Final Report to the California Department of Fish and Wildlife
Agreement P1280004

Population Genetics Study of California's Black Bears

June 30, 2015

Jamie Sherman, PhD Candidate*
Holly Ernest, DVM, PhD*§

*Wildlife Population Heath and Genetics Laboratory
University of California, Davis
One Shields Avenue
Davis, CA 95616

Email: Current: hernest@uwyo.edu
Phone: 307-766-6605

§Current main affiliation:
Wildlife Genomics and Disease Ecology Lab
Department of Veterinary Sciences
University of Wyoming, Laramie

TABLE OF CONTENTS

Population Genetics Study of California Black Bears

Final report to the California Department of Fish and Wildlife
Contract Number P1280004

Executive Summary

Black bears (*Ursus americanus*) are recognized as both an important component of California's ecosystems, and a valuable resource to the people of California. Classified as a game mammal since 1948, black bears have remained a source of recreation for hunters, but also for wildlife viewers as well. Their iconic stature and unique behaviors attract visitors from all over the world to many of California's National Parks each year. Black bear populations have been growing steadily in California for over 20 years. Today, there are estimated to be over 34,000 bears residing in the state. The combination of black bear population and range expansion with human growth and urban development has brought bears and humans closer than ever before, creating an increased potential for human and wildlife conflicts. It is important for the California Department of Fish and Wildlife to have a current and thorough understanding of population demographics, abundance and density in order to scientifically inform black bear management. New advances in population monitoring techniques have made it possible to noninvasively sample and identify individual black bears from as little as a single hair root. Capture-mark-recapture methods using noninvasive hair snags and DNA analysis provide the key individual and population data to support best practice management. This project used noninvasive genetic capture-mark-recapture to estimate the abundance of bears in the Central Coast Ranges, an area that was only colonized by black bears in the past 50 years. Samples were collected in San Luis Obispo and Monterey counties from 2013-2014, from which 67 unique individuals were identified (63 in San Luis Obispo; 4 in Monterey). Population estimation models indicate that there are an estimated 101 bears (confidence interval: 84-134) currently residing in the area sampled in San Luis Obispo County. Population estimates using capture-mark-recapture analysis could not be determined in Monterey County due to low sample sizes. However, since there was an extensive sampling effort with only four individual bears identified by genetics, we hypothesize that Monterey County has an extremely low bear population at this time. Genetic structure and assignment analyses suggest that bears in San Luis Obispo and Monterey counties are part of a single panmictic, or randomly mating, population with origins from the Southern Sierra Nevada Ranges. The results of this study demonstrate the usefulness and applicability of noninvasive genetic capture-mark-recapture for estimating one black bear population in California and provide robust science-based population estimates that can be used as baselines to monitor bears into the future. To further inform black bear management, we estimated the resources (efforts, costs, etc.) necessary to implement capture-mark-recapture methods statewide and make recommendations for the potential approaches to effectively monitor California's black bears into the future.

Introduction and Background

Black bears (*Ursus americanus*) are a widespread game species in North America, including California, where they are not only an important component of local ecosystems but also as a valuable resource to the people of California. Effective monitoring and reliable estimates of population parameters such as abundance and population growth rates are necessary in order to make scientifically informed management decisions (Garshelis 1993; Garshelis and Hristienko 2006; McCall *et al.* 2013), including adjustments to the statewide hunting program and actions towards preventing and responding to human-wildlife conflicts (Harris *et al.* 2011). Dedicated intensive and long-term monitoring programs are rarely implemented due to financial (Long *et al.* 2008) and logistical constraints for physically capturing secretive and wide-ranging species (Harris *et al.* 2011).

Wildlife managers primarily rely on estimates of abundance calculated using sex ratios derived from hunter harvest and mortality data (Pelton 2003; Garshelis and Hristienko 2006). Although these methods are less costly than live-capture or telemetry studies, the reliability of the estimates produced have been challenged (Miller 1989; Kane and Livaitis 1992; Koehler and Pierce 2005; Coster *et al.* 2011). Small sample sizes (Miller 1989) and regional variation in hunter-harvest rates (Diefenback *et al.* 2004) can result in inaccurate demographic and population estimates that do not detect local (small geographic scale) variation in bear density (Ranta *et al.* 2008; Coster *et al.* 2011). For example, in 2013, 1,078 bears, out of the total estimated 34,000, were reported as taken to the California Department of Fish and Wildlife. Ten out of the 58 counties (17%) where bears were hunted, represent over 60% of the total take, further demonstrating the uneven distribution of hunter-take data across the state. In light of these concerns, abundance estimates derived from harvest and mortality data should be verified when possible (Kane and Litvaitis 1992; Garshelis and Hristienko 2006; Coster *et al.* 2011).

Advancements in molecular genetic analysis techniques, such as genetic capture-mark-recapture (CMR) have enabled widespread use of this method to estimate wildlife populations (Coster *et al.* 2011). These methods provide new cost-effective and more reliable monitoring methods that do not require capturing or handling animals, or rely on hunter-take data (Long *et al.* 2008). DNA extracted from hair or scat samples collected noninvasively can be used to identify species, individuals, and their sex. (Garshelis and Noyce 2006). The use of DNA allows for genetic monitoring that provides information on abundance and distribution, but can also be used to describe patterns in population genetics such as, genetic variation (Schwartz *et al.* 2007), population structure (Kendall *et al.* 2009), and dispersal patterns and landscape barriers (Funk *et al.* 2005; Coulon *et al.* 2008).

California's black bear population has increased rapidly over the past 30 years, from an estimate of 10,000 in 1982 to over 34,000 today (California Department of Fish and Wildlife, CDFW 2012). A majority of the state's black bears inhabit north and west of the Sierra Nevada Mountains, however other regions have become increasingly important as both the numbers and ranges of black bears expands throughout the state. Black bears are now found in areas where they were historically excluded, including urban and suburban landscapes (CDFW 2012) and former grizzly bear habitat (Brown

et al. 2009). Black bear population expansion, in conjunction with human population growth and expansion has resulted in greater numbers of human-wildlife conflicts (Hristienko and McDonald 2007). Most conflicts occur in the form of property damage to crops, residential property, pets, livestock, beehives and vehicle collisions, however bears can also pose a threat to personal safety (Hristienko and McDonald 2007).

The grizzly bear (*Ursus arctos*) historically inhabited the Central Coast Ranges of California, (Suckley and Gibbs 1860). Black bears were not believed to inhabit the Central Coast Ranges, including San Luis Obispo and Monterey counties, likely as a result of competitive exclusion from the larger and more powerful grizzly bear (Storer and Tevis 1955; Hall and Kelson 1959; Grinnell *et al.* 1937). After the grizzly bear was extirpated from California in the 1920's due to unregulated hunting, black bears started to colonize new territories. In the Central Coast Ranges, genetic profiles indicate that the area was likely colonized by bears originating from the Southern Sierra Nevada Ranges (Brown *et al*. 2009; California Department of Fish and Game, CDFG 2010). Although bears have been present in the Central Coast Ranges for over 50 years, little is known about the population. Hunting does not take place in either San Luis Obispo or Monterey counties, and therefore all population estimates have been derived from individual sightings and habitat analysis (CDFG 2010).

The overall goal of this project was to gain a better understanding of the black bear population in the Central Coast Ranges, specifically San Luis Obispo and Monterey counties, using noninvasive genetic capture-mark-recapture. Individual objectives include: identify individual black bears by DNA analyses; describe black bear populations' genetic diversity; estimate the population sizes of bears in regions sampled within San Luis Obispo and Monterey counties; and determine the source populations of individual bears observed in each county. In addition, this project presents an evaluation of the resources necessary to implement the noninvasive genetic capture-mark-recapture method used in San Luis Obispo and Monterey on a statewide scale. We present a review of methods commonly used to monitor bear populations and overall recommendations for a California black bear monitoring program. The information generated by this project will inform future adaptive management strategies for the California Department of Fish and Wildlife black bear program.

Methods

Study Area

This study focused on San Luis Obispo and Monterey counties located along the central coast of California (Figure 1). Sampling occurred from June 17 to August 8, 2013 in San Luis Obispo County and from April 28 to July 3, 2014 in Monterey County. San Luis Obispo and Monterey counties are approximately 3,299 (8,544 km^2) and 3,280 (8,495 km^2) land $miles^2$, respectively (U.S. Census Bureau 2014). Both counties are a part of California's Coast Ranges that extend 550 miles (885 km) from the South Fork of the Klamath Province on the north, to the Santa Ynez Mountains on the south (Schoenherr 1992). The coast ranges are divided into two subgroups, by the San Francisco Bay, with San Luis Obispo and Monterey falling into the southern coast ranges (Schoenherr 1992). The Coast Ranges are accentuated by steep slopes, however most peaks are lower than 6,000 feet (1,800 m) in elevation (Schoenherr 1992).

Figure 1. Map of Study Area, including San Luis Obispo and Monterey Counties (adapted from Google Earth)

The climate of San Luis Obispo and Monterey counties is described as Mediterranean with fog and cold temperatures dominating the coastal side of the ranges versus the heat and aridity of the land-facing sides (Schoenherr 1992). The average precipitation for San Luis Obispo and Monterey counties during the study years was 4.45 and 10.45 inches, respectively. The average annual temperature in 2013 in San Luis Obispo was 60.8°F, with a mean temperature of 60.31°F (15.72 °C) and 1.35 inches (3.43 cm) of precipitation during the study months of June to August. The average annual temperature in 2014 in Monterey was 56.6°F (13.67 °C), with a mean temperature of 69.40°F and 0.01 inches (0.25 cm) of precipitation during the study months of May to July (National Oceanic and Atmospheric Administration 2015).

The population of San Luis Obispo County during the study year (2013) was estimated at 274,528 people, or 83.2 people per square mile (U.S. Census Bureau 2014). Monterey County (2014) had a higher population with 428,826 people, and density of 130.7 people per square mile (U.S. Census Bureau 2014). In San Luis Obispo County approximately 83.4% of the population lives in an urban environment (where “urban” is defined as greater than 2500 people), which only makes up 2.96% of the landscape (U.S. Census Bureau 2010). In Monterey County approximately 90% of the population lives in an urban area, which accounts for 3.25% of the landscape (U.S. Census Bureau

2010). Vegetation and land cover vary greatly across both counties. A summary of percent land cover in each county can be found in Table 1.

Table 1. Summary of land cover classifications for San Luis Obispo and Monterey counties (California Department of Forestry and Fire Protection 2012; County of Monterey Planning and Building Inspection 2007)

Land Cover Classification	San Luis Obispo	Monterey
Agriculture	5.69%	12.00%
Bare Soil	0.29%	1.61%
Chaparral	13.56%	0.70%
Grassland	46.66%	34.28%
Montane Hardwood	1.99%	1.39%
Oak Savanna	0.00%	9.51%
Oak Woodland	19.84%	20.10%
Scrub	5.74%	15.23%
Urban	2.96%	3.25%
Other	3.27%	1.94%
	100.00%	100.00%

In addition to the Coast Range sampling described above, three sampling sessions took place in Mono County in the eastern Sierra Nevada from 2010 – 2012. These sampling efforts were part of a larger study conducted by Jonathan Fusaro, Timothy Taylor, Marc Kenyon, and collaborators investigating the populations of bears in the urban landscape of the town of Mammoth Lakes in comparison with the rural landscape of Slinkard Valley Wildlife Area. A written summary of the Mono County study, including population estimates, will be provided by Jonathan Fusaro (Environmental Scientist, Region 6, project lead) per his separate contract with CDFW. Per this contract agreement, population genetic analysis of the bears sampled in Mono County in support of Jonathan Fusaro's work is presented in the following report.

