01	TWIN	25 6	272	13 3	133	152	152	264	26 4	262	262	184	1 9 6	219	22 7	34.1 7472 2	- 117.2 66667
TWI N-02	TWIN	22 4	224	13 3	137	192	192	232	26 4	262	290	184	2 0 0	211	22 3	34.1 7472 2	- 117.2 66667
TWI N-03	TWIN	22 4	284	15 3	153	192	196	264	26 8	262	262	200	2 1 2	211	21 1	34.1 7472 2	- 117.2
TWI N-04	TWIN	22 4	264	13 3	137	160	160	256	26 8	266	270	200	2 1 2	211	22 3	34.1 7472 2	- 117.2
TWI N-05	TWIN	26 4	264	13 3	133	156	168	228	26 4	290	290	184	2	211	21 9	34.1 7472	- 117.2
TWI N-06	TWIN	26 0	292	13 3	133	156	168	256	26 8	266	290	200	2	211	22 7	34.1 7472	- 117.2
TWI N-07	TWIN	26 0	284	12 1	133	160	160	260	26 0	274	282	200	2	219	21 9	34.1 7472	- 117.2
TWI N-08	TWIN	25 2	252	13 3	133	192	192	252	26 4	262	282	200	0 2 0	211	21 1	2 34.1 7472	- 117.2
TWI N-09	TWIN	25 6	268	13 7	141	156	192	264	26 4	254	274	196	0 2 0	211	22 7	2 34.1 7472	- 117.2
TWI N-10	TWIN	22 4	264	13 7	145	156	168	264	26 4	262	274	200	0 2 0	211	21 1	2 34.1 7472	- 117.2
TWI N-11	TWIN	25 6	292	13 3	133	156	192	252	25 2	278	278	200	0 2 0	203	21 5	2 34.1 7472	66667 - 117.2
TWI N-12	TWIN	25 2	280	13 3	137	156	192	236	26 4	270	270	200	0 2 0	211	22 7	2 34.1 7472	66667 - 117.2
CC- 01	CITY CK	23 6	244	14 9	161	152	160	228	22 8	270	270	184	0 1 8	235	23 5	2 34.2 9252	- 117.3 06135

CC- 02	CITY CK	24 4	252	13 7	145	140	164	260	26 0	278	282	184	2 0 0	227	23 5	34.2 9252 6	- 117.3 06135
CC- 03	CITY CK	23 6	236	13 7	149	140	156	244	25 6	274	274	188	1 9 6	227	22 7	34.2 9252 6	- 117.3 06135
CC- 04	CITY CK	24 4	276	14 5	157	156	172	244	24 4	274	278	196	1 9 6	231	23 5	34.2 9252 6	- 117.3 06135
CC- 05	CITY CK	24 4	276	14 1	165	164	172	256	25 6	270	274	184	1 8 8	219	22 7	34.2 9252 6	- 117.3 06135
CC- 06	CITY CK	23 2	272	12 9	149	164	168	244	26 0	274	294	208	2 2 0	227	22 7	34.2 9252 6	- 117.3 06135
CC- 07	CITY CK	24 4	244	14 1	145	152	152	244	24 4	274	274	184	1 8 8	227	23 1	34.2 9252 6	- 117.3 06135
CC- 08	CITY CK	23 2	272	14 1	149	168	168	244	24 4	258	274	196	2 0 4	227	22 7	34.2 9252 6	- 117.3 06135
CC- 09	CITY CK	24 0	276	13 7	153	168	168	244	24 4	274	278	200	2 0 8	227	22 7	34.2 9252 6	- 117.3 06135
CC- 10	CITY CK	24 0	276	14 1	153	156	200	244	25 6	274	274	204	2 2 0	227	22 7	34.2 9252 6	- 117.3 06135
CC- 11	CITY CK	24 4	244	12 5	133	156	168	240	24 8	278	278	188	2 2 0	227	22 7	34.2 9252 6	- 117.3 06135
CCW F-01	CITY CK	23 6	244	14 9	161	164	208	228	22 8	274	274	188	1 9 2	227	23 5	34.2 9252 6	- 117.3 06135
FOR K-1	CITY CK	22 4	224	12 9	137	164	164	236	25 6	282	294	188	1 9 6	235	24 7	34.2 9252 6	- 117.3 06135
FOR K-2	CITY CK	22 4	244	13 7	145	132	172	208	20 8	254	294	200	2 1 2	199	22 7	34.2 9252 6	- 117.3 06135
CAJO -01	CAJON	26 4	276	14 1	141	172	172	232	25 2	254	270	196	2 6 4	199	23 1	34.2 3281 1	- 117.4 27183
CAJO -02	CAJON	25 2	252	13 7	137	156	156	208	22 4	266	282	196	2 3 6	203	23 1	34.2 3281 1	- 117.4 27183
CAJO -03	CAJON	26 8	280	12 5	157	168	172	228	22 8	254	270	196	1 9 6	215	24 7	34.2 3281 1	- 117.4 27183
CAJO -04	CAJON	26 4	268	13 3	153	216	220	240	25 6	254	274	196	2 1	191	22 7	34.2 3281	- 117.4

													2			1	27183
CAJO -05	CAJON	27 6	276	12 5	157	168	172	228	22 8	254	270	196	1 9 6	215	24 7	34.2 3281 1	- 117.4 27183
CAJO -06	CAJON	22 4	264	13 3	145	156	172	232	23 2	254	270	212	2 1 2	227	24 7	34.2 3281 1	- 117.4 27183
CAJO -07	CAJON	22 0	252	14 5	149	176	176	208	22 8	254	270	204	2 0 8	211	23 5	34.2 3281 1	- 117.4 27183
CAJO -08	CAJON	22 4	224	14 1	145	132	172	224	24 0	250	254	188	1 9 6	223	23 1	34.2 3281 1	- 117.4 27183
CAJO -09	CAJON	28 0	280	14 1	141	132	216	228	23 2	254	274	208	2 0 8	199	22 7	34.2 3281 1	- 117.4 27183
CAJO -10	CAJON	27 2	276	13 3	141	176	176	232	24 4	254	266	212	2 1 6	191	22 7	34.2 3281 1	- 117.4 27183
CAJO -11	CAJON	26 0	264	14 5	145	164	164	228	24 0	274	294	196	2 0 4	199	22 7	34.2 3281 1	- 117.4 27183
CAJO -12	CAJON	26 4	280	13 3	145	132	176	228	24 4	266	274	196	2 1 2	227	23 9	34.2 3281 1	- 117.4 27183
CAJO -13	CAJON	25 2	264	14 1	145	168	216	212	22 8	250	270	212	2 1 6	215	22 7	34.2 3281 1	- 117.4 27183
CAJO -14	CAJON	22 4	248	13 3	145	164	180	228	23 2	270	250	204	2 1 2	199	21 1	34.2 3281 1	- 117.4 27183
CAJO -15	CAJON	25 2	272	13 7	141	176	176	236	24 4	270	274	192	1 9 6	227	23 9	34.2 3281 1	- 117.4 27183
CAJO -16	CAJON	24 8	268	12 9	141	132	168	244	24 4	266	270	188	2 1 6	247	24 7	34.2 3281 1	- 117.4 27183
CAJO -17	CAJON	24 8	248	14 1	141	168	168	228	23 6	274	278	188	1 8 8	199	22 7	34.2 3281 1	- 117.4 27183
CAJ W-1	CAJON	22 4	264	13 3	141	176	176	232	23 2	254	266	196	2 1 2	199	22 7	34.2 3281 1	- 117.4 27183
CAJ W-2	CAJON	22 0	272	13 3	145	172	212	244	24 4	254	270	188	1 9 6	227	23 5	34.2 3281 1	- 117.4 27183
CAJ W-3	CAJON	24 8	276	13 3	141	176	180	232	23 6	266	278	196	2 1 2	191	22 7	34.2 3281 1	- 117.4 27183