Sampling Design

Per contract agreement, CDFW designed and carried out the sampling plan in order to provide samples to the UC Davis Wildlife Genetics and Population Health Laboratory, at the University of California, Davis, School of Veterinary Medicine (Davis, CA, USA). CDFW used a systematic grid design to balance sampling effort and minimize capture heterogeneity across the study areas (White *et al.* 1982). The grid was divided into 20 km^2 hexagonal sampling units, which represents a conservative estimate of the average home range size of female black bears in the study area (Novick 1979). Using a Geographic Information Systems (GIS) habitat suitability index model from the California Wildlife Habitat Relationships System (CWHRS) (Airola 1988; CDFG 2010) units comprised of high and medium habitat quality classification zones were identified. Classification zones were determined relative to a bear's life requisites, such as food availability, water and land and canopy cover (California Department of Fish and Game & California Interagency Wildlife Task Group 2002). Those units were given a centroid point directly in the center of the hexagon, representing one sampling station. Each

point was shifted to be within 200 - 500 meters of a road to allow for reasonable sampling access while minimizing the risk of vehicle strikes to animals visiting sampling stations. Written permission from land-owners was obtained for proposed sampling stations located on private property.

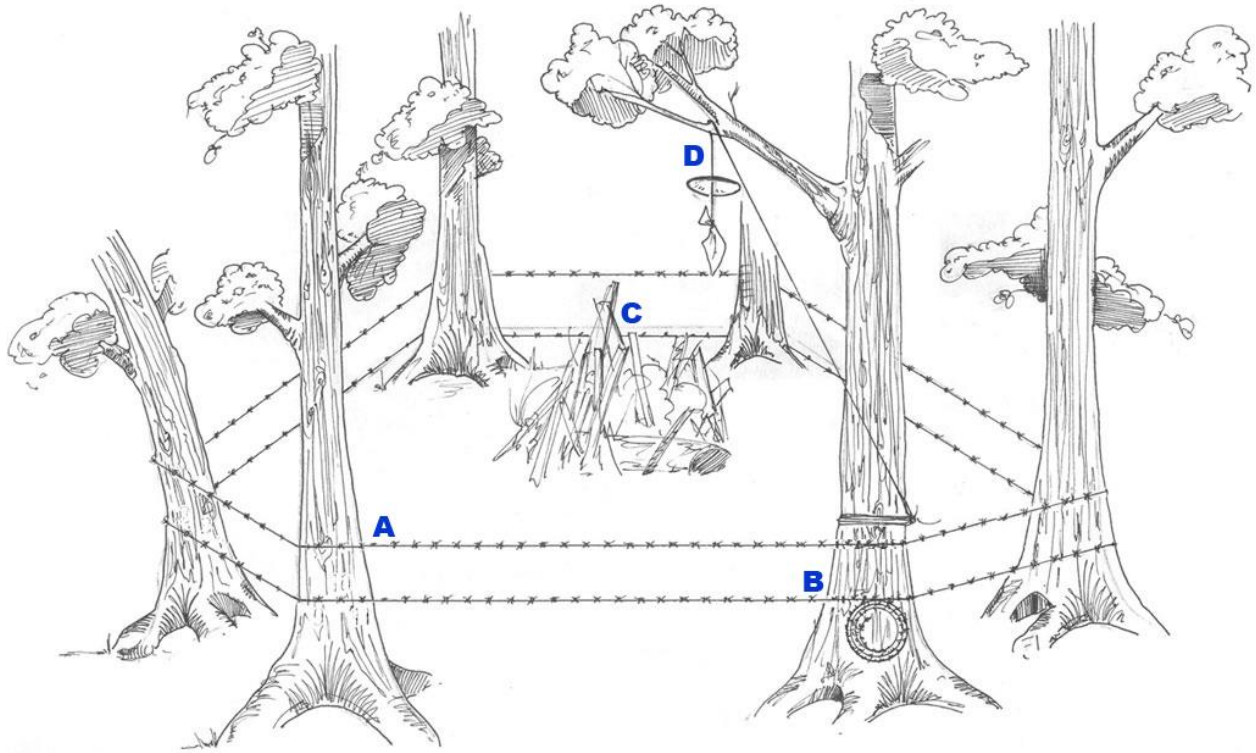

Figure 2. Schematic diagram of sampling station; (A) Upper wire at 70-75 cm from the ground; (B) Lower wire at 20-25 cm from the ground; (C) Debris pile and; (D) Suspended sweet lure. (Diagram adapted from Cascades Carnivore Connectivity Project http://www.cascadesconnectivity.org/)

Each sampling station consisted of one barbed wire corral, made up of two 20-25 meter strands of barbed wire. Strands were strung in a circular fashion at two heights, 35-40 cm for the lower and 70-75 cm for the upper, using naturally occurring trees as anchors (Figure 2). This two-strand design was used in order to maximize the potential for snagging a hair sample from bears of all sizes (Kendall and McKelevy 2008). Sampling stations were baited once weekly by CDFW personnel by pouring a mixture of fermented cattle's blood and fish meal over a debris pile located in the center of the corral. A second scent lure, which rotated weekly between honey, raspberry and anise oils, was suspended above the center of the corral in order to maintain novelty of attractants at sites (Kendall and McKelevy 2008).

Sample Collection

Sampling stations were checked by a CDFW field crew every 7 – 8 days for a sampling period of 8 weeks in San Luis Obispo County and 10 weeks in Monterey County. Barbed wire strands were visually inspected by two people and samples consisting of ≥5 hairs were placed in a labeled coin envelope using sterile tweezers. All tools, and barbs containing samples were sterilized with a lighter after collection. Samples were

stored in a container with desiccant before being transferred to the Wildlife Genetics and Population Health Lab. Opportunistic samples from bears killed for depredation or public safety reasons were collected by CDFW personnel by manually plucking a tuft of at least 10-30 hairs from the carcass and placing in a coin envelope.

Sample Storage and DNA Extraction

Hair samples were stored at room temperature, away from direct sunlight, with silica desiccant beads until DNA extraction. Genomic DNA was isolated from samples with a minimum of one guard hair follicle or at least 3 undercoat hairs with follicles. When sufficient sample was available, DNA was extracted from up to 15 follicles. DNA was extracted in the lab by one of two methods. The first method used the QIAamp DNA Micro kit (QIAGEN Inc., Valencia, CA, USA) following kit protocol for hair. The second method used a hair lysis buffer developed by Maniatis, Fritsch and Sambrook (1989) (Appendix 4). DNA was stored at 4 °C while in use and then transferred to -20 °C for long-term storage.

Genotyping

Each individual bear was genotyped at 14 microsatellite loci, developed by Paetkau and Strobeck (1994); Paetkau *et al.* (1995); Meredith *et al.* (2009), and two sexing loci, developed by Xu *et al.* (2008); Pagès *et al.* (2009). Loci were fluorescently labeled and multiplexed (grouped for polymerase chain reaction, PCR) into four groups based on base pair product size and fluorescent compatibility (Appendix 5). PCR amplifications were carried out using the multiplex PCR protocol for amplification of microsatellite loci with Q solution (QIAGEN Multiplex PCR kit; QIAGEN) (full PCR protocol provided in Appendix 5). PCR products were analyzed using STRand Analysis Software (Toonen and Hughes 2001). Genotypic data from STRand was read twice, by two people blind to the reads of the other, to insure correct and consistent allele calls. All DNA samples were run in at least triplicate in order to check for discrepancies, and each plate of DNA included both negative and positive controls for quality assurance. Samples that did not successfully amplify a bear genotype after the first round of testing were re-extracted (if there was sufficient sample remaining) and tested again. Samples that only amplified canid-specific alleles at G1A and SRY loci were identified as canid based on known reference DNA profiles.

Individual Identification

Samples that produced genotype data for at least 12 of the loci were included in analysis. Genotypes were analyzed using Microsatellite Toolkit (Parks 2001) and Genalex version 6.5 (Peakall and Smouse 2012) software to find matching individuals. Samples with genotypes that matched with no genetic discrepancies were considered to be the same individual.

Census Size Estimation

We estimated population abundance using two types of capture mark recapture models. First, we employed a continuous occasion capture mark recapture model designed for use with noninvasively sampled genetic data. This simple model, implemented in the R package Capwire, is well-suited for estimating abundance from sparse data when recaptures among individuals are not evenly distributed (Miller *et al.* 2005, Pennell *et al.* 2013). The continuous occasion capture mark recapture model has been shown to

provide precise estimates of other bear populations (Robinson *et al.* 2009, Karamanlidis *et al.* 2012). The continuous occasion models implemented in Capwire can be programmed to incorporate assumptions about heterogeneity in probability of capture among individuals and the program output suggests the best-fit model accordingly. There are two available models in Capwire; (1) the Equal Capture Model, which assumes that all individuals have the same probability of being captured or; (2) the Two Innate Rates Model, which assumes that a population contains a mixture of individuals with two distinct capture probabilities, those who are difficult to capture and those who are relatively easy to capture (Miller *et al.* 2005). We chose the best-fit model for each group (males, females, and all combined) using the likelihood ratio test implemented in Capwire.

To validate abundance estimates derived from the continuous occasion model, we performed a Huggins closed capture (discrete occasion) model implemented in the software MARK to estimate abundance using the same capture histories (Huggins 1989, White and Burnham 1999). We collapsed multiple captures of a single individual within a capture occasion into a single capture event to create the discrete input required by the Huggins model. We modeled several simple, biologically plausible scenarios that assume various models for capture probability (*p*) and recapture probability (*c*). We did not vary *p* and *c* with time because bears would not be more or less likely to be sampled during any one occasion during our sampling period. We estimated abundance based on models that assumed an equal *p* and *c* {p=c(.)} and models that assumed p and c were different but constant over time {p(.), c(.)}.

Population Genetics Statistical Analysis

Summary Statistics of genetic diversity including, number of alleles (N_a), expected heterozygosity (H_e) and observed heterozygosity (H_o) were calculated using Microsatellite Toolkit (Parks 2001). Allelic richness (A_R) and tests for deviations from Hardy-Weinberg Equilibrium were performed in GenAlEx version 6.5 (Peakall and Smouse 2012). Deviations from linkage equilibria were tested for in Genepop 4.2.1 (Rousset 2008). Significance for Hardy-Weinberg and linkage equilibrium was determined at alpha = 0.05 using sequential Bonferroni corrections for multiple tests (Rice 1989). Any loci found to deviate significantly from expectations of Hardy-Weinberg or linkage equilibrium over more than two geographic regions were removed from further analysis. The probability of null alleles was tested for using the program ML RELATE (Kalinowski *et al.* 2006).

The probability of identity, or the probability that two individuals will have the same genetic profile at all loci was calculated using GenAlEx version 6.5 (Peakall and Smouse 2012) in two ways: (1) the assumption of random mating and no close relatives in the population (P_{ID}) and (2) the assumption that siblings or close relatives occur in the population (P_{SIB}).

Population Structure

Population structure was determined using a Bayesian genetic clustering algorithm in STRUCTURE version 2.3.4. (Pritchard *et al.* 2000) to determine the likely number of population groups and to probabilistically group individuals without using the known geographic location of sample collection. A population admixture model with a flat prior

was used and assumed that allele frequencies were correlated among populations, and ran 50,000 Markov chain Monte Carlo repetitions following a burnin period of 10,000 repetitions. STRUCTURE is more robust at identifying genetic clusters (K) greater than one, rather than a single panmictic population (Evanno *et al.* 2005). Therefore we performed two analyses, in order to strengthen our results. The first analysis was performed on the entire combined datasets for Mono, San Luis Obispo and Monterey counties (n= 186) to estimate the probability of one through 10 genetic clusters (K), with each run iterated three times. Mono County was included in this analysis as a known genetic cluster different (genetic outgroup) from San Luis Obispo and Monterey counties. A second analysis on the combined San Luis Obispo and Monterey datasets (n=67) was performed as well in order to estimate the probability of one through 10 genetic clusters (K), with each run iterated three times. Using STRUCTURE HARVESTER (Earl 2012) the log probability of the data given K, log Pr(X|K), was averaged across multiple runs for each of the K estimates in order to generate L(K). For each analysis, the K value with highest probability was selected (Pritchard *et al.* 2003; Evanno *et al*. 2005; Waples and Gaggiotti 2006). Individuals were assigned membership to a genetic cluster based upon the highest proportion of ancestry to each inferred cluster.