CAJON	24 8	276	13 3	141	184	184	208	23 6	270	270	196	2 1 2	199	22 7	34.2 3281 1	- 117.4 27183
LYTLE CK	22 0	276	14 1	141	156	156	236	23 6	254	270	188	1 9	227	24 7	34.2 2858 3	- 117.4 73517
LYTLE CK	25 2	276	14 1	141	156	176	232	23 2	270	274	264	2	247	24 7	34.2 2858	- 117.4
LYTLE CK	22 0	264	14 1	141	156	216	228	22 8	254	254	212	4 2 1	211	24 7	34.2 2858	- 117.4
LYTLE CK	26 4	276	14 1	141	156	156	228	23 6	254	254	188	2 1 9	211	21 1	34.2 2858	- 117.4
LYTLE CK	26 4	268	14 1	141	176	176	228	22 8	254	270	188	6 1 8	211	22 7	3 34.2 2858	- 117.4
LYTLE CK	26 4	276	14 1	141	156	156	228	22 8	254	274	208	8 2 6	227	24 7	3 34.2 2858	- 117.4
LYTLE CK	22 0	276	14 1	141	148	152	228	23 2	254	274	188	0 1 8	227	22 7	3 34.2 2858	- 117.4
LYTLE	22 0	276	14 5	145	212	212	236	23 6	254	254	188	8 1 8	227	22 7	3 34.2 2858	73517 - 117.4
LYTLE	22	276	14 1	141	156	216	228	23 6	254	274	212	8 2 6	227	22	3 34.2 2858	73517 - 117.4
LYTLE	22	276	14	141	156	216	228	22	254	254	196	4 2 1	227	24	3 34.2 2858	73517 - 117.4
LYTLE	22	276	14	141	176	216	228	23	254	254	188	2 1 8	211	22	3 34.2 2858	73517 - 117.4
LYTLE	22	252	14	141	156	156	228	23	254	254	188	8 1 8	227	22	3 34.2 2858	73517 - 117.4
LYTLE	22	268	13	141	156	216	228	23	270	274	188	8 1 9	247	24	3 34.2 2858	73517 - 117.4
LYTLE	22	276	13	141	156	176	228	23	254	254	196	6 2 0	211	22	3 34.2 2858	73517 - 117.4
CK LYTLE	0 25	260	7 14	145	172	176	228	2 23	254	254	196	8 2 5	211	7 22	3 34.2 2858	73517 - 117 4
CK LYTLE CK	2 22 0	276	1 13 7	141	156	216	228	6 22 8	254	270	196	- 6 1 9	211	7 21 1	3 34.2 2858	73517
	CAJON LYTLE CK	CAJON 24 LYTLE 22 LYTLE 22 LYTLE 22 LYTLE 22 LYTLE 24 LYTLE 22 LYTLE 24 LYTLE 22 LYTLE 26 LYTLE 26 LYTLE 22 LYTLE 22	CAJON 24 276 LYTLE 22 276 LYTLE 25 276 LYTLE 25 276 LYTLE 26 276 LYTLE 27 276 LYTLE 22 252 LYTLE 22 268 LYTLE 22 276 LYTLE 22 276 LYTLE 22	CAJON 24 8 276 13 1 LYTLE CK 22 2 276 14 1 LYTLE CK 25 2 276 14 1 LYTLE CK 22 2 264 14 1 LYTLE CK 26 2 276 14 1 LYTLE CK 26 2 276 14 1 LYTLE CK 26 2 276 14 1 LYTLE CK 22 2 268 13 LYTLE CK 22 2 260 14 1 LYTLE CK 22 2 260 14 1	CAJON24 827613 3141LYTLE CK22 027614 1141LYTLE CK25 027614 1141LYTLE CK26 026414 1141LYTLE CK26 026814 1141LYTLE CK26 027614 1141LYTLE CK26 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK20 027614 1141LYTLE CK22 027614 1141LYTLE CK22 027614 1141LYTLE CK22 027613 1141	CAJON24 827613 3141184LYTLE CK22 027614 1141156LYTLE CK25 027614 1141156LYTLE CK22 026414 1141156LYTLE CK24 027614 1141156LYTLE CK26 027614 1141156LYTLE CK26 027614 1141156LYTLE CK20 027614 1141156LYTLE CK20 027614 1141156LYTLE CK20 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027613 1141156LYTLE CK22 027614 1141156LYTLE CK22 027614 1141156LYTLE CK22 027613 1141156LYTLE CK22 027614 1141156LYTLE CK22 0 <td< td=""><td>CAJON24 827613 3141184184LYTLE CK22 027614 1141156176LYTLE CK22 027614 1141156176LYTLE CK22 026414 1141156166LYTLE CK24 027614 1141176176LYTLE CK24 027614 1141176161LYTLE CK22 027614 1141156161LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156166LYTLE CK22 027614 1141156166LYTLE CK22 027614 1141156166LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK</td><td>CAJON24 827613 3141184184208LYTLE CK22 227614 1141156156232LYTLE CK22 226414 1141156216228LYTLE CK24 227614 1141156156228LYTLE CK24 227614 1141176176228LYTLE CK24 226814 1141176176228LYTLE CK26 227614 1141156156228LYTLE CK22 227614 1141156126238LYTLE CK22 227614 1141156216238LYTLE CK22 227614 1141156216238LYTLE CK22 227614 1141156216238LYTLE CK22 225214 1141156216228LYTLE CK22 225214 1141156156228LYTLE CK22 226813 3141156156228LYTLE CK22 226813 3141156166228LYTLE CK22 226813 3141156166228LYTLE CK22 226813</td><td>CAJON24 827613 314118418420826 6LYTLE CK2027614 114115615623623 2LYTLE CK2527614 114115617623223 2LYTLE CK2026414 114115621622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2027614 114115615622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2025214 114115621622823 2LYTLE CK2025214 114115615622823 2LYTLE CK2025214 114115615622823 2LYTLE CK2025813 314115615622823 2LYTLE</td><td>CAJON24 827613 314118418420826 623023 6254LYTLE CK22 227614 414115615623623 223 2270LYTLE CK22 226414 414115617623223 2270LYTLE CK26 226414 414115615622823 2254LYTLE CK26 426614 414115615622823 2254LYTLE CK26 427614 414115615622823 2254LYTLE CK26 427614 414115615622823 2254LYTLE CK26 427614 414114815222823 2254LYTLE CK27 427614 414114815222823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LY</td><td>CAION 24 8 276 13 3 141 184 184 208 23 6 270 270 IVTLE CK 20 276 14 1 141 156 156 236 23 6 254 270 IVTLE CK 25 276 14 1 141 156 176 232 23 2 270 274 IVTLE CK 20 264 14 1 141 156 176 228 23 6 254 254 IVTLE CK 26 4 276 14 1 141 156 156 228 23 6 254 270 IVTLE CK 26 6 276 14 1 141 156 156 228 23 6 254 274 IVTLE CK 26 6 276 14 1 141 156 156 228 23 6 254 274 IVTLE CK 20 276 14 1 141 156 156 228 23 6 254 254 IVTLE CK 20 276 14 1 141 156 16 2</td><td>CAUON 24 8 276 13 3 141 184 184 208 25 6 6 270 270 196 LYTLE CK 20 20 276 14 1 141 156 156 236 23 6 254 270 244 245 LYTLE CK 22 20 264 14 1 141 156 216 228 23 23 270 274 264 LYTLE CK 26 26 264 14 1 141 156 156 228 23 23 254 254 148 LYTLE CK 26 24 276 14 1 141 176 176 228 23 23 254 270 188 LYTLE CK 26 26 276 14 1 141 176 176 228 23 23 254 274 208 LYTLE CK 26 20 276 14 1 141 166 156 228 23 23 254 274 188 LYTLE CK 20 20 276 14 1 141 156 216 228 23 23 254 2</td><td>CAION $\frac{24}{8}$ 276 $\frac{13}{3}$ 141 184 126 $\frac{23}{6}$ 270 270 188 $\frac{9}{6}$ L'CK 22 276 $\frac{14}{1}$ 141 156 126 232 23 270 274 264 $\frac{9}{6}$ L'TLE 22 264 141 141 156 126 232 23 274 264 212 $\frac{21}{2}$ L'TLE 22 264 141 141 156 126 232 23 254 254 212 $\frac{19}{2}$ L'TLE 24 276 141 141 156 218 23 254 254 188 $\frac{9}{16}$ L'TLE 24 276 141 141 176 228 23 254 274 288 23 254 274 288 26 274 218 $\frac{8}{18}$ 141 141 141 141 141 141 141 216</td><td>CAION</td><td>CAION $\frac{8}{8}$ 76 $\frac{1}{3}$ 141 184 284 270 270 196 $\frac{1}{4}$ 197 $\frac{7}{4}$ LYTE 20 276 14 141 156 126 23 270 270 188 $\frac{9}{4}$ 247 $\frac{7}{4}$ LYTE 25 276 14 141 156 216 232 270 274 264 $\frac{9}{4}$ 247 $\frac{7}{4}$ LYTE 25 276 14 141 156 216 28 254 254 212 211 211</td><td>CAION $\frac{8}{8}$ 276 $\frac{13}{3}$ 141 184 284 26 270 270 164 $\frac{1}{2}$ 190 $\frac{2}{7}$ $\frac{342}{14}$ LYTLE 20 276 14 141 156 256 23 270 274 264 $\frac{6}{4}$ 277 $\frac{7}{7}$ $\frac{342}{2888}$ LYTLE 20 264 14 141 156 216 22 23 270 274 264 $\frac{2}{7}$ $\frac{2}{7}$</td></td<>	CAJON24 827613 3141184184LYTLE CK22 027614 1141156176LYTLE CK22 027614 1141156176LYTLE CK22 026414 1141156166LYTLE CK24 027614 1141176176LYTLE CK24 027614 1141176161LYTLE CK22 027614 1141156161LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156216LYTLE CK22 027614 1141156166LYTLE CK22 027614 1141156166LYTLE CK22 027614 1141156166LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK22 027613 1141156176LYTLE CK	CAJON24 827613 3141184184208LYTLE CK22 227614 1141156156232LYTLE CK22 226414 1141156216228LYTLE CK24 227614 1141156156228LYTLE CK24 227614 1141176176228LYTLE CK24 226814 1141176176228LYTLE CK26 227614 1141156156228LYTLE CK22 227614 1141156126238LYTLE CK22 227614 1141156216238LYTLE CK22 227614 1141156216238LYTLE CK22 227614 1141156216238LYTLE CK22 225214 1141156216228LYTLE CK22 225214 1141156156228LYTLE CK22 226813 3141156156228LYTLE CK22 226813 3141156166228LYTLE CK22 226813 3141156166228LYTLE CK22 226813	CAJON24 827613 314118418420826 6LYTLE CK2027614 114115615623623 2LYTLE CK2527614 114115617623223 2LYTLE CK2026414 114115621622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2627614 114115615622823 2LYTLE CK2027614 114115615622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2027614 114115621622823 2LYTLE CK2025214 114115621622823 2LYTLE CK2025214 114115615622823 2LYTLE CK2025214 114115615622823 2LYTLE CK2025813 314115615622823 2LYTLE	CAJON24 827613 314118418420826 623023 6254LYTLE CK22 227614 414115615623623 223 2270LYTLE CK22 226414 414115617623223 2270LYTLE CK26 226414 414115615622823 2254LYTLE CK26 426614 414115615622823 2254LYTLE CK26 427614 414115615622823 2254LYTLE CK26 427614 414115615622823 2254LYTLE CK26 427614 414114815222823 2254LYTLE CK27 427614 414114815222823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LYTLE CK27 427614 414115621622823 2254LY	CAION 24 8 276 13 3 141 184 184 208 23 6 270 270 IVTLE CK 20 276 14 1 141 156 156 236 23 6 254 270 IVTLE CK 25 276 14 1 141 156 176 232 23 2 270 274 IVTLE CK 20 264 14 1 141 156 176 228 23 6 254 254 IVTLE CK 26 4 276 14 1 141 156 156 228 23 6 254 270 IVTLE CK 26 6 276 14 1 141 156 156 228 23 6 254 274 IVTLE CK 26 6 276 14 1 141 156 156 228 23 6 254 274 IVTLE CK 20 276 14 1 141 156 156 228 23 6 254 254 IVTLE CK 20 276 14 1 141 156 16 2	CAUON 24 8 276 13 3 141 184 184 208 25 6 6 270 270 196 LYTLE CK 20 20 276 14 1 141 156 156 236 23 6 254 270 244 245 LYTLE CK 22 20 264 14 1 141 156 216 228 23 23 270 274 264 LYTLE CK 26 26 264 14 1 141 156 156 228 23 23 254 254 148 LYTLE CK 26 24 276 14 1 141 176 176 228 23 23 254 270 188 LYTLE CK 26 26 276 14 1 141 176 176 228 23 23 254 274 208 LYTLE CK 26 20 276 14 1 141 166 156 228 23 23 254 274 188 LYTLE CK 20 20 276 14 1 141 156 216 228 23 23 254 2	CAION $\frac{24}{8}$ 276 $\frac{13}{3}$ 141 184 126 $\frac{23}{6}$ 270 270 188 $\frac{9}{6}$ L'CK 22 276 $\frac{14}{1}$ 141 156 126 232 23 270 274 264 $\frac{9}{6}$ L'TLE 22 264 141 141 156 126 232 23 274 264 212 $\frac{21}{2}$ L'TLE 22 264 141 141 156 126 232 23 254 254 212 $\frac{19}{2}$ L'TLE 24 276 141 141 156 218 23 254 254 188 $\frac{9}{16}$ L'TLE 24 276 141 141 176 228 23 254 274 288 23 254 274 288 26 274 218 $\frac{8}{18}$ 141 141 141 141 141 141 141 216	CAION	CAION $\frac{8}{8}$ 76 $\frac{1}{3}$ 141 184 284 270 270 196 $\frac{1}{4}$ 197 $\frac{7}{4}$ LYTE 20 276 14 141 156 126 23 270 270 188 $\frac{9}{4}$ 247 $\frac{7}{4}$ LYTE 25 276 14 141 156 216 232 270 274 264 $\frac{9}{4}$ 247 $\frac{7}{4}$ LYTE 25 276 14 141 156 216 28 254 254 212 211	CAION $\frac{8}{8}$ 276 $\frac{13}{3}$ 141 184 284 26 270 270 164 $\frac{1}{2}$ 190 $\frac{2}{7}$ $\frac{342}{14}$ LYTLE 20 276 14 141 156 256 23 270 274 264 $\frac{6}{4}$ 277 $\frac{7}{7}$ $\frac{342}{2888}$ LYTLE 20 264 14 141 156 216 22 23 270 274 264 $\frac{2}{7}$

													6			3	73517
LCK- 17	LYTLE CK	22 0	276	13 7	141	156	216	232	23 2	254	270	264	2 6 4	211	22 7	34.2 2858 3	- 117.4 73517
LCK- 18	LYTLE CK	25 2	264	14 1	141	156	156	228	22 8	270	270	212	2 6 4	227	22 7	34.2 2858 3	- 117.4 73517
LCK- 19	LYTLE CK	25 2	260	14 5	145	156	156	228	23 6	254	270	188	1 8 8	227	27 7	34.2 2858 3	- 117.4 73517
LCK- 20	LYTLE CK	22 0	276	14 1	141	156	176	232	23 6	254	254	196	2 1 2	227	24 7	34.2 2858 3	- 117.4 73517
LCK- 21	LYTLE CK	26 0	276	14 1	141	156	156	228	23 6	254	254	264	2 6 4	211	22 7	34.2 2858 3	- 117.4 73517
LCK- 22	LYTLE CK	26 4	276	13 7	141	156	176	220	23 2	254	274	196	1 9 6	211	22 7	34.2 2858 3	- 117.4 73517
LCK- 23	LYTLE CK	22 0	264	14 1	141	156	156	228	23 2	254	274	188	2 1 2	227	24 7	34.2 2858 3	- 117.4 73517
LCK- 24	LYTLE CK	22 0	268	14 1	141	156	156	228	23 2	274	274	196	1 9	211	22 7	34.2 2858 3	- 117.4
LCK- 25	LYTLE CK	22 0	220	13 3	141	156	156	232	23 2	254	282	188	2 1 2	247	24 7	34.2 2858 3	- 117.4 73517
LCK- 26	LYTLE CK	26 4	276	14 1	141	156	176	232	23 6	254	274	188	1 8 8	227	22 7	34.2 2858 3	- 117.4 73517
LCK- 27	LYTLE CK	26 4	276	14 1	141	152	152	224	22 8	254	270	196	2 1 2	227	24 7	34.2 2858 3	- 117.4 73517
LCK- 28	LYTLE CK	22 0	280	14 1	141	172	172	220	22 8	254	274	196	2 6 0	211	24 7	34.2 2858 3	- 117.4 73517
CATC R-01	CATTL E CAN	24 4	284	12 5	137	160	164	244	24 8	258	262	212	2 1 6	219	23 1	34.2 3	- 117.7 78832
CATC R-02	CATTL E CAN	22 8	252	12 5	161	152	156	252	26 8	278	278	228	2 2 8	203	20 7	34.2 3	- 117.7 78832
CATC R-03	CATTL E CAN	25 2	264	12 5	141	160	172	232	25 6	270	274	224	2 2 8	231	23 9	34.2 3	- 117.7 78832
EFSG R-01	EF SAN GAB	24 0	244	13 7	141	160	164	276	26 0	266	298	220	2 2 8	227	23 9	34.2 5805 6	- 118.1 03611

EFSG R-02	EF SAN GAB	24 4	252	13 7	145	160	164	276	34 0	278	298	224	2 3 2	199	23 1	34.2 5805	- 118.1 03611	
NFS GR- 01	NF SAN GAB	26 4	272	13 3	141	152	172	232	25 6	266	294	196	2 0	187	23 5	34.2 5805	- 118.1 03611	
NFS GR-	NF SAN GAB	24 0	264	12 9	145	156	172	252	26 4	266	294	196	2 3 2	235	25 5	34.2 5805	- 118.1 03611	
FISH- 1	FISH	25 2	260	13 3	133	152	168	248	25 2	266	270	212	2 2 8	203	23 1	34.1 6937 2	- 117.9 25931	
FISH- 2	FISH	27 6	288	13 3	133	152	172	268	26 8	262	274	224	2 4	223	23 1	34.1 6937	- 117.9	
FISH- 3	FISH	22 0	264	13 3	137	152	152	220	22 0	270	270	224	4 2 4	203	20 3	34.1 6937 2	- 117.9	
FISH- 4	FISH	26 0	284	13 3	133	152	176	248	24 8	266	266	208	4 2 1 2	203	20 3	34.1 6937 2	- 117.9 25931	
FISH- 5	FISH	26 0	260	12 9	133	152	172	248	24 8	262	262	240	2 4	215	21 9	34.1 6937 2	- 117.9	
WFS GR- 01	WF SAN GAB	25 2	260	13 7	153	160	164	260	26 0	278	278	192	2 4 0	223	23 5	34.2 5805	- 118.1 03611	
WFS GR- 01	WF SAN GAB	25 2	260	13 7	153	160	164	260	26 0	278	278	192	2 4 0	223	23 5	34.2 5805 6	- 118.1 03611	
HAIN -1	HAIN	26 0	260	11 7	145	148	148	232	27 6	270	274	216	2 3 6	203	20 3	34.2 3678 8	- 118.2 82027	
HAIN -2	HAIN	26 0	268	14 5	145	132	152	232	27 6	270	274	204	2 3 6	219	21 9	34.2 3678 8	- 118.2 82027	
PINE -1	PINE	28 0	280	12 9	137	128	132	244	27 6	314	314	180	1 7 2	227	22 7	37.1 7277 8	- 118.2 59444	
PINE -2	PINE	27 2	280	19 3	197	128	128	248	28 0	238	238	176	1 8 4	175	29 1	37.1 7277 8	- 118.2 59444	
PINE -3	PINE	27 2	276	13 3	133	128	132	244	28 0	262	294	200	2 2 8	215	21 5	37.1 7277 8	118.2 59444	
MAR V-1	MARVI N	24 8	256	12 1	169	128	128	264	29 6	258	258	172	1 7 6	207	19 1	37.4 7078 3	118.4	
MAR V-2	MARVI N	26 8	272	12 9	129	128	128	256	30 0	258	274	168	2 0	187	18 7	37.4 7078	- 118.4	

													8			3	02549
MAR V-3	MARVI N	24 4	284	12 9	225	128	128	256	30 4	274	282	172	2 9 8	199	19 9	37.4 7078 3	- 118.4 02549
BRIZ- 1	BRIZZ OLARI	28 0	292	17 7	177	108	108	196	24 8	274	282	160	1 6 0	231	23 1	35.3 1361 8	- 120.6 51393
BRIZ- 2	BRIZZ OLARI	29 2	308	17 7	177	112	112	256	25 6	230	242	160	1 6 0	223	22 3	35.3 1361 8	- 120.6 51393
BRIZ- 3	BRIZZ OLARI	24 8	292	17 7	177	108	108	196	19 6	274	274	160	1 6 0	235	19 1	35.3 1361 8	- 120.6 51393
STEN -1	STENN ER	26 0	284	17 3	181	108	108	256	25 6	242	242	160	1 6 0	195	22 7	35.3 2760 3	- 120.6 65568
STEN -2	STENN ER	26 4	276	17 7	177	116	116	196	22 0	242	242	160	1 6 0	191	22 7	35.3 2760 3	- 120.6 65568
STEN -3	STENN ER	27 6	276	14 5	177	108	112	256	26 4	242	242	160	1 6 0	199	19 9	35.3 2760 3	- 120.6
SLO- 1	SAN LUIS OBI	27 2	272	16 5	165	108	108	256	25 6	242	242	160	1 6 0	199	19 9	35.2 9758	- 120.6 26288
SLO- 2	SAN LUIS OBI	29 2	300	13 7	145	108	108	256	30 0	242	242	160	1 6 0	191	22 7	35.2 9758 5	- 120.6 26288
SLO- 3	SAN LUIS OBI	28 4	288	16 1	177	108	108	216	22 4	242	242	160	1 6 0	227	27 7	35.2 9758 5	- 120.6 26288
DBC- 01	DAVY BROW N	25 2	256	18 9	189	108	112	276	28 0	242	242	160	1 6 0	175	22 3	34.7 7207 8	- - 119.9 43624
DBC- 02	DAVY BROW N	25 2	252	17 3	185	108	112	204	22 8	242	242	160	1 6 0	233	23 3	34.7 7207 8	- 119.9 43624
CUY R-01	CUYA	25 6	276	14 9	149	112	112	200	23 6	242	242	160	1 6 0	219	21 9	35.0 0464	- 119.8 2442
CUY R-02	CUYA MA	25 2	272	18 5	185	108	112	232	27 6	242	242	160	1 6 0	187	23 5	35.0 0464	- 119.8
MZN A-01	MANZ ANA	25 6	292	17 7	185	108	108	196	19 6	242	242	160	1 6 0	219	21 9	34.7 7043	- 119.9
MZN A-02	MANZ ANA	24 8	252	14 9	173	108	112	232	23 6	242	242	160	1 6 0	219	21 9	34.7 7043 6	- 119.9 36469

MNC R-01	MANZ ANA	25 6	256	17 3	189	108	108	232	23 6	242	242	160	1 6 0	219	21 9	34.7 7043 6	- 119.9 36469
MNC R-02	MANZ ANA	24 8	292	17 3	189	108	112	236	23 6	242	242	160	1 6 0	219	21 9	34.7 7043 6	- 119.9 36469

7	123	2	108	15	1	123											
			SANTA ANA	SAI GABF	N RIEL	SOCAL											
Sample	Рор	RHOS 5		RHOS 9		RHOS 25		RHOS 23		RHOS 14		RHOS 8		RHOS 29		х	Y
INDCK- 01	SANTA ANA	232	232	137	13 7	144	18 0	236	23 6	270	27 4	204	21 2	203	21 9	33.7619 64	- 116.8839 11
INDCK- 02	SANTA ANA	232	236	137	15 7	148	14 8	236	23 6	270	27 8	192	21 2	199	19 9	33.7619 64	- 116.8839 11
INDCK- 03	SANTA ANA	232	256	149	15 7	148	14 8	236	23 6	250	27 0	204	20 4	219	23 5	33.7619 64	- 116.8839 11
INDCK- 04	SANTA ANA	232	232	145	14 9	148	14 8	236	23 6	270	27 4	192	20 4	235	23 5	33.7619 64	- 116.8839 11
INDCK- 05	SANTA ANA	232	232	137	13 7	128	14 8	236	24 4	262	26 6	192	20 4	199	21 9	33.7619 64	- 116.8839 11
INDCK- 06	SANTA ANA	232	232	149	15 7	148	14 8	236	23 6	270	27 0	192	19 2	195	19 9	33.7619 64	- 116.8839 11
INDCK- 07	SANTA ANA	256	256	137	13 7	184	21 6	236	23 6	270	27 0	192	19 2	199	21 9	33.7619 64	- 116.8839 11
MILL-1	SANTA ANA	252	256	137	14 1	156	16 0	232	25 2	282	28 2	192	20 0	219	22 7	34.0777 89	- 117.0994 5
MILL-2	SANTA ANA	252	264	137	14 1	160	16 0	232	25 2	274	28 2	216	21 6	203	20 7	34.0777 89	- 117.0994 5
MILL-3	SANTA ANA	264	276	137	13 7	160	16 0	228	25 2	282	28 2	192	26 4	207	21 5	34.0777 89	- 117.0994 5
MILL-4	SANTA	236	276	137	13	140	17	232	23	274	28	200	22	199	21	34.0777	-

GENALEX MICROSATELLITE DATA FILE – SOUTHERN CALIFRONIA WATERSHEDS

	ANA				7		2		2		2		0		5	89	117.0994 5
MILL-5	SANTA ANA	228	272	133	13 7	140	16 0	252	25 2	274	28 2	200	21 6	203	21 5	34.0777 89	- 117.0994 5
PC-01	SANTA ANA	252	252	133	13 7	140	18 8	228	22 8	238	23 8	192	19 6	223	22 7	34.1089 17	- 117.1507 5
PC-02	SANTA ANA	236	280	137	13 7	116	11 6	256	25 6	238	23 8	192	19 6	223	22 7	34.1089 17	- 117.1507 5
PC-03	SANTA ANA	228	264	137	14 1	180	19 2	248	24 8	238	23 8	204	22 0	227	22 7	34.1089 17	- 117.1507 5
PC-04	SANTA ANA	252	264	137	15 7	140	15 2	224	24 8	302	30 2	200	20 4	203	21 5	34.1089 17	- 117.1507 5
PC-05	SANTA ANA	252	276	141	15 7	140	16 0	256	25 6	278	28 6	204	22 4	223	22 3	34.1089 17	- 117.1507 5
PC-06	SANTA ANA	252	280	137	15 7	140	18 0	256	25 6	254	28 2	204	26 8	195	21 9	34.1089 17	- 117.1507 5
PC-07	SANTA ANA	264	280	137	13 7	140	18 0	232	23 2	274	25 8	220	26 8	203	22 7	34.1089 17	- 117.1507 5
PC-08	SANTA ANA	252	280	133	14 5	152	15 2	228	25 6	286	28 6	192	20 4	231	20 3	34.1089 17	- 117.1507 5
PC-09	SANTA ANA	252	280	137	13 7	152	19 2	232	23 2	254	27 4	196	20 4	203	22 7	34.1089 17	- 117.1507 5
PC-10	SANTA ANA	276	276	133	13 7	140	14 0	228	22 8	254	26 6	192	22 0	203	24 7	34.1089 17	- 117.1507 5
PC-11	SANTA ANA	228	268	137	14 1	168	16 8	244	24 4	278	28 2	200	20 4	227	27 7	34.1089 17	- 117.1507 5
PC-12	SANTA	228	268	133	13	168	19	248	24	266	27	192	22	195	20	34.1089	-