To further assess and visualize genetic relationships among the three counties, a principal coordinate analysis (PCoA) via covariance matrices with data standardization was performed (Orlóci 1978). In GenAlEx, a pairwise, individual-by-individual genetic distance matrix was generated and then used to create the PCoA. The PCoA process located four major axes of variation within the data set, and because each successive axis explains proportionately less of the total genetic variation, the first two axes were used to reveal the major separation among individuals.

Population Source Genetic Assignment

Since the black bear populations in San Luis Obispo and Monterey counties were likely founded within the past 50 years (CDFG 2010) it was important to identify the most likely source populations of each of the 67 individuals identified in the two counties. The USEPOPOINFO model in STRUCTURE version 2.3.4. (Pritchard *et al.* 2000) was employed in order to determine the probability that an individual belongs to a given population. The same parameters used to detect population structure were used with the addition of USE POPINFO = 1, MIGRPRIOR of v=0.05 and MAXPOPS of K = 4, as recommended by Pritchard *et al.* (2000). The four genetic clusters described by Brown *et al.* (2009) (North Coast/Klamath; Northern Sierra Nevada/Cascade; Central Sierra Nevada/ Southern California; and Southern Sierra Nevada/Central Coast) were used as the four possible source populations (K). The San Luis Obispo and Monterey datasets (n = 67) were combined with the Brown *et al.* (2009) dataset (n = 496) at 5 loci used in common in both studies (G1A, G1D, G10H, G10L, G10o). The 17 Central Coast bears identified by Brown *et al.* (2009) were removed from the Southern Sierra Nevada/Central Coast genetic cluster for this analysis in order to prevent inflated or false assignment probabilities resulting from the genetic similarity between the 2009 San Luis Obispo and the 2013-2014 San Luis Obispo and Monterey bears.

Bottleneck

To test the likelihood that the Central Coast population underwent a recent genetic bottleneck, the allele frequency distribution was examined and tested for a heterozygosity excess using a one-tailed Wilcoxon sign-rank test implemented in BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996). The analysis can detect whether the reduction of alleles occurred faster than the overall heterozygosity, which is a common characteristic of populations that have experienced a recent reduction of effective population size (Cornuet and Luikart 1996; Luikart *et al.* 1998). These tests were performed using the two-phase (TPM, 70% step-wise mutation model and 30% IAM) model of microsatellite evolution and 10,000 iterations. We estimated inbreeding coefficients (F_{IS}) for each locus in our focal population in Arlequin (version 3.5; Excoffier and Lischer 2010).

Effective Population Size

Effective population size (N_e) was estimated using the linkage disequilibrium method of (Hill 1981; Waples 2006) as implemented in NeEstimator V2.01 (Do *et al.* 2014). The linkage disequilibrium method has been demonstrated to produce the most powerful and precise estimates of N_e, particularly from single-sample data of small populations of 200 or less (Waples and Do 2010). A P_{crit} value of 0.02, which excludes alleles that occur as only one copy, was chosen in order to maximize precision while limiting bias (Waples and Do 2010). Jackknife and parametric methods were used to determine 95% confidence intervals.

Statewide Monitoring Cost Analysis

Field work/sample collection expenses were estimated by the California Department of Fish and Wildlife, based on actual budgets used in the San Luis Obispo and Monterey counties studies. Laboratory expenses were estimated by the Wildlife Genetics and Population Health Lab at UC Davis. It is important to note that all costs presented are estimates and are subject to change.

We determined the total area to be sampled using a GIS habitat suitability index model from the California Wildlife Habitat Relationships System (CWHRS) (Airola 1988; CDFW 2010). Similar to methods used in San Luis Obispo and Monterey counties, we calculated the total area (in square kilometers) of high and medium black bear habitat quality across the entire state of California. Habitat suitability was determined relative to a bear's life requisites, such as food availability, water and land and canopy cover (California Wildlife Habitat Relationships System 2002).

The total cost to conduct a noninvasive genetic capture-mark-recapture study across all the high and medium bear habitat in California was estimated by extrapolating the field and laboratory expenses to the statewide scale.

Results

Samples Collected

Table 2 presents a summary of all the samples collected in Mono, San Luis Obispo and Monterey counties from 2010-2014.

Table 2. Summary of all samples received from Mono, San Luis Obispo and Monterey counties. CMR = Capture-Mark-Recapture and represent hairs collected from hair snags. Opportunistic = samples collected outside of CMR study (i.e. natural rubs, live handling, etc.). SVWA = Slinkard Valley Wildlife Area, TML = Town of Mammoth Lakes. Dead Bears = bears killed by authorities for Depredation or Public Safety.

Contract Year	Sample Type	Location	Year	# of Samples Received
1	Dead Bears	Mono	2011-2012	31
	CMR	SVWA	2011	62
	CMR	TML	2011	71
	Opportunistic	Mono	2010-2012	82
	CMR	SVWA	2010	57
	CMR	SVWA	2012	100
	CMR	TML	2012	104
2	CMR	San Luis Obispo	2013	179
	Dead Bears	San Luis Obispo	2013	7
	Opportunistic	San Luis Obispo	2013	1
3	CMR	Monterey	2014	219
	Dead Bears	Monterey	2013	1
	Opportunistic	Monterey	2014	0

Individual Identification

San Luis Obispo County - A total of 187 samples were collected in San Luis Obispo County from June 17, 2013 to August 8, 2013 (Table 2). Of the 187 samples tested, 119 classified as bear genotype and of those, 63 unique individuals were identified. Twenty-nine of those unique individuals were captured by hair snare and DNA analysis in more than one time period. Forty-two of the samples were identified as non-target species, primarily canids. An additional 26 samples could not be PCR-amplified due to poor or insufficient sample quality, such as too few hairs, damaged follicles or degradation of DNA from exposure to sunlight and other environmental factors. These samples typically contained fewer than 3 guard hair roots. It should be noted that species identifications (bear or non-bear) could not be determined for poor quality samples.

Fifty-four sampling sites were employed throughout San Luis Obispo County, providing a sample coverage across 1,080 km^2. Bears were observed by DNA identification at

only 21 of those sites. Most notably, five of the sampling sites were visited by five or more different bears within the 8 week study period. According to GIS models California Wildlife Habitat Relationships System (CWHRS) (Airola 1988; CDFG 2010), San Luis Obispo County has approximately 2,905 km^2 of suitable bear habitat, thus 37% of bear habitat was sampled. The main factor that limited sampling coverage reported by CDFW was the lack of access of CDFW personnel to private lands in the northern half of the county. The limited coverage and geographic locations of sample sites was taken into account when conducting further analysis, including population estimation.

Monterey County - A total of 219 hair samples were collected in Monterey County from April 28, to July 3, 2014 (Table 2). One additional sample was collected from a dead bear in Monterey County in 2013. Of the 220 samples tested, only 16 amplified a bear genotype, resulting in four unique individuals, all of which were DNA identified as males. Each individual was observed only once within the sampling season. Seventy-two sampling stations were deployed across the county, however bears were only observed at two stations. Two individuals were recorded at one sampling station (Fort Hunter Liggett), during two different sessions, and the third individual was observed at a different sampling location (Los Padres National Forest), approximately 10 km away. The two sampling stations where bears were observed were located in the southern half of Monterey County. The fourth individual, also male, was collected from the dead bear removed for public safety reasons in 2013. According to GIS models, Monterey County has approximately 2,471 km^2 of suitable bear habitat, of which 58% was sampled. One hundred and four of the samples received were identified as non-target species, primarily canines and pigs (*Sus scrofa*). An additional 99 samples could not be amplified due to poor quality, such as too few hairs, damaged follicles or degradation of DNA from exposure to sunlight, but more likely unidentified non-target species, such as small rodents, raccoons, opossums, etc. These samples typically contained fewer than 3 guard hair roots. It should be noted that species identifications (bear or non-bear) could not be determined for poor quality samples.

Mono County– A total of 507 samples were collected from Mono County between the years of 2010-2012. Of those, successful genotypes were obtained from 392 samples, and 163 unique individuals were identified. Of the 163 unique individuals, 50 were female, 109 were male, and four could not be identified to sex.

Census Size Estimation

The program Capwire returned two sets of population estimates for the area sampled in San Luis Obispo County, one from the Two Innate Rates Model (TIRM) and the other from the Equal Capture Model (ECM) (Table 3). The likelihood ratio test indicated that the TIRM was the best-fit model for the estimates of females and the total population. The ECM, however, was a slightly better fit for males. The TIRM is most commonly used in ursid studies, as individuals tend to occupy semi-discrete areas, or home ranges. This aspect introduces individual capture heterogeneity into grid based studies, such as the one performed here (Woods *et al.* 1999; Bellemain *et al.* 2005; Miller *et al.* 2005). We therefore believe that the estimates of males, females and the total population generated under the TIRM are generally biologically and statistically more robust than the ECM, despite the likelihood ratio test for males.

Table 3. Comparison of population estimates for the two models produces in Capwire. Numbers highlighted in bold indicate the best fit model as indicated by the likelihood ratio test.

Model	Males	95% CI	Females	95% CI	Total	95% CI
ECM	**34**	**28 - 41**	35	28 - 43	73	61 - 85
TIRM	41	34 - 62	**50**	**36 - 76**	**101**	**84 - 134**

In addition to analysis using Capwire, we employed the program MARK to estimate the abundance of bear in the San Luis Obispo study area. Figure 3 shows the comparison of the estimates generated in both programs. Although providing similar results, we believe that the Capwire estimates represent the most accurate estimation of black bears in San Luis Obispo County study area. Capwire provided tighter confidence intervals and the males are probably overestimated in MARK.

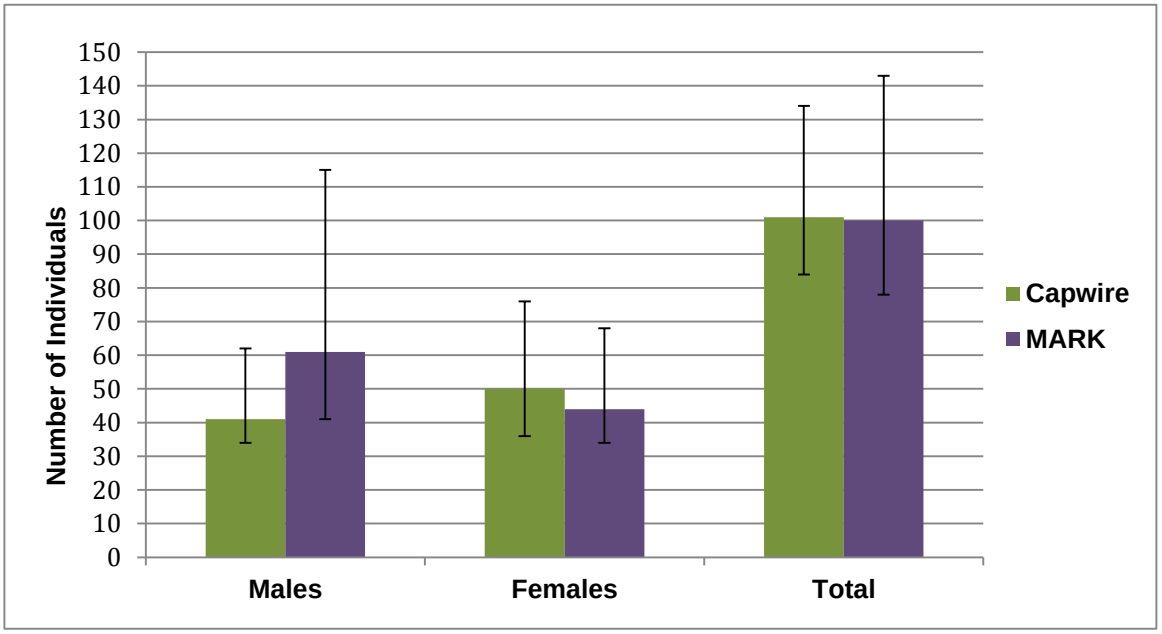

Figure 3. A comparison of Capwire and MARK census size estimates for San Luis Obispo County. Estimates are broken down by males, females and a combined total and presented with 95% confidence intervals. Capwire estimates presented above were derived from the TIRM model.