	ANA				7		2		8		8		0		3	17	117.1507 5
PC-13	SANTA ANA	236	280	137	14 5	136	18 4	248	24 8	246	27 4	200	20 0	215	22 7	34.1089 17	- 117.1507 5
PC-14	SANTA ANA	252	280	137	13 7	168	18 0	252	25 6	274	27 4	196	20 4	215	21 5	34.1089 17	- 117.1507 5
PC-15	SANTA ANA	252	276	141	14 1	168	16 8	228	25 6	290	29 0	200	20 0	175	17 5	34.1089 17	- 117.1507 5
PC-16	SANTA ANA	236	252	141	16 1	148	16 8	232	25 2	282	28 2	192	20 0	199	20 3	34.1089 17	- 117.1507 5
PLNG-1	SANTA ANA	236	276	137	14 1	140	18 4	248	24 8	266	29 0	200	20 4	203	20 3	34.1089 17	- 117.1507 5
PLNG-2	SANTA ANA	252	268	157	15 7	140	17 2	228	25 6	258	29 0	204	26 8	215	21 9	34.1089 17	- 117.1507 5
PLNG-3	SANTA ANA	276	276	137	14 5	140	18 4	244	24 4	266	28 6	192	20 4	219	22 7	34.1089 17	- 117.1507 5
PLNG-4	SANTA ANA	264	276	137	16 1	172	17 2	244	24 4	266	26 6	204	22 0	215	21 9	34.1089 17	- 117.1507 5
PLUN-1	SANTA ANA	276	276	157	16 1	152	17 2	256	25 6	266	26 6	196	22 4	203	21 9	34.1089 17	- 117.1507 5
TWIN- 01	SANTA ANA	256	272	133	13 3	152	15 2	264	26 4	262	26 2	184	19 6	219	22 7	34.1747 22	- 117.2666 67
TWIN- 02	SANTA ANA	224	224	133	13 7	192	19 2	232	26 4	262	29 0	184	20 0	211	22 3	34.1747 22	- 117.2666 67
TWIN- 03	SANTA ANA	224	284	153	15 3	192	19 6	264	26 8	262	26 2	200	21 2	211	21 1	34.1747 22	- 117.2666 67
TWIN-	SANTA	224	264	133	13	160	16	256	26	266	27	200	21	211	22	34.1747	-

04	ANA				7		0		8		0		2		3	22	117.2666 67
TWIN- 05	SANTA ANA	264	264	133	13 3	156	16 8	228	26 4	290	29 0	184	20 0	211	21 9	34.1747 22	- 117.2666 67
TWIN- 06	SANTA ANA	260	292	133	13 3	156	16 8	256	26 8	266	29 0	200	20 0	211	22 7	34.1747 22	- 117.2666 67
TWIN- 07	SANTA ANA	260	284	121	13 3	160	16 0	260	26 0	274	28 2	200	20 0	219	21 9	34.1747 22	- 117.2666 67
TWIN- 08	SANTA ANA	252	252	133	13 3	192	19 2	252	26 4	262	28 2	200	20 0	211	21 1	34.1747 22	- 117.2666 67
TWIN- 09	SANTA ANA	256	268	137	14 1	156	19 2	264	26 4	254	27 4	196	20 0	211	22 7	34.1747 22	- 117.2666 67
TWIN- 10	SANTA ANA	224	264	137	14 5	156	16 8	264	26 4	262	27 4	200	20 0	211	21 1	34.1747 22	- 117.2666 67
TWIN- 11	SANTA ANA	256	292	133	13 3	156	19 2	252	25 2	278	27 8	200	20 0	203	21 5	34.1747 22	- 117.2666 67
TWIN- 12	SANTA ANA	252	280	133	13 7	156	19 2	236	26 4	270	27 0	200	20 0	211	22 7	34.1747 22	- 117.2666 67
CC-01	SANTA ANA	236	244	149	16 1	152	16 0	228	22 8	270	27 0	184	18 4	235	23 5	34.2925 26	- 117.3061 35
CC-02	SANTA ANA	244	252	137	14 5	140	16 4	260	26 0	278	28 2	184	20 0	227	23 5	34.2925 26	- 117.3061 35
CC-03	SANTA ANA	236	236	137	14 9	140	15 6	244	25 6	274	27 4	188	19 6	227	22 7	34.2925 26	- 117.3061 35
CC-04	SANTA ANA	244	276	145	15 7	156	17 2	244	24 4	274	27 8	196	19 6	231	23 5	34.2925 26	- 117.3061 35
CC-05	SANTA	244	276	141	16	164	17	256	25	270	27	184	18	219	22	34.2925	-

	ANA				5		2		6		4		8		7	26	117.3061 35
CC-06	SANTA ANA	232	272	129	14 9	164	16 8	244	26 0	274	29 4	208	22 0	227	22 7	34.2925 26	- 117.3061 35
CC-07	SANTA ANA	244	244	141	14 5	152	15 2	244	24 4	274	27 4	184	18 8	227	23 1	34.2925 26	- 117.3061 35
CC-08	SANTA ANA	232	272	141	14 9	168	16 8	244	24 4	258	27 4	196	20 4	227	22 7	34.2925 26	- 117.3061 35
CC-09	SANTA ANA	240	276	137	15 3	168	16 8	244	24 4	274	27 8	200	20 8	227	22 7	34.2925 26	- 117.3061 35
CC-10	SANTA ANA	240	276	141	15 3	156	20 0	244	25 6	274	27 4	204	22 0	227	22 7	34.2925 26	- 117.3061 35
CC-11	SANTA ANA	244	244	125	13 3	156	16 8	240	24 8	278	27 8	188	22 0	227	22 7	34.2925 26	- 117.3061 35
CCWF- 01	SANTA ANA	236	244	149	16 1	164	20 8	228	22 8	274	27 4	188	19 2	227	23 5	34.2925 26	- 117.3061 35
FORK-1	SANTA ANA	224	224	129	13 7	164	16 4	236	25 6	282	29 4	188	19 6	235	24 7	34.2925 26	- 117.3061 35
FORK-2	SANTA ANA	224	244	137	14 5	132	17 2	208	20 8	254	29 4	200	21 2	199	22 7	34.2925 26	- 117.3061 35
CAJO-01	SANTA ANA	264	276	141	14 1	172	17 2	232	25 2	254	27 0	196	26 4	199	23 1	34.2328 11	- 117.4271 83
CAJO-02	SANTA ANA	252	252	137	13 7	156	15 6	208	22 4	266	28 2	196	23 6	203	23 1	34.2328 11	- 117.4271 83
CAJO-03	SANTA ANA	268	280	125	15 7	168	17 2	228	22 8	254	27 0	196	19 6	215	24 7	34.2328 11	- 117.4271 83
CAJO-04	SANTA	264	268	133	15	216	22	240	25	254	27	196	21	191	22	34.2328	-

	ANA				3		0		6		4		2		7	11	117.4271 83
CAJO-05	SANTA ANA	276	276	125	15 7	168	17 2	228	22 8	254	27 0	196	19 6	215	24 7	34.2328 11	- 117.4271 83
CAJO-06	SANTA ANA	224	264	133	14 5	156	17 2	232	23 2	254	27 0	212	21 2	227	24 7	34.2328 11	- 117.4271 83
CAJO-07	SANTA ANA	220	252	145	14 9	176	17 6	208	22 8	254	27 0	204	20 8	211	23 5	34.2328 11	- 117.4271 83
CAJO-08	SANTA ANA	224	224	141	14 5	132	17 2	224	24 0	250	25 4	188	19 6	223	23 1	34.2328 11	- 117.4271 83
CAJO-09	SANTA ANA	280	280	141	14 1	132	21 6	228	23 2	254	27 4	208	20 8	199	22 7	34.2328 11	- 117.4271 83
CAJO-10	SANTA ANA	272	276	133	14 1	176	17 6	232	24 4	254	26 6	212	21 6	191	22 7	34.2328 11	- 117.4271 83
CAJO-11	SANTA ANA	260	264	145	14 5	164	16 4	228	24 0	274	29 4	196	20 4	199	22 7	34.2328 11	- 117.4271 83
CAJO-12	SANTA ANA	264	280	133	14 5	132	17 6	228	24 4	266	27 4	196	21 2	227	23 9	34.2328 11	- 117.4271 83
CAJO-13	SANTA ANA	252	264	141	14 5	168	21 6	212	22 8	250	27 0	212	21 6	215	22 7	34.2328 11	- 117.4271 83
CAJO-14	SANTA ANA	224	248	133	14 5	164	18 0	228	23 2	270	25 0	204	21 2	199	21 1	34.2328 11	- 117.4271 83
CAJO-15	SANTA ANA	252	272	137	14 1	176	17 6	236	24 4	270	27 4	192	19 6	227	23 9	34.2328 11	- 117.4271 83
CAJO-16	SANTA ANA	248	268	129	14 1	132	16 8	244	24 4	266	27 0	188	21 6	247	24 7	34.2328 11	- 117.4271 83
CAJO-17	SANTA	248	248	141	14	168	16	228	23	274	27	188	18	199	22	34.2328	-

	ANA				1		8		6		8		8		7	11	117.4271 83
CAJW-1	SANTA ANA	224	264	133	14 1	176	17 6	232	23 2	254	26 6	196	21 2	199	22 7	34.2328 11	- 117.4271 83
CAJW-2	SANTA ANA	220	272	133	14 5	172	21 2	244	24 4	254	27 0	188	19 6	227	23 5	34.2328 11	- 117.4271 83
CAJW-3	SANTA ANA	248	276	133	14 1	176	18 0	232	23 6	266	27 8	196	21 2	191	22 7	34.2328 11	- 117.4271 83
CAJW-4	SANTA ANA	248	276	133	14 1	184	18 4	208	23 6	270	27 0	196	21 2	199	22 7	34.2328 11	- 117.4271 83
LCKC-01	SANTA ANA	220	276	141	14 1	156	15 6	236	23 6	254	27 0	188	19 6	227	24 7	34.2285 83	- 117.4735 17
LCKC-02	SANTA ANA	252	276	141	14 1	156	17 6	232	23 2	270	27 4	264	26 4	247	24 7	34.2285 83	- 117.4735 17
LCKC-03	SANTA ANA	220	264	141	14 1	156	21 6	228	22 8	254	25 4	212	21 2	211	24 7	34.2285 83	- 117.4735 17
LCKC-04	SANTA ANA	264	276	141	14 1	156	15 6	228	23 6	254	25 4	188	19 6	211	21 1	34.2285 83	- 117.4735 17
LCKC-05	SANTA ANA	264	268	141	14 1	176	17 6	228	22 8	254	27 0	188	18 8	211	22 7	34.2285 83	- 117.4735 17
LCKC-06	SANTA ANA	264	276	141	14 1	156	15 6	228	22 8	254	27 4	208	26 0	227	24 7	34.2285 83	- 117.4735 17
LCKC-07	SANTA ANA	220	276	141	14 1	148	15 2	228	23 2	254	27 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCKC-08	SANTA ANA	220	276	145	14 5	212	21 2	236	23 6	254	25 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCKC-09	SANTA	220	276	141	14	156	21	228	23	254	27	212	26	227	22	34.2285	-

	ANA				1		6		6		4		4		7	83	117.4735 17
LCKC-10	SANTA ANA	220	276	141	14 1	156	21 6	228	22 8	254	25 4	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-11	SANTA ANA	220	276	141	14 1	176	21 6	228	23 2	254	25 4	188	18 8	211	22 7	34.2285 83	- 117.4735 17
LCK-12	SANTA ANA	220	252	141	14 1	156	15 6	228	23 2	254	25 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCK-13	SANTA ANA	220	268	137	14 1	156	21 6	228	23 6	270	27 4	188	19 6	247	24 7	34.2285 83	- 117.4735 17
LCK-14	SANTA ANA	220	276	137	14 1	156	17 6	228	23 2	254	25 4	196	20 8	211	22 7	34.2285 83	- 117.4735 17
LCK-15	SANTA ANA	252	260	141	14 5	172	17 6	228	23 6	254	25 4	196	25 6	211	22 7	34.2285 83	- 117.4735 17
LCK-16	SANTA ANA	220	276	137	14 1	156	21 6	228	22 8	254	27 0	196	19 6	211	21 1	34.2285 83	- 117.4735 17
LCK-17	SANTA ANA	220	276	137	14 1	156	21 6	232	23 2	254	27 0	264	26 4	211	22 7	34.2285 83	- 117.4735 17
LCK-18	SANTA ANA	252	264	141	14 1	156	15 6	228	22 8	270	27 0	212	26 4	227	22 7	34.2285 83	- 117.4735 17
LCK-19	SANTA ANA	252	260	145	14 5	156	15 6	228	23 6	254	27 0	188	18 8	227	27 7	34.2285 83	- 117.4735 17
LCK-20	SANTA ANA	220	276	141	14 1	156	17 6	232	23 6	254	25 4	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-21	SANTA ANA	260	276	141	14 1	156	15 6	228	23 6	254	25 4	264	26 4	211	22 7	34.2285 83	- 117.4735 17
LCK-22	SANTA	264	276	137	14	156	17	220	23	254	27	196	19	211	22	34.2285	-

	ANA				1		6		2		4		6		7	83	117.4735 17
LCK-23	SANTA ANA	220	264	141	14 1	156	15 6	228	23 2	254	27 4	188	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-24	SANTA ANA	220	268	141	14 1	156	15 6	228	23 2	274	27 4	196	19 6	211	22 7	34.2285 83	- 117.4735 17
LCK-25	SANTA ANA	220	220	133	14 1	156	15 6	232	23 2	254	28 2	188	21 2	247	24 7	34.2285 83	- 117.4735 17
LCK-26	SANTA ANA	264	276	141	14 1	156	17 6	232	23 6	254	27 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCK-27	SANTA ANA	264	276	141	14 1	152	15 2	224	22 8	254	27 0	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-28	SANTA ANA	220	280	141	14 1	172	17 2	220	22 8	254	27 4	196	26 0	211	24 7	34.2285 83	- 117.4735 17
CATCR- 01	SAN GABRIEL	244	284	125	13 7	160	16 4	244	24 8	258	26 2	212	21 6	219	23 1	34.23	- 117.7788 32
CATCR- 02	SAN GABRIEL	228	252	125	16 1	152	15 6	252	26 8	278	27 8	228	22 8	203	20 7	34.23	- 117.7788 32
CATCR- 03	SAN GABRIEL	252	264	125	14 1	160	17 2	232	25 6	270	27 4	224	22 8	231	23 9	34.23	- 117.7788 32
EFSGR- 01	SAN GABRIEL	240	244	137	14 1	160	16 4	276	26 0	266	29 8	220	22 8	227	23 9	34.2580 56	- 118.1036 11
EFSGR- 02	SAN GABRIEL	244	252	137	14 5	160	16 4	276	34 0	278	29 8	224	23 2	199	23 1	34.2580 56	- 118.1036 11
NFSGR- 01	SAN GABRIEL	264	272	133	14 1	152	17 2	232	25 6	266	29 4	196	20 8	187	23 5	34.2580 56	- 118.1036 11
NFSGR-	SAN	240	264	129	14	156	17	252	26	266	29	196	23	235	25	34.2580	-

02	GABRIEL				5		2		4		4		2		5	56	118.1036 11
FISH-1	SAN GABRIEL	252	260	133	13 3	152	16 8	248	25 2	266	27 0	212	22 8	203	23 1	34.1693 72	- 117.9259 31
FISH-2	SAN GABRIEL	276	288	133	13 3	152	17 2	268	26 8	262	27 4	224	24 4	223	23 1	34.1693 72	- 117.9259 31
FISH-3	SAN GABRIEL	220	264	133	13 7	152	15 2	220	22 0	270	27 0	224	24 4	203	20 3	34.1693 72	- 117.9259 31
FISH-4	SAN GABRIEL	260	284	133	13 3	152	17 6	248	24 8	266	26 6	208	21 2	203	20 3	34.1693 72	- 117.9259 31
FISH-5	SAN GABRIEL	260	260	129	13 3	152	17 2	248	24 8	262	26 2	240	24 4	215	21 9	34.1693 72	- 117.9259 31
WFSGR- 01	SAN GABRIEL	252	260	137	15 3	160	16 4	260	26 0	278	27 8	192	24 0	223	23 5	34.2580 56	- 118.1036 11
HAIN-1	SAN GABRIEL	260	260	117	14 5	148	14 8	232	27 6	270	27 4	216	23 6	203	20 3	34.2367 88	- 118.2820 27
HAIN-2	SAN GABRIEL	260	268	145	14 5	132	15 2	232	27 6	270	27 4	204	23 6	219	21 9	34.2367 88	- 118.2820 27

0.2	123	3	7 SAN	52 SAN	64 SAN	1	12 3										
Sample	Рор	Rhos 5	JACINTO	BERNARDINO Rhos 9	GABRIEL	Rhos 25		Rhos 23		Rhos 14		Rhos 8		Rhos 29		х	Y
INDCK- 01	SAN JACINTO	232	232	137	137	144	18 0	236	23 6	270	27 4	204	21 2	203	21 9	33.7619 64	- 116.8839 11
INDCK- 02	SAN JACINTO	232	236	137	157	148	14 8	236	23 6	270	27 8	192	21 2	199	19 9	33.7619 64	- 116.8839 11
INDCK- 03	SAN JACINTO	232	256	149	157	148	14 8	236	23 6	250	27 0	204	20 4	219	23 5	33.7619 64	- 116.8839 11
INDCK- 04	SAN JACINTO	232	232	145	149	148	14 8	236	23 6	270	27 4	192	20 4	235	23 5	33.7619 64	- 116.8839 11
INDCK- 05	SAN JACINTO	232	232	137	137	128	14 8	236	24 4	262	26 6	192	20 4	199	21 9	33.7619 64	- 116.8839 11
INDCK- 06	SAN JACINTO	232	232	149	157	148	14 8	236	23 6	270	27 0	192	19 2	195	19 9	33.7619 64	- 116.8839 11
INDCK- 07	SAN JACINTO	256	256	137	137	184	21 6	236	23 6	270	27 0	192	19 2	199	21 9	33.7619 64	- 116.8839 11
MILL-1	SAN BERNARDINO	252	256	137	141	156	16 0	232	25 2	282	28 2	192	20 0	219	22 7	34.0777 89	- 117.0994 5
MILL-2	SAN BERNARDINO	252	264	137	141	160	16 0	232	25 2	274	28 2	216	21 6	203	20 7	34.0777 89	- 117.0994 5
MILL-3	SAN BERNARDINO	264	276	137	137	160	16 0	228	25 2	282	28 2	192	26 4	207	21 5	34.0777 89	- 117.0994 5
MILL-4	SAN BERNARDINO	236	276	137	137	140	17 2	232	23 2	274	28 2	200	22 0	199	21 5	34.0777 89	- 117.0994 5

GENALEX MICROSATELLITE DATA FILE – MOUNTAIN RANGES

MILL-5	SAN BERNARDINO	228	272	133	137	140	16 0	252	25 2	274	28 2	200	21 6	203	21 5	34.0777 89	- 117.0994 5
PC-01	SAN BERNARDINO	252	252	133	137	140	18 8	228	22 8	238	23 8	192	19 6	223	22 7	34.1089 17	- 117.1507 5
PC-02	SAN BERNARDINO	236	280	137	137	116	11 6	256	25 6	238	23 8	192	19 6	223	22 7	34.1089 17	- 117.1507 5
PC-03	SAN BERNARDINO	228	264	137	141	180	19 2	248	24 8	238	23 8	204	22 0	227	22 7	34.1089 17	- 117.1507 5
PC-04	SAN BERNARDINO	252	264	137	157	140	15 2	224	24 8	302	30 2	200	20 4	203	21 5	34.1089 17	- 117.1507 5
PC-05	SAN BERNARDINO	252	276	141	157	140	16 0	256	25 6	278	28 6	204	22 4	223	22 3	34.1089 17	- 117.1507 5
PC-06	SAN BERNARDINO	252	280	137	157	140	18 0	256	25 6	254	28 2	204	26 8	195	21 9	34.1089 17	- 117.1507 5
PC-07	SAN BERNARDINO	264	280	137	137	140	18 0	232	23 2	274	25 8	220	26 8	203	22 7	34.1089 17	- 117.1507 5
PC-08	SAN BERNARDINO	252	280	133	145	152	15 2	228	25 6	286	28 6	192	20 4	231	20 3	34.1089 17	- 117.1507 5
PC-09	SAN BERNARDINO	252	280	137	137	152	19 2	232	23 2	254	27 4	196	20 4	203	22 7	34.1089 17	- 117.1507 5
PC-10	SAN BERNARDINO	276	276	133	137	140	14 0	228	22 8	254	26 6	192	22 0	203	24 7	34.1089 17	- 117.1507 5
PC-11	SAN BERNARDINO	228	268	137	141	168	16 8	244	24 4	278	28 2	200	20 4	227	27 7	34.1089 17	- 117.1507 5
PC-12	SAN BERNARDINO	228	268	133	137	168	19 2	248	24 8	266	27 8	192	22 0	195	20 3	34.1089 17	- 117.1507 5
PC-13	SAN BERNARDINO	236	280	137	145	136	18 4	248	24 8	246	27 4	200	20 0	215	22 7	34.1089 17	- 117.1507 5
PC-14	SAN BERNARDINO	252	280	137	137	168	18 0	252	25 6	274	27 4	196	20 4	215	21 5	34.1089 17	- 117.1507

PC-15	SAN BERNARDINO	252	276	141	141	168	16 8	228	25 6	290	29 0	200	20 0	175	17 5	34.1089 17	- 117.1507 5
PC-16	SAN BERNARDINO	236	252	141	161	148	16 8	232	25 2	282	28 2	192	20 0	199	20 3	34.1089 17	- 117.1507 5
PLNG-1	SAN BERNARDINO	236	276	137	141	140	18 4	248	24 8	266	29 0	200	20 4	203	20 3	34.1089 17	- 117.1507 5
PLNG-2	SAN BERNARDINO	252	268	157	157	140	17 2	228	25 6	258	29 0	204	26 8	215	21 9	34.1089 17	- 117.1507 5
PLNG-3	SAN BERNARDINO	276	276	137	145	140	18 4	244	24 4	266	28 6	192	20 4	219	22 7	34.1089 17	- 117.1507 5
PLNG-4	SAN BERNARDINO	264	276	137	161	172	17 2	244	24 4	266	26 6	204	22 0	215	21 9	34.1089 17	- 117.1507 5
PLUN-1	SAN BERNARDINO	276	276	157	161	152	17 2	256	25 6	266	26 6	196	22 4	203	21 9	34.1089 17	- 117.1507 5
TWIN- 01	SAN BERNARDINO	256	272	133	133	152	15 2	264	26 4	262	26 2	184	19 6	219	22 7	34.1747 22	- 117.2666 67
TWIN- 02	SAN BERNARDINO	224	224	133	137	192	19 2	232	26 4	262	29 0	184	20 0	211	22 3	34.1747 22	- 117.2666 67
TWIN- 03	SAN BERNARDINO	224	284	153	153	192	19 6	264	26 8	262	26 2	200	21 2	211	21 1	34.1747 22	- 117.2666 67
TWIN- 04	SAN BERNARDINO	224	264	133	137	160	16 0	256	26 8	266	27 0	200	21 2	211	22 3	34.1747 22	- 117.2666 67
TWIN- 05	SAN BERNARDINO	264	264	133	133	156	16 8	228	26 4	290	29 0	184	20 0	211	21 9	34.1747 22	- 117.2666 67
TWIN- 06	SAN BERNARDINO	260	292	133	133	156	16 8	256	26 8	266	29 0	200	20 0	211	22 7	34.1747 22	- 117.2666 67
TWIN- 07	SAN BERNARDINO	260	284	121	133	160	16 0	260	26 0	274	28 2	200	20 0	219	21 9	34.1747 22	- 117.2666 67
TWIN- 08	SAN BERNARDINO	252	252	133	133	192	19 2	252	26 4	262	28 2	200	20 0	211	21 1	34.1747 22	- 117.2666 67
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TWIN- 09	SAN BERNARDINO	256	268	137	141	156	19 2	264	26 4	254	27 4	196	20 0	211	22 7	34.1747 22	- 117.2666 67
TWIN- 10	SAN BERNARDINO	224	264	137	145	156	16 8	264	26 4	262	27 4	200	20 0	211	21 1	34.1747 22	- 117.2666 67
TWIN- 11	SAN BERNARDINO	256	292	133	133	156	19 2	252	25 2	278	27 8	200	20 0	203	21 5	34.1747 22	- 117.2666 67
TWIN- 12	SAN BERNARDINO	252	280	133	137	156	19 2	236	26 4	270	27 0	200	20 0	211	22 7	34.1747 22	- 117.2666 67
CC-01	SAN BERNARDINO	236	244	149	161	152	16 0	228	22 8	270	27 0	184	18 4	235	23 5	34.2925 26	- 117.3061 35
CC-02	SAN BERNARDINO	244	252	137	145	140	16 4	260	26 0	278	28 2	184	20 0	227	23 5	34.2925 26	- 117.3061 35
CC-03	SAN BERNARDINO	236	236	137	149	140	15 6	244	25 6	274	27 4	188	19 6	227	22 7	34.2925 26	- 117.3061 35
CC-04	SAN BERNARDINO	244	276	145	157	156	17 2	244	24 4	274	27 8	196	19 6	231	23 5	34.2925 26	- 117.3061 35
CC-05	SAN BERNARDINO	244	276	141	165	164	17 2	256	25 6	270	27 4	184	18 8	219	22 7	34.2925 26	- 117.3061 35
CC-06	SAN BERNARDINO	232	272	129	149	164	16 8	244	26 0	274	29 4	208	22 0	227	22 7	34.2925 26	- 117.3061 35
CC-07	SAN BERNARDINO	244	244	141	145	152	15 2	244	24 4	274	27 4	184	18 8	227	23 1	34.2925 26	- 117.3061 35
CC-08	SAN BERNARDINO	232	272	141	149	168	16 8	244	24 4	258	27 4	196	20 4	227	22 7	34.2925 26	- 117.3061 35
CC-09	SAN BERNARDINO	240	276	137	153	168	16 8	244	24 4	274	27 8	200	20 8	227	22 7	34.2925 26	- 117.3061 35
CC-10	SAN BERNARDINO	240	276	141	153	156	20 0	244	25 6	274	27 4	204	22 0	227	22 7	34.2925 26	- 117.3061