The total estimated population of black bears in the San Luis Obispo County study area was 101 (95% CI: 84 – 134). When categorized by sex, we estimate there to be 41 (95% CI: 34-62) males and 50 (95% CI: 36 – 76) females. In comparison, the minimum number of individuals observed over the 8-week study in the study region in San Luis Obispo County was 63 total, 33 males and 30 females. Table 4 presents a summary of all the population estimates generated for San Luis Obispo County study area. Population estimates could not be determined for Monterey County due to insufficient sample size. A total of three individuals were observed over the 10 week study period, plus one additional individual removed for public safety reasons in 2013, all of which all were male.

Table 4. Summary of census size estimates for the San Luis Obispo County study area. The numbers highlighted in bold represent the recommended abundance estimates, as indicated by the biology of the system and the statistical robustness of the given model.

Model	Males	95% CI	Females	95% CI	Total	95% CI
Capwire - ECM	34	28 - 41	35	28 - 43	73	61 - 85
Capwire - TIRM	**41**	**34 - 62**	**50**	**36 - 76**	**101**	**84 - 134**
MARK	61	41 - 115	44	34 - 68	100	78 - 143
Minimum Number	33	N/A	30	N/A	63	N/A

Population Genetics Statistical Analysis

Fourteen of the loci used were polymorphic in Mono County and thirteen loci were polymorphic in across San Luis Obispo and Monterey counties. One of the fourteen loci, G10B, was discarded because it was determined to significantly depart from expectations of Hardy-Weinberg equilibrium. All subsequent analyses were performed with 13 loci. There was no evidence of linkage disequilibria or null alleles in the remaining 13 loci. In earlier analyses (preliminary reports) of Mono County samples, there were no differences in individual identifications between the use of 13 vs. 14 loci.

The average probabilities of identity under the assumptions of either random mating (PID_{RM}) or mating among siblings (PID_{SIBS}) for Mono County (n = 28) were 5.9×10^{-10} and 1.4×10^{-4} and for San Luis Obispo County (n = 8) were 6.5×10^{-5} and 1.3×10^{-2}, respectively. Neither, PID_{RM} or PID_{SIBS} could be determined for Monterey County (n = 1) due to insufficient sample size. These small values indicate that our panel of loci provided a high resolution to accurately distinguish individuals (Waits *et al.* 2001; Woods *et al.* 1999). For example, given this data the probability of two bears having the same genotype at all 13 loci was less than 1 in 1.6 million in Mono County, and less than 1 in 15,000 in San Luis Obispo County. The disparity in resolution between the two counties was primarily due to the smaller sample size and lower genetic diversity observed in San Luis Obispo County samples. However, given the overall smaller population of San Luis Obispo County, we are confident that our 13 loci panel was sufficiently sensitive at distinguishing unique individuals in San Luis Obispo and Monterey counties.

Table 5. Summary statistics of genetic diversity for San Luis Obispo, Monterey and Mono counties. *Central Coast includes the combination of San Luis Obispo and Monterey counties.

Population	Year	N	H_E	H_E SD	H_O	H_O SD	N_a	N_a SD	A_r	A_r SD
Central Coast*	2013-2014	67	0.39	0.08	0.39	0.02	3.54	1.81	2.06	0.28
San Luis Obispo	2013	63	0.40	0.08	0.38	0.02	3.57	1.74	2.04	0.25
Monterey	2014	4	0.39	0.09	0.46	0.06	2.31	1.25	2.16	0.25
Mono	2010-2012	163	0.59	0.06	0.59	0.01	7.54	3.93	3.06	0.41

Summary statistics determined for the Central Coast (San Luis Obispo and Monterey counties) were compared to the reported statistics published by Brown *et al.* (2009) in order to infer whether population genetic indices changed over time (Tables 5 and 6). Summary statistics values for the Central Coast from this study were not significantly different from the summary statistics reported by Brown *et al.* (2009) (alpha = 0.05), indicating that the genetic diversity of bears in that region has remained relatively constant.

Table 6. Comparison of Central Coast (2013-2014) summary statistics with reported values from Brown *et al.* (2009). * Indicates date when values published, sample collection dates vary.

Population	Year	N	H_E	H_E SD	H_O	H_O SD	N_a	N_a SD	A_r	A_r SD
Central Coast	2013-2014	67	0.39	0.08	0.39	0.02	3.54	1.81	2.06	0.28
Central Coast	2009*	17	0.41	0.07	0.40	0.03	3.08	1.31	2.04	0.25

Population Structure

Bayesian clustering analysis on bears from Mono (used as a genetic outgroup as noted in Methods), San Luis Obispo and Monterey counties (n = 230) indicated that there were two main genetic groups, cluster A and cluster B (Table 7). The 163 individuals from Mono County assigned to population A with an average probability assignment of 0.977 (SD = 0.05). However, six of the Mono County individuals (~4%) assigned to population A with a probability less than 0.90, including two individuals who assigned with a probability less than 0.65. The 63 individuals from San Luis Obispo County and the four individuals from Monterey County assigned to population B with an average probability assignment of 0.980 (SD = 0.06). Two of the individuals from San Luis Obispo County showed more variable assignment, with probabilities of assignment of 0.754 and 0.56. Both individuals were observed in the southern half of the county, at adjacent sampling stations. The genetic assignment of these individuals suggest that there was a dispersal event or genetic exchange from the Sierra Nevada's to the Central Coast. Probability assignments for each individual are presented graphically in Figure 4.

Table 7. STRUCTURE HARVESTER output Evanno method results for the Mono, San Luis Obispo and Monterey counties dataset. Yellow highlight indicates the largest value of Delta *K* and indicates the number of *K* groups that best fit the data.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	3	-5593.133333	0.404145	—	—	—
2	3	-4954.700000	1.852026	638.433333	529.766667	286.047113
3	3	-4846.033333	2.514624	108.666667	66.700000	26.524841
4	3	-4804.066667	10.885924	41.966667	14.633333	1.344244
5	3	-4776.733333	34.035472	27.333333	3.633333	0.106751
6	3	-4753.033333	18.169847	23.700000	49.866667	2.744474
7	3	-4779.200000	36.319554	-26.166667	40.466667	1.114184
8	3	-4764.900000	38.381767	14.300000	4.766667	0.124191
9	3	-4755.366667	13.282445	9.533333	80.933333	6.093256
10	3	-4826.766667	92.109138	-71.400000	—	—

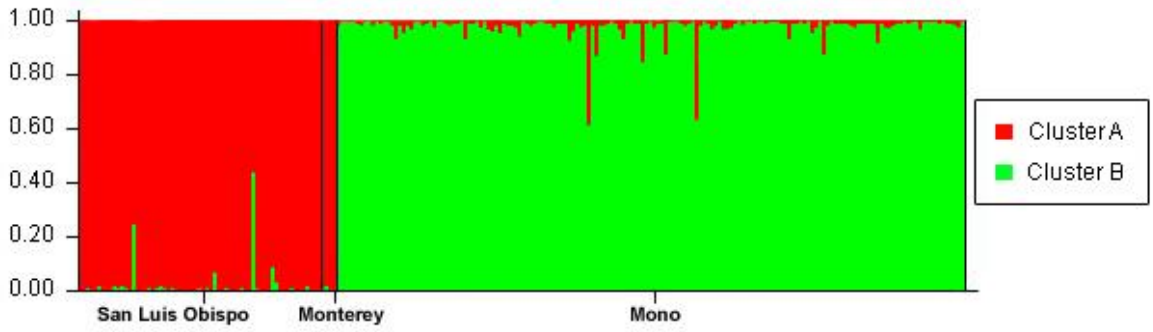

Figure 4. STUCTURE bar plot displaying the genetic clustering relationship of black bears in Mono, San Luis Obispo and Monterey counties. Note that vertical pattern of red (San Luis Obispo plus Monterey genotypes) and green (Mono) genetic clusters indicates strong population structure. Note that each individual fine line (represents a bear genotype and its proportional assignment to each of two genetic clusters).

When the San Luis Obispo and Monterey datasets were analyzed alone, no significant structure was identified. Figure 5A demonstrates that when data were tested at two genetic clusters (K = 2), the horizontal pattern to the STRUCTURE bar plot reflects lack of genetic structure: each individual would have a ~50% chance of being assigned to the red cluster and a ~50% chance of assigning to the green cluster. Similarly and confirming lack of genetic structure in the San Luis Obispo and Monterey data sets, Figure 5B shows when tested at three genetic clusters (K = 3), each individual would have ~30% probability of belonging to each cluster. If genetic structure

existed, there would be an uneven distribution of probability assignments among the individuals and a vertical bar pattern to the plot. It is, however, important to note that the sample size for Monterey (n=4) is very small and, substructure may be discerned with the addition of more samples.

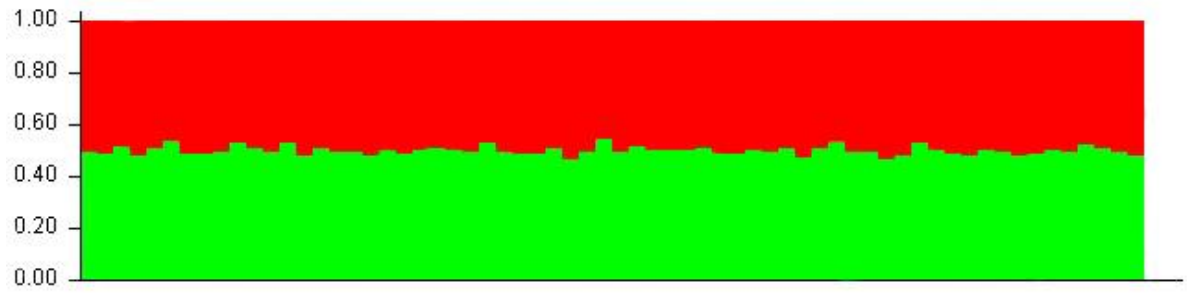

Figure 5A. STUCTURE bar plot (testing at K = 2, genetic clusters) displaying the lack of genetic clustering relationship of black bears within in the data set for San Luis Obispo and Monterey counties.

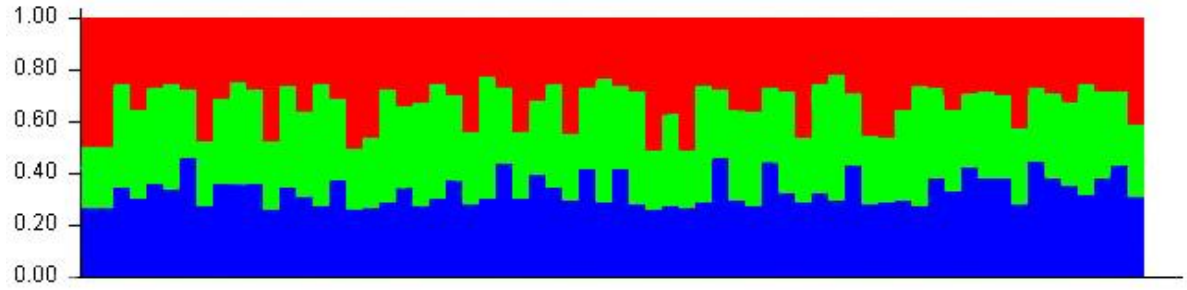

Figure 5B. STUCTURE bar plot (testing at K = 3, genetic clusters) displaying the lack of genetic clustering relationship of black bears within in the data set for San Luis Obispo and Monterey counties.

Principal coordinate analysis of genetic profiles from Mono, San Luis Obispo and Monterey counties (n = 230) (Figure 6A) allowed for graphical examination of the first two major axes of multivariate genetic variation of bears in the three study areas. The PCoA reinforced the STRUCTURE findings of two distinct genetic clusters, cluster A consisting of individuals from Mono County and cluster B consisting of individuals from San Luis Obispo and Monterey counties.