CC-11	SAN BERNARDINO	244	244	125	133	156	16 8	240	24 8	278	27 8	188	22 0	227	22 7	34.2925 26	- 117.3061 35
CCWF- 01	SAN BERNARDINO	236	244	149	161	164	20 8	228	22 8	274	27 4	188	19 2	227	23 5	34.2925 26	- 117.3061 35
FORK-1	SAN BERNARDINO	224	224	129	137	164	16 4	236	25 6	282	29 4	188	19 6	235	24 7	34.2925 26	- 117.3061 35
FORK-2	SAN BERNARDINO	224	244	137	145	132	17 2	208	20 8	254	29 4	200	21 2	199	22 7	34.2925 26	- 117.3061 35
CAJO-01	SAN GABRIEL	264	276	141	141	172	17 2	232	25 2	254	27 0	196	26 4	199	23 1	34.2328 11	- 117.4271 83
CAJO-02	SAN GABRIEL	252	252	137	137	156	15 6	208	22 4	266	28 2	196	23 6	203	23 1	34.2328 11	- 117.4271 83
CAJO-03	SAN GABRIEL	268	280	125	157	168	17 2	228	22 8	254	27 0	196	19 6	215	24 7	34.2328 11	- 117.4271 83
CAJO-04	SAN GABRIEL	264	268	133	153	216	22 0	240	25 6	254	27 4	196	21 2	191	22 7	34.2328 11	- 117.4271 83
CAJO-05	SAN GABRIEL	276	276	125	157	168	17 2	228	22 8	254	27 0	196	19 6	215	24 7	34.2328 11	- 117.4271 83
CAJO-06	SAN GABRIEL	224	264	133	145	156	17 2	232	23 2	254	27 0	212	21 2	227	24 7	34.2328 11	- 117.4271 83
CAJO-07	SAN GABRIEL	220	252	145	149	176	17 6	208	22 8	254	27 0	204	20 8	211	23 5	34.2328 11	- 117.4271 83
CAJO-08	SAN GABRIEL	224	224	141	145	132	17 2	224	24 0	250	25 4	188	19 6	223	23 1	34.2328 11	- 117.4271 83
CAJO-09	SAN GABRIEL	280	280	141	141	132	21 6	228	23 2	254	27 4	208	20 8	199	22 7	34.2328 11	- 117.4271 83
CAJO-10	SAN GABRIEL	272	276	133	141	176	17 6	232	24 4	254	26 6	212	21 6	191	22 7	34.2328 11	- 117.4271 83

CAJO-11	SAN GABRIEL	260	264	145	145	164	16 4	228	24 0	274	29 4	196	20 4	199	22 7	34.2328 11	- 117.4271 83
CAJO-12	SAN GABRIEL	264	280	133	145	132	17 6	228	24 4	266	27 4	196	21 2	227	23 9	34.2328 11	- 117.4271 83
CAJO-13	SAN GABRIEL	252	264	141	145	168	21 6	212	22 8	250	27 0	212	21 6	215	22 7	34.2328 11	- 117.4271 83
CAJO-14	SAN GABRIEL	224	248	133	145	164	18 0	228	23 2	270	25 0	204	21 2	199	21 1	34.2328 11	- 117.4271 83
CAJO-15	SAN GABRIEL	252	272	137	141	176	17 6	236	24 4	270	27 4	192	19 6	227	23 9	34.2328 11	- 117.4271 83
CAJO-16	SAN GABRIEL	248	268	129	141	132	16 8	244	24 4	266	27 0	188	21 6	247	24 7	34.2328 11	- 117.4271 83
CAJO-17	SAN GABRIEL	248	248	141	141	168	16 8	228	23 6	274	27 8	188	18 8	199	22 7	34.2328 11	- 117.4271 83
CAJW-1	SAN GABRIEL	224	264	133	141	176	17 6	232	23 2	254	26 6	196	21 2	199	22 7	34.2328 11	- 117.4271 83
CAJW-2	SAN GABRIEL	220	272	133	145	172	21 2	244	24 4	254	27 0	188	19 6	227	23 5	34.2328 11	- 117.4271 83
CAJW-3	SAN GABRIEL	248	276	133	141	176	18 0	232	23 6	266	27 8	196	21 2	191	22 7	34.2328 11	- 117.4271 83
CAJW-4	SAN GABRIEL	248	276	133	141	184	18 4	208	23 6	270	27 0	196	21 2	199	22 7	34.2328 11	- 117.4271 83
LCKC-01	SAN GABRIEL	220	276	141	141	156	15 6	236	23 6	254	27 0	188	19 6	227	24 7	34.2285 83	- 117.4735 17
LCKC-02	SAN GABRIEL	252	276	141	141	156	17 6	232	23 2	270	27 4	264	26 4	247	24 7	34.2285 83	- 117.4735 17
LCKC-03	SAN GABRIEL	220	264	141	141	156	21 6	228	22 8	254	25 4	212	21 2	211	24 7	34.2285 83	- 117.4735 17
LCKC-04	SAN GABRIEL	264	276	141	141	156	15 6	228	23 6	254	25 4	188	19 6	211	21 1	34.2285 83	- 117.4735

LCKC-05	SAN GABRIEL	264	268	141	141	176	17 6	228	22 8	254	27 0	188	18 8	211	22 7	34.2285 83	- 117.4735 17
LCKC-06	SAN GABRIEL	264	276	141	141	156	15 6	228	22 8	254	27 4	208	26 0	227	24 7	34.2285 83	- 117.4735 17
LCKC-07	SAN GABRIEL	220	276	141	141	148	15 2	228	23 2	254	27 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCKC-08	SAN GABRIEL	220	276	145	145	212	21 2	236	23 6	254	25 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCKC-09	SAN GABRIEL	220	276	141	141	156	21 6	228	23 6	254	27 4	212	26 4	227	22 7	34.2285 83	- 117.4735 17
LCKC-10	SAN GABRIEL	220	276	141	141	156	21 6	228	22 8	254	25 4	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-11	SAN GABRIEL	220	276	141	141	176	21 6	228	23 2	254	25 4	188	18 8	211	22 7	34.2285 83	- 117.4735 17
LCK-12	SAN GABRIEL	220	252	141	141	156	15 6	228	23 2	254	25 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCK-13	SAN GABRIEL	220	268	137	141	156	21 6	228	23 6	270	27 4	188	19 6	247	24 7	34.2285 83	- 117.4735 17
LCK-14	SAN GABRIEL	220	276	137	141	156	17 6	228	23 2	254	25 4	196	20 8	211	22 7	34.2285 83	- 117.4735 17
LCK-15	SAN GABRIEL	252	260	141	145	172	17 6	228	23 6	254	25 4	196	25 6	211	22 7	34.2285 83	- 117.4735 17
LCK-16	SAN GABRIEL	220	276	137	141	156	21 6	228	22 8	254	27 0	196	19 6	211	21 1	34.2285 83	- 117.4735 17
LCK-17	SAN GABRIEL	220	276	137	141	156	21 6	232	23 2	254	27 0	264	26 4	211	22 7	34.2285 83	- 117.4735 17
LCK-18	SAN GABRIEL	252	264	141	141	156	15 6	228	22 8	270	27 0	212	26 4	227	22 7	34.2285 83	- 117.4735 17

LCK-19	SAN GABRIEL	252	260	145	145	156	15 6	228	23 6	254	27 0	188	18 8	227	27 7	34.2285 83	- 117.4735 17
LCK-20	SAN GABRIEL	220	276	141	141	156	17 6	232	23 6	254	25 4	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-21	SAN GABRIEL	260	276	141	141	156	15 6	228	23 6	254	25 4	264	26 4	211	22 7	34.2285 83	- 117.4735 17
LCK-22	SAN GABRIEL	264	276	137	141	156	17 6	220	23 2	254	27 4	196	19 6	211	22 7	34.2285 83	- 117.4735 17
LCK-23	SAN GABRIEL	220	264	141	141	156	15 6	228	23 2	254	27 4	188	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-24	SAN GABRIEL	220	268	141	141	156	15 6	228	23 2	274	27 4	196	19 6	211	22 7	34.2285 83	- 117.4735 17
LCK-25	SAN GABRIEL	220	220	133	141	156	15 6	232	23 2	254	28 2	188	21 2	247	24 7	34.2285 83	- 117.4735 17
LCK-26	SAN GABRIEL	264	276	141	141	156	17 6	232	23 6	254	27 4	188	18 8	227	22 7	34.2285 83	- 117.4735 17
LCK-27	SAN GABRIEL	264	276	141	141	152	15 2	224	22 8	254	27 0	196	21 2	227	24 7	34.2285 83	- 117.4735 17
LCK-28	SAN GABRIEL	220	280	141	141	172	17 2	220	22 8	254	27 4	196	26 0	211	24 7	34.2285 83	- 117.4735 17
CATCR- 01	SAN GABRIEL	244	284	125	137	160	16 4	244	24 8	258	26 2	212	21 6	219	23 1	34.23	- 117.7788 32
CATCR- 02	SAN GABRIEL	228	252	125	161	152	15 6	252	26 8	278	27 8	228	22 8	203	20 7	34.23	- 117.7788 32
CATCR- 03	SAN GABRIEL	252	264	125	141	160	17 2	232	25 6	270	27 4	224	22 8	231	23 9	34.23	- 117.7788 32
EFSGR- 01	SAN GABRIEL	240	244	137	141	160	16 4	276	26 0	266	29 8	220	22 8	227	23 9	34.2580 56	- 118.1036 11
EFSGR- 02	SAN GABRIEL	244	252	137	145	160	16 4	276	34 0	278	29 8	224	23 2	199	23 1	34.2580 56	- 118.1036

NFSGR- 01	SAN GABRIEL	264	272	133	141	152	17 2	232	25 6	266	29 4	196	20 8	187	23 5	34.2580 56	- 118.1036 11
NFSGR- 02	SAN GABRIEL	240	264	129	145	156	17 2	252	26 4	266	29 4	196	23 2	235	25 5	34.2580 56	- 118.1036 11
FISH-1	SAN GABRIEL	252	260	133	133	152	16 8	248	25 2	266	27 0	212	22 8	203	23 1	34.1693 72	- 117.9259 31
FISH-2	SAN GABRIEL	276	288	133	133	152	17 2	268	26 8	262	27 4	224	24 4	223	23 1	34.1693 72	- 117.9259 31
FISH-3	SAN GABRIEL	220	264	133	137	152	15 2	220	22 0	270	27 0	224	24 4	203	20 3	34.1693 72	- 117.9259 31
FISH-4	SAN GABRIEL	260	284	133	133	152	17 6	248	24 8	266	26 6	208	21 2	203	20 3	34.1693 72	- 117.9259 31
FISH-5	SAN GABRIEL	260	260	129	133	152	17 2	248	24 8	262	26 2	240	24 4	215	21 9	34.1693 72	- 117.9259 31
WFSGR- 01	SAN GABRIEL	252	260	137	153	160	16 4	260	26 0	278	27 8	192	24 0	223	23 5	34.2580 56	- 118.1036 11
HAIN-1	SAN GABRIEL	260	260	117	145	148	14 8	232	27 6	270	27 4	216	23 6	203	20 3	34.2367 88	- 118.2820 27
HAIN-2	SAN GABRIEL	260	268	145	145	132	15 2	232	27 6	270	27 4	204	23 6	219	21 9	34.2367 88	- 118.2820 27