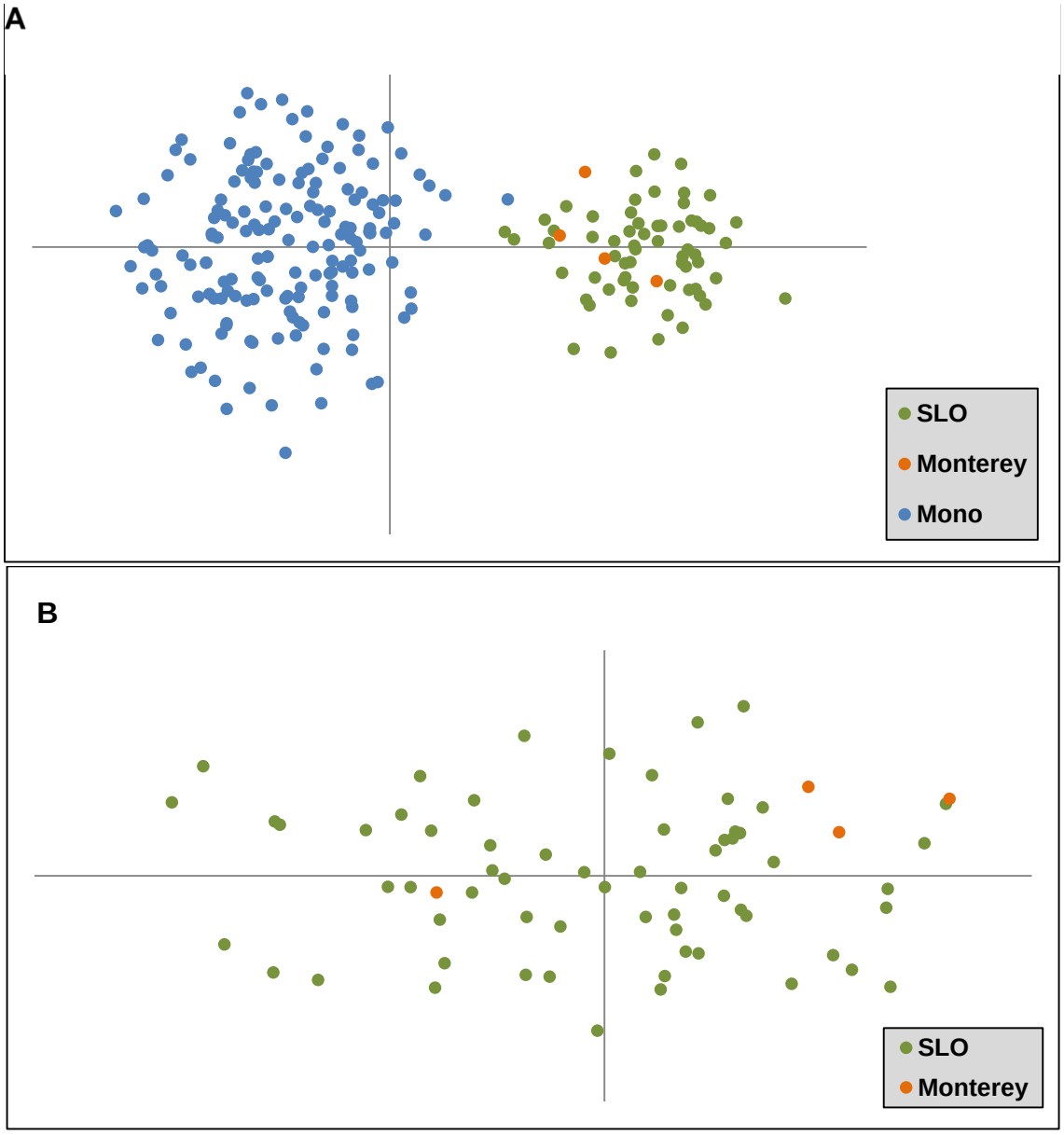

Figure 6. Principal coordinate analysis (PCoA) constructed using genetic covariance matrices (Genalex) for (A) 230 bear genetic profiles from Mono, San Luis Obispo and Monterey counties; and (B) 67 bear genetic profiles from San Luis Obispo and Monterey counties. Each point is color coded to its sampling region and represents a single unique individual.

The PCoA of San Luis Obispo and Monterey counties' bears alone also supported the STRUCTURE finding that there was no significant genetic structure to distinguish bears from the two counties (Figure 6B). However, it is important to note that the sample size of bears from Monterey County was very small, and it is possible that more genetic structure would have been illuminated with the addition of more individuals.

Population Source Genetic Assignment

The 67 individuals from San Luis Obispo and Monterey counties assigned to the Southern Sierra Nevada/Central Coast population, as identified by Brown *et al.* (2009),

with an average probability of assignment of 0.980 (SD: 0.009). The sample size of Central Coast bears in the Brown *et al.* (2009) paper (n = 17) was very small, and therefore this finding further supports their hypothesis that bears migrating from the Southern Sierra Nevada Range's founded Central Coast Range population.

Bottleneck and Inbreeding

There was no evidence in our data of a genetic bottleneck in the Central Coast population (San Luis Obispo and Monterey counties) of bears according to the one-tailed Wilcoxon sign-rank test for heterozygosity excess (P=0.976). Allele distributions showed a normal L shape, providing further evidence of a no recent bottleneck according to our data. There was also no evidence of significant levels of inbreeding (alpha =0.05) in either of the counties.

Effective Population Size

The effective population size (N_E) estimate using the linkage disequilibrium method in NeEstimator was 50.3 in the Mono County study area, with relatively tight parametric and jackknife confidence intervals (Table 8). The effective population size estimate for the San Luis Obispo County study area was 69.9, with wide confidence intervals (Table 8.). Since San Luis Obispo County had a smaller and less heterozygous population in comparison with Mono County, we believe that the N_E estimate may be overestimated and should be used with caution due to bias potentially created by potentially unidentified genetic substructure, genetic admixture, age structure and/or a small sample size (Luikart *et al.* 2010).

Table 8. Effective population size estimates (N_E) for San Luis Obispo and Mono counties.

Location	N_E	Parametric CI	Jackknife CI
San Luis Obispo	69.9	38.3 - 188.2	36.6 - 215.7
Mono	50.3	43.1 - 58.9	40.3 – 63.4

Review of Population Monitoring Methods

Although black bears are widely distributed throughout North America much more effort has been directed at developing and implementing methods of monitoring of grizzly bear populations. As a result, there is a scientific need to evaluate and improve methods of how black bear populations are monitored. The following review presents a summary of common techniques used for monitoring bear populations, including their strengths, weaknesses and their applicability to black bears.

<u>Presence/Absence Models - Sign Surveys</u>

Sign surveys are implemented by skillfully searching multiple transects within a sampling area for signs of black bear presence, such as tracks, scat, hair, or clawed trees. Identification of tracks is most accurate in areas of snow or mud, however they can be difficult to spot along other substrates (Heinmeyer *et al.* 2008). Accuracy of scat detection is highly variable and dependent on the observer. When relying on visual scat detection, it is important to measure the accuracy of the observer by confirming scat identities with DNA analyses (Boitani and Powell 2012). Data obtained from sign surveys cannot be used to generate population abundance estimates, however

occupancy modeling allows researchers to combine detection/non-detection histories with spatial modeling to estimate and predict species' occurrence across the landscape (MacKenzie and Nichols 2004; MacKenzie *et al.* 2006).

Presence/Absence Models - Bait Station Survey

The use of bait station surveys evolved from pre-baiting techniques used in the Great Smoky Mountains National Park (Marcum 1974, Eagar 1977) to improve the efficiency of black bear trapping for tagging and radiocollaring (Johnson and Pelton 1980). The technique was mirrored after scent-station surveys for other fur-bearers (Roughton and Sweeny 1982), in which changes in the proportion of bait removed, serve as an index of changes in the overall population size. Bait station survey were first implemented in the Great Smoky Mountains National Park in 1981 and later adopted by more than 15 wildlife management agencies in North America (Garshelis 1990). This method is relatively inexpensive to implement and requires fewer logistical factors than other monitoring techniques. However, environmental variables, such as natural food source availability have been shown to have a confounding effect on bait station visitation, therefore limiting the accuracy of this technique for measuring black bear populations (Clark *et al.* 2005).

Hunter-Harvest Models

Population abundance estimates can be generated from hunter-harvest data. The theory behind this method is that harvest numbers, sex-ratios and hunter effort will depend on the abundance of the overall hunted population (Paloheimo and Fraser 1981). This method uses the age-at-harvest and sex-ratio data to estimate the rate of harvest mortality, which is then extrapolated to develop a population index (Fraser *et al.* 1982). This method is inexpensive and requires minimal effort, however estimates can often be inaccurate as a result of low harvest rates, biased take (males more likely to be harvested), assumptions of stable age distribution, and therefore managers should be cautious when interpreting hunter-harvest estimates (Miller 1990).

Capture-Mark-Recapture Models - Camera Traps

Photographing wildlife using remotely triggered game cameras first emerged in 1877 (Guggisberg 1977), but was not widely adopted until the invention of infrared triggers in the 1980s (Boitani and Powell 2012). Game cameras are small, lightweight and digital media technology allows a single camera to store thousands of photos at once. Individuals are "marked" either by the identification of previously placed ear tags (Mace *et al.* 1994), or with newer technologies photos can be scrutinized for body size, color, markings, scars and facial morphometrics and "recaptured" by subsequent photos (Boitani and Powell 2012). Mace *et al.* (1994) found this technique to be very effective for monitoring grizzly bear populations, however required the use of ear tags for accurate individual identification. Equipment, and effort required for setting up camera traps is minimal, however placing ear tags on individuals is both labor and cost intensive (Mace *et al.* 1994). This method has great potential for the future with the advancement of morphometric identification techniques that do not require physical capture/ear tag placement (Boitani and Powell 2012).

Capture-Mark-Recapture Models - Radio Telemetry

Radio telemetry involves the application of transmitters assigned a unique frequency (often ear tags or collars) to individuals, which are followed via aerial surveys (Miller 1987) and/or GPS information downloads (White and Garrott 2012). Population estimates are derived using capture-mark-recapture methods in which, the unique radio frequency marks an animal, and the detection of that frequency during a follow-up survey is considered a recapture (Miller *et al.* 1997). "Recapturing" individuals using this technique can be limited by lost transmitters (due to dead batteries or falling off) (Sellers and Miller 1994) and in heavily forested or canyon-like habitats (Miller *et al.* 1997). Radio telemetry, and specifically GPS-based technology, can be very expensive due to costs and effort associated with physical capture, which is required to affix transmitters on individuals, collar hardware, and cloud storage of GPS data. However the costs of recapture can be varied by altering the frequency of follow-up flights and distance flown (Miller *et al. 1997),* the number of GPS points recorded per day, and/or the type GPS information download methods (White and Garrott 2012).

Capture-Mark-Recapture Models – Noninvasive Genetic Sampling

Noninvasive genetic sampling was first introduced in 1992 to obtain samples from rare and elusive brown bears (*Ursus arctos*) in Europe (Höss *et al*. 1992; Taberlet and Bouvet 1992). Since that time, noninvasive genetic methods have been implemented in a variety of important applications for many species (see Waits and Patkaeu 2005 for review). Hair and scat are the two most common genetic samples collected for black bear population studies (Boitani and Powell 2012). Hairs are often obtained via a snagging mechanism and scat is collected along sampling transects. DNA is obtained from hairs through extraction of individual hair follicles. DNA can be extracted from a single hair root, however, yields are increased with the inclusion of multiple hairs. Although extracting DNA from multiple hairs increases DNA concentrations, it can, however, result in "contamination" by including hairs from multiple individuals in a single sample (Alpers *et al.* 2003; Roon *et al.* 2005). Fecal DNA is obtained from epithelial cells sloughed from the intestinal lining, found on the outer surface of scat samples (Gorman and Trowbridge 1989; Barja *et al.* 2005). Fecal DNA can be difficult to isolate due to low cell count and other substances within the scat that inhibit DNA amplification (Boitani and Powell 2012).

The use of hair snag stations were first published by Woods *et al.* (1999), for capture-mark-recapture analysis of free ranging black and brown bears. As a part of the study, four different hair snag station designs were evaluated, and the recommended barbed-wire corral design has subsequently been used in studies all over the world. Woods *et al.* (1999) also reported that although field supplies (wire, scent lures, hand tools, etc.) were inexpensive, the cost of personnel and laboratory analysis can be significant.