STRUCTURE PARAMETER SET: SIMULATION CONFIGURATION

Parameter Set: 100K_1MIL

Running Length

Length of Burnin Period: 100000

Number of MCMC Reps after Burnin: 1000000

Ancestry Model Info

Use Admixture Model

* Use Sampling Location Information

* Use Population IDs as Sampling Location Information

* Infer Alpha

* Initial Value of ALPHA (Dirichlet Parameter for Degree of Admixture): 1.0

* Use Same Alpha for all Populations

* Use a Uniform Prior for Alpha

** Maximum Value for Alpha: 10.0

** SD of Proposal for Updating Alpha: 0.025

Frequency Model Info

Allele Frequencies are Correlated among Pops

* Assume Different Values of Fst for Different Subpopulations

* Prior Mean of Fst for Pops: 0.01

* Prior SD of Fst for Pops: 0.05

* Use Constant Lambda (Allele Frequencies Parameter)

* Value of Lambda: 1.0

Advanced Options

Estimate the Probability of the Data Under the Model

Frequency of Metropolis update for Q: 10





Absolute value of the 2nd order rate of change of the likelihood distribution (mean)



DeltaK = mean(|L''(K)|) / sd(L(K))



Absolute value of the 2nd order rate of change of the likelihood distribution (mean)

CLUMPP K=3 INDIVIDUAL PARAMETER FILE

This is the file that sets the parameters for the program CLUMPP, version 1.1 # Everything after "#" will be ignored by the program. Parameters are: # K, C, R, M, W, GREEDY OPTION, REPEATS, PERMUTATIONFILE, PRINT PERMUTED DATA, # PERMUTED_DATAFILE, PRINT_EVERY_PERM and EVERY_PERMFILE. # All parameter names shall be followed by at least one blank space and then # the parameter-value. # ------ Main parameters -----_____ DATATYPE 0 # The type of data to be read in. # 0 = individual data inthe file # specified by INDFILE, 1 = population # data in the file specified by # POPFILE. INDFILE K3.indfile # The name of the individual datafile. # Required if DATATYPE = 0. POPFILE k3.popfile # The name of the population datafile. # Required if DATATYPE = 1. OUTFILE k3.outfile # The average cluster membership # coefficients across the permuted runs # are printed here. MISCFILE k3.miscfile # The parameters used and a summary of # the results are printed here.

к 3 # Number of clusters. C 146 # Number of individuals or populations. R 25 # Number of runs. м 2 # Method to be used (1 =FullSearch, # 2 = Greedy, 3 =LargeKGreedy). # Weight by the number of W 1 individuals # in each population as specified in # the datafile (1 if yes, 0 if no). s 2 # Pairwise matrix similarity statistic # to be used. $1 = G_{, 2} =$ G'. # - Additional options for the Greedy and LargeKGreedy algorithm (M = 2 or 3) -GREEDY OPTION 2 # 1 = All possible input orders, # 2 = random input orders, # 3 = pre-specified input orders. REPEATS 1000 # If GREEDY OPTION = 2, then REPEATS # determines the number of random input # orders to be tested. If GREEDY OPTION # = 3, then REPEATS is the number of # input orders in PERMUTATIONFILE. PERMUTATIONFILE arabid.permutationfile # The permutations of the runs in # PERMUTATIONFILE will be used, if

GREEDY OPTION = 3.

_____ PRINT PERMUTED DATA 1 # Print the permuted data (clusters) in # INDFILE or POPFILE to # PERMUTED DATAFILE (0 = don't print, # 1 = print into one file, 2 = print# into separate files for each run). PERMUTED_DATAFILE arabid.perm_datafile# The permuted data (clusters) will be # printed to this file (if # PRINT PERMUTED DATA = 2, several # files with the extensions " 1" to # " R" will be created). PRINT EVERY PERM 0 # Print every tested permutation of the # runs and the corresponding value of # SSC to a file specified by # EVERY PERMFILE (0 = don't print, # 1 = print).# Note that printing may result in a # very large file. EVERY PERMFILE arabid.every permfile # Every tested permutation of the runs # and the corresponding SSC will be # printed here. PRINT RANDOM INPUTORDER 0 # Print random input orders of runs to # RANDOM INPUTORDER (0 =don't print,

1 = print). This option is only # available if GREEDY OPTION = 2. RANDOM_INPUTORDERFILE arabid.random_inputorderfile # Every random input order # of the runs (generated by CLUMPP if # GREEDY OPTION = 2) will be printed # here. # ------ Advanced options ------_____ OVERRIDE WARNINGS 0 # This option allows the user to # override non-crucial warnings from # the program (0 allow warnings, 1 do # not issue non-crucial warnings). ORDER BY RUN 1 # Permute the clusters of the output # files by the specified run. (0 to # not specify a run, 1 to R specifies # a run in the INDFILE or POPFILE). # ----- Additional comments -----_____ # The term ''permutation'' is used in two different contexts, permutations of # membership coefficients, or clusters, and permutations of runs. # For example, if the datafile has has data A B C D E (each letter indicates a # column corresponding to a cluster), then permutation 3 2 5 1 4 of the # clusters means C B E A D.

```
# Permutation 4 1 2 3 of runs 1-4 would mean start with run 4,
then run 1, then
\# run 2, and then run 3.
# ----- Command line arguments -----
_____
# -i INDFILE
# -p POPFILE
# -0 OUTFILE
# -j MISCFILE
# −k K
# -c C
# -r R
# −m M
# -w W
# -s S
# ______
_____
```

CLUMPP K=3 POPULATION PARAMETER FILE

This is the file that sets the parameters for the program CLUMPP, version 1.1 # Everything after "#" will be ignored by the program. Parameters are: # K, C, R, M, W, GREEDY OPTION, REPEATS, PERMUTATIONFILE, PRINT PERMUTED DATA, # PERMUTED_DATAFILE, PRINT_EVERY_PERM and EVERY_PERMFILE. # All parameter names shall be followed by at least one blank space and then # the parameter-value. # ------ Main parameters -----_____ DATATYPE 1 # The type of data to be read in. # 0 = individual data inthe file # specified by INDFILE, 1 = population # data in the file specified by # POPFILE. INDFILE K3.popfile # The name of the individual datafile. # Required if DATATYPE = 0. POPFILE k3.popfile # The name of the population datafile. # Required if DATATYPE = 1. OUTFILE k3.outfile # The average cluster membership # coefficients across the permuted runs # are printed here. MISCFILE k3.miscfile # The parameters used and a summary of # the results are printed here.

к 3 # Number of clusters. С З # Number of individuals or populations. R 25 # Number of runs. м 2 # Method to be used (1 =FullSearch, # 2 = Greedy, 3 =LargeKGreedy). # Weight by the number of W 1 individuals # in each population as specified in # the datafile (1 if yes, 0 if no). s 2 # Pairwise matrix similarity statistic # to be used. $1 = G_{, 2} =$ G'. # - Additional options for the Greedy and LargeKGreedy algorithm (M = 2 or 3) -GREEDY OPTION 2 # 1 = All possible input orders, # 2 = random input orders, # 3 = pre-specified input orders. REPEATS 1000 # If GREEDY OPTION = 2, then REPEATS # determines the number of random input # orders to be tested. If GREEDY OPTION # = 3, then REPEATS is the number of # input orders in PERMUTATIONFILE. PERMUTATIONFILE arabid.permutationfile # The permutations of the runs in # PERMUTATIONFILE will be used, if

GREEDY OPTION = 3.

_____ PRINT PERMUTED DATA 1 # Print the permuted data (clusters) in # INDFILE or POPFILE to # PERMUTED DATAFILE (0 = don't print, # 1 = print into one file, 2 = print# into separate files for each run). PERMUTED_DATAFILE arabid.perm_datafile# The permuted data (clusters) will be # printed to this file (if # PRINT PERMUTED DATA = 2, several # files with the extensions " 1" to # " R" will be created). PRINT EVERY PERM 0 # Print every tested permutation of the # runs and the corresponding value of # SSC to a file specified by # EVERY PERMFILE (0 = don't print, # 1 = print).# Note that printing may result in a # very large file. EVERY PERMFILE arabid.every permfile # Every tested permutation of the runs # and the corresponding SSC will be # printed here. PRINT RANDOM INPUTORDER 0 # Print random input orders of runs to # RANDOM INPUTORDER (0 = don't print,

1 = print). This option is only # available if GREEDY OPTION = 2. RANDOM_INPUTORDERFILE arabid.random_inputorderfile # Every random input order # of the runs (generated by CLUMPP if # GREEDY OPTION = 2) will be printed # here. # ------ Advanced options ------_____ OVERRIDE WARNINGS 0 # This option allows the user to # override non-crucial warnings from # the program (0 allow warnings, 1 do # not issue non-crucial warnings). ORDER BY RUN 1 # Permute the clusters of the output # files by the specified run. (0 to # not specify a run, 1 to R specifies # a run in the INDFILE or POPFILE). # ----- Additional comments -----_____ # The term ''permutation'' is used in two different contexts, permutations of # membership coefficients, or clusters, and permutations of runs. # For example, if the datafile has has data A B C D E (each letter indicates a # column corresponding to a cluster), then permutation 3 2 5 1 4 of the # clusters means C B E A D.

```
# Permutation 4 1 2 3 of runs 1-4 would mean start with run 4,
then run 1, then
\# run 2, and then run 3.
# ----- Command line arguments -----
_____
# -i INDFILE
# -p POPFILE
# -0 OUTFILE
# -j MISCFILE
# −k K
# -c C
# -r R
# −m M
# -w W
# -s S
# ______
_____
```

CLUMPP K=2 INDIVIDUAL PARAMETER FILE

This is the file that sets the parameters for the program CLUMPP, version 1.1 # Everything after "#" will be ignored by the program. Parameters are: # K, C, R, M, W, GREEDY OPTION, REPEATS, PERMUTATIONFILE, PRINT PERMUTED DATA, # PERMUTED_DATAFILE, PRINT_EVERY_PERM and EVERY_PERMFILE. # All parameter names shall be followed by at least one blank space and then # the parameter-value. # ------ Main parameters -----_____ DATATYPE 0 # The type of data to be read in. # 0 = individual data inthe file # specified by INDFILE, 1 =population # data in the file specified by # POPFILE. INDFILE k2.indfile # The name of the individual datafile. # Required if DATATYPE = 0. POPFILE k2.popfile # The name of the population datafile. # Required if DATATYPE = 1. OUTFILE k2.outfile # The average cluster membership # coefficients across the permuted runs # are printed here. MISCFILE k2.miscfile # The parameters used and a summary of # the results are printed here.

К 2 # Number of clusters. C 123 # Number of individuals or populations. # Number of runs. R 15 м 2 # Method to be used (1 =FullSearch, # 2 = Greedy, 3 =LargeKGreedy). # Weight by the number of W 1 individuals # in each population as specified in # the datafile (1 if yes, 0 if no). s 2 # Pairwise matrix similarity statistic # to be used. $1 = G_{, 2} =$ G'. # - Additional options for the Greedy and LargeKGreedy algorithm (M = 2 or 3) -GREEDY OPTION 2 # 1 = All possible input orders, # 2 = random input orders, # 3 = pre-specified input orders. REPEATS 1000 # If GREEDY OPTION = 2, then REPEATS # determines the number of random input # orders to be tested. If GREEDY OPTION # = 3, then REPEATS is the number of # input orders in PERMUTATIONFILE. PERMUTATIONFILE arabid.permutationfile # The permutations of the runs in # PERMUTATIONFILE will be used, if

GREEDY OPTION = 3.