Alternatively, hair can be collected using rub stations that are placed opportunistically within a given study area by identifying natural rub sites, such as smoothed bark or claw-marked trees, and attaching a mechanism to collect hair (i.e. barbed wire) (Kendall *et al.* 2008). In practice, Sawaya *et al.* (2012) found that black bears may actively avoid rub stations, and therefore are not recommended for black bear population monitoring studies.

Scat can be one of the most readily collected animal by-product samples for many

wildlife species. It can be acquired without disturbing individuals and does not require the time-intensive set-up and removal of designated sampling stations. Typically scat collection is performed by walking transects within a sampling area and searching visually (Boitani and Powell 2012). Researchers can also increase scat collection rates over large remote areas by using specially trained scat detection dogs (*Canis familiaris*), however, this can largely increase the overall cost of a study (Wasser *et al.* 2004). In addition, scat laboratory DNA analysis is complicated and typically more time and resource intensive due to DNA degradation, trace concentrations of DNA, plant or other PCR inhibitors present in scat, and contamination with prey tissues present in scat (Wasser *et al.* 1997).

Statewide Monitoring Cost Analysis

Field Work Expenses

Estimated expenses associated with the field-work required to implement a noninvasive genetic, hair snag, capture-mark-recapture study were provided by the California Department of Fish and Wildlife. Expenses can be broken down into personnel, travel and supplies. Personnel includes the staff required to design a study and coordinate logistics, and a field crew. Supplies include, but are not limited to sampling station equipment - barbed wire, flagging tape, paint, signage, gloves and machetes; hair collection supplies – envelopes, labels, forceps, lighters, printing and desiccant beads; and bait supplies – 50 gallon barrels, Tyvek suits, respirators, fish meal, cattle blood, action packers, bleach, and 1 liter bottles. Estimates presented in Table 9 represent the costs of implementing a one-year, 12-week capture-mark-recapture study in one county (approximately 65 sampling stations or 1,300 km^2), provided by the California Department of Fish and Wildlife.

Table 9. Field Work Expenses

Item	Cost	Quantity	Total Cost
Personnel			
Scientific Aid	$26,411.40	1.5	$39,617.10
Environmental Scientist	$73,740	0.35	$25,809.22
Travel			
Fuel	$3.85 / gallon	2,000	$7,700
Vehicle repair	$2,000	1	$2,000
Supplies	$7,500	1	$7,500
Total			**$82,626.32**

Laboratory Expenses

Once hair samples have been collected by the California Department of Fish and Wildlife, they must be transferred to a laboratory for DNA analysis and individual identification. The expenses associated with capture-mark-recapture methods and analysis include, but are not limited to the costs of sample preparation and archiving, DNA extraction, polymerase chain reaction for the generation of individual genotypes,

genetics statistical analysis, database maintenance, population modeling and report write-up. Table 10 presents a current estimate (June 2015) of the approximate cost for one year of laboratory analysis (up to 400 hair samples) and data generation. It is important to note that these costs represent a rough estimate and are subject to variation and change based on the choice of laboratory, costs of reagents, salary scales and the overall size of the project. Laboratory analysis must be contracted out of the California Department of Fish and Wildlife to a lab capable of handling the volume of samples and producing accurate data, statistical analysis and interpretation resulting in a detailed report useful for CDFW bear management. Indirect (overhead) Costs represent the administrative and other fees associated with contracting out to an institutional lab. Approximate annual cost estimates are provided by the Wildlife Genomics and Disease Ecology at the University of Wyoming (UW), Laramie (Ernest research lab, formerly at UC Davis) assuming up to 400 samples per year and study design integrated between CDFW and UW. Per-sample costs may be reduced with each additional sample once base funding is acquired for each year, as personnel costs represent a large portion of the budget and adding samples up to a certain point, adds diminishing effort, time, and funding requirements.

Table 10. Laboratory Expenses

Item	Total Cost
Personnel	$64,000
Travel	$1,500
Supplies	$40,000
Indirect Costs	$46,356
Total	**$151,856**

Total Cost Simulation

The total cost, including field and laboratory expenses, to implement a noninvasive genetic capture-mark-recapture study across 65-twenty square kilometer traps is approximately $234,482. This value represents the approximate cost to sample regions in one county for one year.

Table 11. Black Bear High and Medium Habitat Classifications for California.

Habitat Classification	**Area (km^2)**
High	74,314
Medium	41,685
Total	115,999

In order to sample all high and medium quality bear habitat across the entire state of California, approximately 115,999 km^2 (Table 11) for one sampling season, it would require nearly 5,800-twenty square kilometer traps. This equates to 178 field crews (2 people per crew), 2,600 hair samples and a very approximate estimate over $20 million.

Realistically, it is difficult to access, and therefore sample all of the high and medium quality habitat in a county due to private land access and other geographical features. For example, in San Luis Obispo County, field crews were only able to access 37% of the total habitat and 58% in Monterey County. If we assume, that we are able to sample 50% of the total high and medium bear habitat in California, it would still cost over $10 million (approximate estimation).

For these reasons, we recommend a stratified approach for monitoring bear populations, by first identifying and covering regions of high priority, followed by more stable populations. Detailed recommendations are provided below.

Discussion

Black bears in the Central Coast Ranges of California have remained of interest to the California Department of Fish and Wildlife for over 50 years. In the absence of hunting, and therefore harvest-driven population estimates, wildlife managers have sought other methods to estimate bear numbers and population dynamics in the region. This study investigated the abundance and population genetics of black bears in two counties in the Central Coast Ranges, San Luis Obispo and Monterey.

During two sampling periods in 2013 and 2014, we identified 67 unique individual bears. Sixty-three of those individuals were identified through hair snag DNA “capture” or opportunistically in San Luis Obispo County and four individuals in Monterey County. Approximately 58% of high and medium quality bear habitat was sampled in Monterey County as opposed to 36% in San Luis Obispo County. In addition, the same field and laboratory methods were followed for each county. Therefore, it is unlikely that bear detection in Monterey County was lower as a result of sampling coverage or DNA methodology. It is more likely that there are very few bears inhabiting Monterey County. This study was not designed to investigate the movement or dispersal patterns of specific bears in the Central Coast Ranges, which would require multiple years of sampling and the application of GPS collars, so we can only hypothesize why there are few bears in Monterey County.

Brown *et al.* (2009) hypothesized that black bears in the Central Coast Ranges are an extension of populations from the southern Sierra Nevada and Tehachapi Ranges. Our data supports this hypothesis, and as San Luis Obispo County is geographically located south of Monterey County, it is likely that animals dispersing from the southern Sierra Nevada and Tehachapi Ranges would immigrate to San Luis Obispo County before Monterey County. Given that black bears have inhabited these counties for only about 50 years, it is possible that they have not dispersed far enough north to establish a large population in Monterey. In addition, all four individuals captured in Monterey County were males, which are more likely to disperse and travel long distances than females. Since only 63 individuals were identified in San Luis Obispo County, that area, as well as Monterey County may be under carrying capacity, which lessens the pressure for black bear expansion into Monterey. Geographic barriers, such as anthropogenic constructs of highways, urban developments, and waterways, as well as natural topographic barriers may be slowing or preventing northward dispersal and colonization of bears in Monterey County.

We used two different models to estimate the abundance of bears in the sampled region in San Luis Obispo County in order to assess accuracy and precision of our estimates. The total population estimates generated by both models were nearly identical, 101 for Capwire and, 100 for MARK. The estimates generated by Capwire had tighter confidence intervals than MARK. There are several advantages to the Capwire results over MARK results. For example, the models implemented in Capwire, allowed inclusion of multiple captures of an individual within the same week, whereas the MARK model collapses multiple captures from the same week to into one capture event, causing the loss of capture data (Miller *et al.* 2005). Also, estimating abundance precisely using many of the closed-capture models implemented in MARK requires high capture probabilities and low capture heterogeneity among individuals, so data with many single captures, like our data, are not ideal for these models (White *et al.* 1982; Boulanger *et al.* 2004).

In 2010, the California Department of Fish and Wildlife estimated the population of bears in San Luis Obispo County using a combination of the Habitat Suitability Index model and data from locations where the Department had confirmed bear sightings (i.e. vehicle-induced mortalities, depredation occurrences, visual observations, etc). Confirmed bear sightings were used to calculate an average bear density/mi^2 for each Habitat Suitability Index category (high, medium or low) in the county, and verified by comparing the results with published literature and local experts. The land-area of each category was multiplied by its respective estimated density in order to generate an estimate of the bear population in the entire county. The results of this model indicated that approximately 1,067 bears occupied suitable habitats in San Luis Obispo County (CDFW 2010). The difference between the Habitat Suitability Index Model estimates and the noninvasive genetic capture-mark-recapture estimates demonstrates the importance of current data driven models rather than simulation based studies.

For this study, sampling occurred for one season (8-10 weeks) in each county. As a result, there were low recapture rates in San Luis Obispo and Monterey counties. Low recapture rates can widen the confidence intervals of abundance estimates, and sometimes preclude the calculation of estimates altogether (Boulanger *et al.* 2004). We were unable to analyze the dataset using Spatially Explicit Capture-Mark-Recapture Models due to low recapture rates (Royal *et al.* 2013). Furthermore, we were not able to generate density estimates using these methods either (Gardner *et al.* 2010). Recapture rates could be increased in the future by conducting more than one sampling season over consecutive years, particularly in regions with small numbers of bears.

Multiple sampling periods over consecutive years, in a single region, can also be beneficial to increase sample sizes for genetic analyses. The greater the number of individuals that represent a population, the higher the resolution to detect subtle genetic differentiation, population structure, etc. In addition, increasing the number of genetic markers used for genotyping or the inclusion of whole genome data in the future may provide more accurate population estimates, effective population size, and evidence of population bottlenecks or underlying population genetic structure, among other population indicators.

Beyond San Luis Obispo and Monterey counties, California's black bear population has grown over the past thirty years, from an approximate estimate of 10,000 in 1982, to over 30,000 today. In order to manage this rapidly growing population it is important to understand population genetics, demographics, abundance, and density, particularly in areas with increased risks for human interaction. The California Department of Fish and Wildlife currently informs management decisions and the hunting program based on population information generated from hunter-take data each year. Population estimates are derived from a method which projects the percent of the population harvested from the sex and age composition of harvested bears (Fraser 1982, 1984). This method has significant limitations when applied to generating statewide population information.

In 2012, legislation that banned the use of hunting dogs was passed, resulting in a 45.1% decrease in bears taken from 2012 to 2013 (Ypema and Garcia 2015). This decrease probably does not represent an overall decrease in the population, but rather an increase in the effort required per take. Furthermore, the methods used to estimate the overall statewide population rely heavily on observed sex-ratios, which are often biased towards males due to hunter selection for size and the increased probability of being encountered as a result of their larger home ranges (Litvaitis and Kane 1994; Kane 1989). The proportion of hunter take varies greatly between each county, with a few northern counties generally representing a high proportion of the yearly harvest. Extrapolating that data across the entire state likely generates population information that may not be representative of bear populations statewide.

We present an analysis of the resources and costs necessary to implement a noninvasive genetics capture-mark-recapture study to estimate the total number of bears that currently reside in the entire state of California. Our analysis indicates that the costs required to sample all of the high and medium quality bear habitat in a large state such as California, is not realistic for most management agencies. Therefore, alternative sampling methods must be employed, which take into consideration the tradeoffs between optimizing the number of samples collected, or the precision of an estimate, with the funding and logistics necessary to carry out a study. The results of this study may be used to make recommendations for a statewide monitoring plan for California Department of Fish and Wildlife Black Bear Management Program.

Management Implications and Recommendations

This project has provided data to support the value of noninvasive genetic capture-mark-recapture models for estimating the abundance of black bear populations in California. We have provided the first baseline estimate of black bears residing in San Luis Obispo County, and although a population estimate could not be generated for Monterey County, our data suggests that very few bears inhabit the region. The numbers of bears observed in both counties was lower than predicted by the California Department of Fish and Wildlife. Furthermore, the genetic diversity of bears from San Luis Obispo and Monterey counties was lower, in comparison with reported values for other bear populations in California (Brown *et al.* 2009). The combination of these findings, indicate that population and genetic monitoring should continue in this region, but do not warrant additional interventions. Population structure analysis supported the hypothesis that the Central Coast Range population was founded by dispersed individuals from the southern Sierra Nevada Ranges.

Noninvasive genetic sampling and analysis provide a powerful tool for proactive monitoring and management of black bear populations. However, as demonstrated by our evaluation of cost and effort required to implement these methods across the entire black bear range in the state of California, forward scientific planning of the most efficient use of [multiple] sampling strategies may be necessary in order to achieve management goals within a finite budget. This analysis could serve as a guide for designing future scientifically rigorous statewide black bear monitoring efforts to track long-term population abundance and health trends in California.

Using the data generated by this project, alongside published literature and expert opinions, we make the following recommendations to improve and grow the California Department of Fish and Wildlife Black Bear Management Program:

1) Bear Management Units. Using the current understanding of California's range of black bear habitats, density estimations and genetic clusters described by Brown *et al.* (2009), we propose the establishment of four Bear Management Units. These units include: 1) North Coast/Klammath; 2) Cascade/Sierra Nevada; 3) Central Coast and; 4) Southern California. Table 12 presents the California counties belonging to each Bear Management Unit. The state of California has diverse populations of bears, including issues of habitat choice/availability, genetic structure, and overall sizes. By establishing Bear Management Units, based on scientifically similar bear life history characteristics, the California Department of Fish and Wildlife can more effectively monitor and manage bears across the state.

Table 12. Proposed Bear Management Units

North Coast/Klamath	**Cascade/Sierra Nevada**	**Central Coast**	**Southern California**
Del Norte	Kern	San Luis Obispo	Santa Barbara
Siskiyou	Tulare	Monterey	Ventura
Modoc	Inyo	Santa Cruz	Los Angeles
Humboldt	Fresno	San Benito	Orange
Trinity	Madera	Santa Clara	San Diego
Shasta	Mariposa	Alameda	Riverside
Lassen	Tuolumne		San Bernardino
Mendocino	Mono		
Sonoma	Calaveras		
Glenn	Amador		
Lake	Alpine		
Colusa	El Dorado		
Napa	Placer		
Yolo	Nevada		
Marin	Yuba		
Solano	Sierra		
western half of Tehama	Butte		
	Plumas		
	eastern half of Tehama		

2) Accuracy of Hunter-Harvest Derived Population Estimates. Although population estimates derived from hunter-harvest data may not provide an accurate representation of bear populations across the entire state of California, these estimates may be useful in areas where a high density of hunting occurs. The methods required to generate population estimates from hunter-harvest data are inexpensive relative to costs of other methods, and therefore, if accurate, may be superior to more costly and time intensive capture-mark-recapture efforts in selected areas. However, before this can be determined, it is important to directly compare the accuracy and precision of population estimates derived using both methods in the same regions. We suggest that the California Department of Fish and Wildlife conduct a multi-year study to evaluate the accuracy of hunter harvest derived population estimates in the North Coast/Klamath and Cascade/Sierra Nevada (proposed) Bear Management Units. Both units have high numbers of bears taken by hunters each year (over 400), and therefore would be the areas most likely to be accurately represented by hunter-harvest population estimates. A study would be conducted by picking at least two representative counties with a hunter take of 45 or greater, from each Bear Management Unit, and conducting a noninvasive genetic capture-mark-recapture study in each county for two or more consecutive sampling seasons. Population estimates generated from the capture-mark-recapture studies would then be compared with hunter-harvest estimates in those counties order to evaluate the accuracy of each method. It is important that a study include more than one county from each unit over multiple years in order to have a larger representation of the bear management and reduce bias created by time.

3) Long-Term Statewide Monitoring Plan. Generating point population estimates is important for managers to gain an understanding of the *current* population. However, it is also important for managers to be able to monitor population trends, by identifying trends in numbers over time, and enabling detection of population census changes. Early detection of a population decline, or increase, is integral for managers to be able to respond with adaptive management techniques and interventions. We suggest the development of methodical multi-year sampling plans for each proposed Bear Management Unit. As our simulation indicates, it would be time and cost prohibitive to implement a noninvasive genetic capture-mark-recapture study in every single county in California where bears are present. Therefore it will be necessary to identify representative counties for each Bear Management Unit that will be monitored more intensively. These counties should be sampled consecutively for 2-3 years in order to establish a baseline population estimate. It is important to include multiple consecutive years of sampling, in order to reduce potential bias created by food availability, climate, drought, etc. Once baseline population estimates have been established for each Bear Management Unit, re-sampling should occur at least once every 1-2 generations, or every 5-8 years for black bears.

4) Opportunistic Sample Collection, Storage and Database Management. Maintaining an archive of genetic samples and data, both current and historical, can be important for many population studies. We recommend that the California Department of Fish and Wildlife collect and archive a DNA sample (i.e. hair, blood, or cheek swab) from all bears that are handled. These bears could include depredation, road kill, relocation and rehabilitation animals. Samples should be stored appropriately along with their supplemental information including age, sex and location. Establishing and maintaining

this archive and database will be vital for future studies by enabling sufficient sample sizes and allowing for the inclusion of historical samples for comparison.

Acknowledgements
The research was supported by the California Department of Fish and Wildlife. We extend thanks to the field crews who carried implemented the field portion of this study in San Luis Obispo and Monterey. We would also like to thank all the private land-owners, Forest Service, State Parks and UC Reserves in San Luis Obispo and Monterey counties who allowed access to their land for the duration of the study. Thank you to Michael Buchalski, Jeff Cann, Deana Clifford, Kristi Cripe, Lisa Dalbeck, Johnathan Draper, Tracy Drazenovich, Jonathan Fusaro, Jesse Garcia, Ben Gonzales, Bruce Hoar, Marc Kenyon, Zachary Lounsberry, Bob Stafford, Craig Stowers, Pam Swift, Tim Taylor, Rocky Thompson, Todd Tognazzini and the Veterinary Genetics Laboratory and UC Davis for their intellectual and technical assistance. We appreciate all the assistance provided by various staff including but not limited to, biologists, scientific aides, GIS specialists, environmental scientists and wardens.

Glossary

Allele: One of two or more versions of a gene that can occur at the same location (locus) on homologous (paired) chromosomes. A population can have many alleles for a particular locus, but an individual can carry no more than two alleles (one from mother and one from father) at a diploid locus.

Amplify: To increase in the frequency of a gene, as a result of DNA replication, processes, such as by polymerase chain reaction polymerase chain reaction or gene duplication.

CMR: Capture-Mark-Recapture; here represented by hair snare samples.

Genotype: The specific set of alleles inherited at a locus, or across multiple loci.

Hardy-Weinberg equilibrium (HWE): Given certain simplifying assumptions such as no genetic drift, random mating, non-overlapping generations, no selection and no (genetic) migration, the genotype frequencies in an infinite population can be predicted from the gene frequencies, *p* and *q* by the formula:

$$p^2 + 2pq + q^2$$

A population will achieve Hardy-Weinberg equilibrium (HWE) in a single generation (unless one of the assumptions listed above is violated). We test for HWE by comparing observed and expected genotype frequencies.

Linkage Disequilibrium: the non-random association of alleles at adjacent loci. When a particular allele at one locus is statistically associated with a specific allele at a second locus (more often than expected if the loci were segregating independently in a population) the loci are in disequilibrium.

Locus: (plural is loci) The physical site or location of a specific gene on a chromosome— often used as synonymous with 'gene' in the broad sense, meaning a stretch of DNA being analyzed for variability (e.g., a microsatellite locus).

Microsatellite: Short tandem repeats (e.g., ACACACACACAC) of nucleotide sequences -- the tandem units can be dinucleotides (AC), trinucleotides (ACC) or tetranucleotides (ACCC). The apparent mutation process is by slippage replication errors, where the repeats allow matching via excision or addition of repeats. Because this sort of slippage replication is more likely than point mutations, microsatellite loci tend to be highly variable.

Multiplex: Groups of microsatellites run together in PCR.

Polymerase Chain Reaction: A procedure that produces multiple copies of a short segment of DNA through cycles of: 1) denaturation (heat-induced separation of double-stranded DNA into single strands); 2) annealing (binding of specific primers on either end of the target segment); and 3) elongation (extension of the primer sequences over the target segment with DNA polymerase).

Primer: Short, pre-existing single-stranded polynucleotide chain to which new nucleotides can be added. The primer anneals to DNA of the organism of interest and promotes copying of the template, starting from the primer site. To amplify DNA one uses a forward and reverse primer pair. Some primer sequences may be conserved across wide taxonomic gaps (e.g., across families), while others may differ even among congeners.

Probability of Identity, (P(Id): The probability that two unrelated individuals drawn at random from a population will have the same genotype at multiple loci. In Excel worksheet it can be read as: 1 in [PID] unrelated bears drawn at random from Mono County would have the exact same genotype.

Probability of Identity of Siblings (PI sibs): The probability that two siblings drawn at random from a population will have the same genotype at multiple loci. In Excel worksheet it can be read as: 1 in [PID_{sibs}] sibling bears drawn at random from Mono County would have the exact same genotype.

SVWA: Slinkard Valley Wildlife Area

TML: Town of Mammoth Lakes

* Glossary adapted from

http://www.nwfsc.noaa.gov/publications/techmemos/tm37/glossary.htm
http://www.dorak.info/genetics/popgen.html
http://www.uwyo.edu/dbmcd/popecol/Maylects/PopGenGloss.html
https://www3.nationalgeographic.com/genographic/glossary.html
http://www.ornl.gov/sci/techresources/Human_Genome/glossary/

Literature Cited:

Airola, D.A. 1988. Guide to the California wildlife habitat relationships system. California Department of Fish and Game, Sacramento, CA, USA.

Alpers, D.L., A.C. Taylor, P. Sunnucks, S.A. Bellman, and W.B. Sherwin. 2003. Pooling hair samples to increase DNA yield for PCR. *Conservation Genetics*. 4:779-788

Barja, I., F.J. Miguel, and F. Bárcena. 2005. Faecal marking behavior of Iberian wolf in different zones of their territory. *Folia Zoologica*. 54:21-29.

Bellemain, E.V.A., J.E. Swenson, D. Tallmon, S. Brunberg, and P. Taberlet. 2005. Estimating Population Size of Elusive Animals with DNA from Hunter-Collected Feces: Four Methods for Brown Bears. *Conservation Biology*. *19*(1):150-161.

Boitani, L., and R.A. Powell. 2012. Carnivore ecology and conservation: a handbook of techniques. Oxford University Press. Oxford, UK.

Boulanger, J., B.N. McLellan, J.G. Woods, M.F. Proctor, and C. Strobeck, C. 2004. Sampling design and bias in DNA-based capture-mark-recapture population and density estimates of grizzly bears. *Journal of Wildlife Management*. *68*(3):457-469.

Brown, S.K., J.M. Hull, D. Updike, S. Fain, and H.B. Ernest. 2009. Black Bear Population Genetics in California: Signatures of Population Structure, Competitive Release and Historical Translocation. *Journal of Mammalogy*. 90:1066–1074.

California Department of Fish and Game & California Interagency Wildlife Task Group. 2002. California wildlife habitat relationships system. Wildlife and Habitat Data Analysis Branch, Sacramento, CA, USA.

California Department of Fish and Game. 2010. Draft Environmental Document. Sections 265, 365, 366, 367.5, 401, 708, Title 14, California Code of Regulations, Regarding Bear Hunting. January 27, 2010. State of California. The Natural Resources Agency.

California Department of Fish and Wildlife. 2012. California Department of Fish and Wildlife black bear population report. California Department of Fish and Wildlife, Sacramento, CA, USA.

California Department of Forestry and Fire Protection. 2012. San Luis Obispo Fire Plan. Available at: http://cdfdata.fire.ca.gov/pub/fireplan/fpupload/fpppdf1337.pdf.

Clark, J.D., F.T. Van Manen, and M.R. Pelton. 2005. Bait stations, hard mast, and black bear population growth in Great Smoky Mountains National Park. *Journal of Wildlife Management*. 69(4):1633-1640.