_____ PRINT PERMUTED DATA 1 # Print the permuted data (clusters) in # INDFILE or POPFILE to # PERMUTED DATAFILE (0 = don't print, # 1 = print into one file, 2 = print# into separate files for each run). PERMUTED_DATAFILE arabid.perm_datafile# The permuted data (clusters) will be # printed to this file (if # PRINT PERMUTED DATA = 2, several # files with the extensions " 1" to # " R" will be created). PRINT EVERY PERM 0 # Print every tested permutation of the # runs and the corresponding value of # SSC to a file specified by # EVERY PERMFILE (0 = don't print, # 1 = print).# Note that printing may result in a # very large file. EVERY PERMFILE arabid.every permfile # Every tested permutation of the runs # and the corresponding SSC will be # printed here. PRINT RANDOM INPUTORDER 0 # Print random input orders of runs to # RANDOM INPUTORDER (0 = don't print,

1 = print). This option is only # available if GREEDY OPTION = 2. RANDOM_INPUTORDERFILE arabid.random_inputorderfile # Every random input order # of the runs (generated by CLUMPP if # GREEDY OPTION = 2) will be printed # here. # ------ Advanced options ------_____ OVERRIDE WARNINGS 0 # This option allows the user to # override non-crucial warnings from # the program (0 allow warnings, 1 do # not issue non-crucial warnings). ORDER BY RUN 1 # Permute the clusters of the output # files by the specified run. (0 to # not specify a run, 1 to R specifies # a run in the INDFILE or POPFILE). # ----- Additional comments -----_____ # The term ''permutation'' is used in two different contexts, permutations of # membership coefficients, or clusters, and permutations of runs. # For example, if the datafile has has data A B C D E (each letter indicates a # column corresponding to a cluster), then permutation 3 2 5 1 4 of the # clusters means C B E A D.

```
# Permutation 4 1 2 3 of runs 1-4 would mean start with run 4,
then run 1, then
\# run 2, and then run 3.
# ----- Command line arguments -----
_____
# -i INDFILE
# -p POPFILE
# -0 OUTFILE
# -j MISCFILE
# −k K
# -c C
# -r R
# −m M
# -w W
# -s S
# ______
_____
```

CLUMPP K=2 POPULATION

This is the file that sets the parameters for the program CLUMPP, version 1.1 # Everything after "#" will be ignored by the program. Parameters are: # K, C, R, M, W, GREEDY OPTION, REPEATS, PERMUTATIONFILE, PRINT PERMUTED DATA, # PERMUTED DATAFILE, PRINT EVERY PERM and EVERY PERMFILE. # All parameter names shall be followed by at least one blank space and then # the parameter-value. # ------ Main parameters ------_____ DATATYPE 1 # The type of data to be read in. # 0 = individual data in the file # specified by INDFILE, 1 = population # data in the file specified by # POPETLE. INDFILE k2.indfile # The name of the individual datafile. # Required if DATATYPE = 0. # The name of the POPFILE k2.popfile population datafile. # Required if DATATYPE = 1. OUTFILE k2.outfile # The average cluster membership # coefficients across the permuted runs # are printed here. MISCFILE k2.miscfile # The parameters used and a summary of # the results are printed here. к 2 # Number of clusters.

C 13 # Number of individuals or populations. R 15 # Number of runs. M 2 # Method to be used (1 = FullSearch, # 2 = Greedy, 3 =LargeKGreedy). W 1 # Weight by the number of individuals # in each population as specified in # the datafile (1 if yes, 0 if no). S 2 # Pairwise matrix similarity statistic # to be used. $1 = G_{1} = 2 =$ G'. # - Additional options for the Greedy and LargeKGreedy algorithm (M = 2 or 3) -# 1 = All possible input GREEDY OPTION 2 orders, # 2 = random input orders, # 3 = pre-specified input orders. REPEATS 1000 # If GREEDY OPTION = 2, then REPEATS # determines the number of random input # orders to be tested. If GREEDY OPTION # = 3, then REPEATS is the number of # input orders in PERMUTATIONFILE. PERMUTATIONFILE arabid.permutationfile # The permutations of the runs in # PERMUTATIONFILE will be used, if # GREEDY OPTION = 3.

_____ PRINT PERMUTED DATA 1 # Print the permuted data (clusters) in # INDFILE or POPFILE to # PERMUTED DATAFILE (0 =don't print, # 1 = print into one file, 2 = print# into separate files for each run). PERMUTED DATAFILE arabid.perm datafile# The permuted data (clusters) will be # printed to this file (if # PRINT PERMUTED DATA = 2, several # files with the extensions " 1" to # " R" will be created). PRINT_EVERY_PERM 0 # Print every tested permutation of the # runs and the corresponding value of # SSC to a file specified by # EVERY PERMFILE (0 = don't print, # 1 = print).# Note that printing may result in a # very large file. EVERY PERMFILE arabid.every permfile # Every tested permutation of the runs # and the corresponding SSC will be # printed here. PRINT RANDOM INPUTORDER 0 # Print random input orders of runs to # RANDOM INPUTORDER (0 =don't print, # 1 = print). This option is only

available if GREEDY OPTION = 2. RANDOM INPUTORDERFILE arabid.random inputorderfile # Every random input order # of the runs (generated by CLUMPP if # GREEDY OPTION = 2) will be printed # here. # ------ Advanced options ------_____ OVERRIDE WARNINGS 0 # This option allows the user to # override non-crucial warnings from # the program (0 allow warnings, 1 do # not issue non-crucial warnings). # Permute the clusters of ORDER BY RUN 1 the output # files by the specified run. (0 to # not specify a run, 1 to R specifies # a run in the INDFILE or POPFILE). # ------ Additional comments ------_____ # The term ''permutation'' is used in two different contexts, permutations of # membership coefficients, or clusters, and permutations of runs. # For example, if the datafile has has data A B C D E (each letter indicates a # column corresponding to a cluster), then permutation 3 2 5 1 4 of the # clusters means C B E A D. # Permutation 4 1 2 3 of runs 1-4 would mean start with run 4, then run 1, then

DISTRUCT PARAMETER FILE K=3

PARAMETERS FOR THE PROGRAM distruct. YOU WILL NEED TO SET THESE IN ORDER TO RUN THE PROGRAM.

Data settings

#define INFILE_POPQ k3.popq // (str) input file of population q's #define INFILE_INDIVQ k3.indivq // (str) input file of individual q's #define INFILE_LABEL_BELOW k3.watersheds // (str) input file of labels for below figure #define INFILE_LABEL_ATOP k3.regions // (str) input file of labels for atop figure #define INFILE_CLUST_PERM k3.perm // (str) input file of permutation of clusters to print #define OUTFILE k3.ps //(str) name of output file

#define K 3 // (int) number of clusters

```
#define NUMPOPS 3 // (int) number of pre-defined populations
#define NUMINDS 146 // (int) number of individuals
Main usage options
#define PRINT_INDIVS 1 // (B) 1 if indiv q's are to be
printed, 0 if only population q's
#define PRINT_LABEL_ATOP 0 // (B) print labels above figure
#define PRINT_LABEL_BELOW 1 // (B) print labels below figure
#define PRINT SEP 1 // (B) print lines to separate
populations
Figure appearance
#define FONTHEIGHT 6 // (d) size of font
#define DIST ABOVE 5 // (d) distance above plot to place text
#define DIST_BELOW -7 // (d) distance below plot to place text
#define BOXHEIGHT 36 // (d) height of the figure
#define INDIVWIDTH 1.5 // (d) width of an individual
Extra options
#define ORIENTATION 0 // (int) 0 for horizontal orientation
(default)
                     // 1 for vertical orientation
                     11
                                 2 for reverse horizontal
orientation
                            // 3 for reverse vertical
orientation
#define XORIGIN 75 // (d) lower-left x-coordinate of
fiqure
#define YORIGIN 250
                      // (d) lower-left y-coordinate of
figure
#define XSCALE 1 // (d) scale for x direction
#define YSCALE 1 // (d) scale for y direction
#define ANGLE LABEL ATOP 60 // (d) angle for labels atop figure
(in [0,180])
#define ANGLE LABEL BELOW 60 // (d) angle for labels below
figure (in [0,180])
#define LINEWIDTH RIM 3 // (d) width of "pen" for rim of box
#define LINEWIDTH SEP 0.3 // (d) width of "pen" for separators
between pops and for tics
#define LINEWIDTH IND 0.3 // (d) width of "pen" used for
individuals
#define GRAYSCALE 0 // (B) use grayscale instead of
colors
#define ECHO DATA 1
                               // (B) print some of the data to
the screen
```

```
#define REPRINT DATA 1
                               // (B) print the data as a
comment in the ps file
#define PRINT INFILE NAME 0
                                // (B) print the name of
INFILE POPQ above the figure
                                11
                                     this option is meant for
use only with ORIENTATION=0
#define PRINT COLOR BREWER 1
                                // (B) print ColorBrewer settings
in the output file
                                11
                                       this option adds 1689
lines and 104656 bytes to the output
                                //
                                       and is required if using
ColorBrewer colors
Command line options:
-d drawparams
-K K
-M NUMPOPS
-N NUMINDS
-p input file (population q's)
-i input file (individual q's)
-a input file (labels atop figure)
-b input file (labels below figure)
-c input file (cluster permutation)
-o output file
```

DISTRUCT PARAMETER FILE K=2

PARAMETERS FOR THE PROGRAM distruct. YOU WILL NEED TO SET THESE IN ORDER TO RUN THE PROGRAM. "(int)" means that this takes an integer value. "(B)" means that this variable is Boolean (1 for True, and 0 for False) "(str)" means that this is a string (but not enclosed in quotes) "(d)" means that this is a double (a real number). Data settings #define INFILE_POPQ k2.popq // (str) input file of population q's

#define INFILE INDIVQ k2.indivq // (str) input file of individual q's #define INFILE LABEL BELOW k2.names // (str) input file of labels for below figure #define INFILE LABEL ATOP k2.regions // (str) input file of labels for atop figure #define INFILE CLUST PERM k2.perm // (str) input file of permutation of clusters to print #define OUTFILE k2.ps //(str) name of output file #define K 2 // (int) number of clusters #define NUMPOPS 13 // (int) number of pre-defined populations #define NUMINDS 123 // (int) number of individuals Main usage options #define PRINT INDIVS 1 // (B) 1 if indiv q's are to be printed, 0 if only population q's #define PRINT_LABEL_ATOP 0 // (B) print labels above figure #define PRINT LABEL BELOW 1 // (B) print labels below figure #define PRINT SEP ____ 1 // (B) print lines to separate populations Figure appearance #define FONTHEIGHT 6 // (d) size of font #define DIST ABOVE 5 // (d) distance above plot to place text #define DIST BELOW -7 // (d) distance below plot to place text #define BOXHEIGHT 36 // (d) height of the figure #define INDIVWIDTH 1.5 // (d) width of an individual Extra options #define ORIENTATION 0 // (int) 0 for horizontal orientation (default) // 1 for vertical orientation 2 for reverse horizontal orientation // 3 for reverse vertical orientation #define XORIGIN 75 // (d) lower-left x-coordinate of figure // (d) lower-left y-coordinate of #define YORIGIN 250 fiqure #define XSCALE 1 // (d) scale for x direction
#define YSCALE 1 // (d) scale for y direction

#define ANGLE LABEL ATOP 60 // (d) angle for labels atop figure (in [0,180]) #define ANGLE LABEL BELOW 60 // (d) angle for labels below figure (in [0,180]) #define LINEWIDTH RIM 3 // (d) width of "pen" for rim of box #define LINEWIDTH SEP 0.3 // (d) width of "pen" for separators between pops and for tics #define LINEWIDTH IND 0.3 // (d) width of "pen" used for individuals #define GRAYSCALE 0 // (B) use grayscale instead of colors #define ECHO DATA 1 // (B) print some of the data to the screen #define REPRINT DATA 1 // (B) print the data as a comment in the ps file #define PRINT INFILE NAME 0 // (B) print the name of INFILE POPQ above the figure 11 this option is meant for use only with ORIENTATION=0 #define PRINT COLOR BREWER 1 // (B) print ColorBrewer settings in the output file // this option adds 1689 lines and 104656 bytes to the output and is required if using 11 ColorBrewer colors Command line options: -d drawparams -K K -M NUMPOPS -N NUMINDS -p input file (population q's) -i input file (individual q's) -a input file (labels atop figure) -b input file (labels below figure) -c input file (cluster permutation)

-o output file
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