Coster, S.S., A.I. Kovach, P.J. Pekins, A.B. Cooper, and A. Timmins. 2011. Genetics mark-recapture population estimation in black bears and issues of scale. *Journal of Wildlife Management*. 75(5):1128-1136.

Cornuet, J.M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*. 144(4):2001-2014.

Coulon, A., J.W. Fitzpatrick, R. Bowman, B.M. Stith, C.A. Makarewich, L.M. Stenzler, and I.J. Lovette. 2008. Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). *Molecular Ecology*. 17:1685–1701.

County of Monterey Planning and Building Inspection. 2007. Biological Resources. Available at: http://www.co.monterey.ca.us/planning/gpu/2007_GPU_DEIR_Sept_2008/Text/Sec_04.9_Biological_Resources.pdf.

Diefenback, D.R., J.L. Laake, and G.L. Alt. 2004. Spatio-Temporal and demographic variation in the harvest of black bears: implications for population estimation. *Journal of Wildlife Management*. 68:947–959.

Do, C., R.S. Waples, D. Peel, G.M. Macbeth, B.J. Tillett, and J.R. Ovenden. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*. 14:209-214.

Eagar, D.C. 1977. Radioisotope feces tagging as a population estimator of black bear density in the Great Smoky Mountains National Park. Thesis, University of Tennessee, Knoxville, USA.

Earl, D.A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*. *4*(2):359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*. 14(8):2611-2620.

Excoffier, L. and H.E.L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. 10:564-567.

Fraser, D., J.F. Gardner, G.B. Kolenosky, and S. Strathearn. 1982. Estimation of harvest rate of black bears from age and sex data. *Wildlife Society Bulletin*. 53-57.

Fraser, D. 1984. A simple relationship between removal rate and age-sex composition of removals for certain animal populations. *Journal of Applied Ecology*. 21:97-101.

Funk, W.C., M.S. Blouin, P.S. Corn, B.A. Maxell, D.S. Pilliod, S. Amish, and F. W. Allendorf. 2005. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology.* 14:483–493.

Gardner, B., J.A. Royle, M.T. Wegan, R.E. Rainbolt, and P.D. Curtis PD. 2010. Estimating Black Bear Density Using DNA Data From Hair Snares. *Journal of Wildlife Management*. 74:318–325.

Garshelis, D.L. 1990. Monitoring effects of harvest on black bear populations in North America: a review and evaluation of techniques. *Eastern Workshop on Black Bear Research and Management*. 10:120–144.

Garshelis, D.L. 1993. Monitoring black bear populations: pitfalls and recommendations. *Western Black Bear Workshop.* 4:123–144.

Garshelis, D.L. and H. Hristienko. 2006. State and provincial estimates of American black bear numbers versus assessments of population trend. *Ursus.* 17:1–7.

Garshelis, D.L. and K.N. Noyce. 2006. Discerning biases in a large scale mark-recapture population estimate for black bears. *Journal of Wildlife Management*. 7:1634–1643.

Gorman, M.L. and B.J. Trowbridge. 1989. The role of odor in the social lives of carnivores. In *Carnivore behavior, ecology, and evolution* (pp. 57-88). Springer US.

Grinnell, J., J.S. Dixon, and J.M. Lindsdale. 1937. Fur-bearing mammals of California. Volume 1. University of California Press, Berkeley, CA, USA.

Guggisberg, C.A.W. 1977. Early wildlife photographers. Taplinger Publishing Company.
Heinemeyer, K.S., T.J. Ulizio, and R.L. Harrison. 2008. Natural sign: tracks and scats. In *Noninvasive survey methods for carnivores*. 45-74.

Hall, R. and K. Kelson. 1959. The Mammals of North America. John Wiley and Sons Press. New York, NY, USA.

Harris, R.B., C.C. Schwartz, R.D. Mace, M.A. Haroldson. 2011. Study design and sampling intensity for demographic analyses of bear populations. *Ursus*. 22:24-36.

Hill, W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. *Genetical Research*. 38(03): 209-216.

Höss, M., M. Kohn, S. Pääbo, F. Knauer, and W. Schröder.1992. Excrement analysis by PCR. *Nature*. 359:199.

Hristienko, H. and J.E. Mcdonald Jr. 2007. Going into the 21st century: a perspective on trends and controversies in the management of the American black bear. *Ursus*. 18(1):72-88.

Huggins, R.M. 1989. On the statistical analysis of capture experiments. *Biometrika*. *76*(1):133-140.

Johnson, K.G. and M.R. Pelton. 1980. Prebaiting and snaring techniques for black bears. *Wildlife Society Bulletin*. 8:46–54.

Kalinowski, S.T., A.P. Wagner, and M.L. Taper. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*. 6(2):576-579.

Kane, D.M. 1989. Factors influencing the exploitation and vulnerability of black bears in northern New Hampshire. M.S. Thesis, University of New Hampshire, Durham, New Hampsire, USA. 40pp.

Kane, D.M. and J.A. Litvaitis. 1992. Age and sex composition of live-captured and hunter-killed samples of black bears. *Journal of Mammalogy.* 73:215–217.

Karamanlidis, A.A., M. Straka, E. Drosopoulou, M. de Gabriel Hernand, I. Kocija, L. Paule, and Z. Scouras. 2012. Genetic diversity, structure, and size of an endangered brown bear population threatened by highway construction in the Pindos Mountains, Greece. *European Journal of Wildlife Research*. 58(3):511-522.

Kendall, K.C. and K.S. McKelvey. 2008. Chapter 6: Hair collection. In: Long, R. A., P. MacKay, J.C. Ray, and W.J. Zielinski, editors. Noninvasive survey methods for North American carnivores. Island Press. Washington D.C., USA.

Kendall, K.C., J.B. Stetz, D.A. Roon, L.P. Waits, J. Boulanger, and D. Paetkau. 2008. Grizzly bear density in Glacier National Park, Montana. *Journal of Wildlife Management.* 72:1693–1705.

Kendall, K.C., J.B. Stetz, J. Boulanger, A.C. Macleod, D. Paetkau, and G.C. White. 2009. Demography and genetic structure of a recovering grizzly bear population. *Journal of Wildlife Management*. 73(1): 3-17.

Koehler, G.M. and D.J. Pierce. 2005. Survival, cause-specific mortality, sex, and ages of American black bears in Washington state, USA. *Ursus.* 16:157–166.

Livaitis. J.A and D.M. Kane. 1994. Relationship of hunting technique and hunter selectivity to composition of black bear harvest. *Wildlife Society Bulletin*. 22(60):1-60.

Long R.A., P. Mackay, W.J. Zielinski, J.C. Ray, editors. 2008. Noninvasive survey methods for carnivores. Island Press. Washington, D.C., USA.

Luikart, G., F.W. Allendorf, J.M. Cornuet, and W.B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*. 89(3):238-247.

Luikart, G., N. Ryman, D.A. Tallmon, M.K. Schwartz, and F.W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics*. 11(2):355-373.

Mace, R.D., S.C. Minta, T.L. Manley, and K.E. Aune. 1994. Estimating grizzly bear population size using camera sightings. *Wildlife Society Bulletin*. 74-83.

MacKenzie, D.I. and J.D. Nichols. 2004. Occupancy as a surrogate for abundance estimation. *Animal Biodiversity and Conservation*. 27(1):461-467.

MacKenzie, D.I. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor University Press. Cold Harbor, NY, USA.

Marcum, L.C. 1974. An evaluation of radioactive feces tagging as a technique for determining densities of black bears in Great Smoky Mountains National Park. Thesis, University of Tennessee, Knoxville, USA.

McCall, B.S., M.S. Mitchell, M.K. Schwartz, J. Hayden, S.A. Cushman, P. Zager, and W.F. Kasworm. 2013. Combined use of mark-recapture and genetic analyses reveals response of a black bear population to changes in food productivity. *Journal of Wildlife Management*. 77(8):1572-1582.

Meredith, E.P., J.A. Rodzen, J.D. Banks, and K.C. Jones. 2009. Characterization of 29 tetranucleotide microsatellite loci in black bear (*Ursus americanus*) for use in forensic and population applications. *Conservation Genetics*. 10:693-696.

Miller, S.D. 1987. Susitna hydroelectric project final report. Big game studies. Vol. 6-black bear and brown bear. Alaska Department of Fish and Game, Anchorage. 276pp.

Miller, S.D. 1989. Population management of bears in North America. *International Conference on Bear Research and Management.* 8:357–373.

Miller, S.D. 1990. Population management of bears in North America. In: *Bears: Their Biology and Management*. 357-373.

Miller, S.D., G.C. White, R.A. Sellers, H.V. Reynolds, J.W. Schoen, K. Titus, ... and C.C. Schwartz. 1997. Brown and black bear density estimation in Alaska using radiotelemetry and replicated mark-resight techniques. *Wildlife Monographs*. 3-55.

Miller, C.R., P. Joyce, and L.P. Waits. 2005. A new method for estimating the size of small populations from genetic mark–recapture data. *Molecular Ecology*. 14(7):1991-2005.

National Oceanic Atmospheric Administration. 2015. Annual Climatological Summary. National Climatic Data Center. Asheville, NC, USA.

Novick, H.J. 1979. Home range and habitat preferences of black bears (Ursus americanus) in the San Bernardino Mountains of southern California. Thesis, California State Polytechnic University, Pomona, California, USA.

Orlóci, L. 1978. Ordination by resemblance matrices. In *Ordination of Plant Communities* (pp. 239-275). Springer Netherlands.

Pagès, M., C. Maudet, E. Bellemain, P. Taberlet, S. Hughes, and C. Hänni. 2009. A system for sex determination from degraded DNA: a useful tool for palaeogenetics and conservation genetics of ursids. *Conservation Genetics*. 10:897-907.

Paloheimo, J. E. and D. Fraser. 1981. Estimation of harvest rate and vulnerability from age and sex data. *The Journal of Wildlife Management*. 45(4):948-958

Parks, S.D.E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin (reference for Microsatellite toolkit software)

Paetkau, D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*. 3(5):489-495.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*. 4(3):347-354.

Peakall, R. and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*. 28:2537-2539.

Pelton, M.R. 2003. Black bear (*Ursus americanus*). *in* Feldhammer, G. A., B.C. Thompson, and J.A. Chapman, editors. Wild mammals of North America. John Hopkins University Press. Baltimore, Maryland, USA.

Pennell, M.W., C.R. Stansbury, L.P. Waits, and C.R. Miller. 2013. Capwire: a R package for estimating population census size from non-invasive genetic sampling. *Molecular Ecology resources*. 13(1):154-157.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155(2):945-959.

Pritchard, J.K., W. Wen, and D. Falush. 2003. Documentation for structure software: version 2.

Ranta, E., J. Lindstrom, H. Linden, and P. Helle. 2008. How reliable are harvesting data for analyses of spatio-temporal population dynamics? *Oikos*. 117:1461–1468.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*. 223-225.

Robinson, S.J., L.P. Waits, and I.D. Martin. 2009. Estimating abundance of American black bears using DNA-based capture-mark-recapture models. *Ursus*. 20(1):1-11.

Roon, D.A., M.E. Thomas, K.C. Kendall, and L.P. Waits. 2005. Evaluating mixed samples as a source of error in non-invasive genetic studies using microsatellites. *Molecular Ecology*. 14(1):195-201.

Roughton, R.D. and M.D. Sweeny. 1982. Refinements in scent-station methodology for assessing trends in carnivore populations. *Journal of Wildlife Management*. 46:217–229.

Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*. *8*(1):103-106.

Royle, J.A., R.B. Chandler, R. Sollmann, and B. Gardner. 2013. *Spatial capture-recapture*. Academic Press.

Sawaya, M.A., J.B. Stetz, A.P. Clevenger, M.L. Gibeau, S.T. Kalinowski. 2012. Estimating Grizzly and Black Bear Population Abundance and Trend in Banff National Park Using Noninvasive Genetic Sampling. *PLoS ONE*. 7(5): e34777.

Schoenherr, A.A. 1992. A natural history of California (Vol. 56). University of California Press. Berkeley, CA, USA.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution.* 22:25–33.

Sellers, R.A. and L.D. Miller. 1994. Brown bear population parameters at McNeil River, Alaska. *International Conference for Bear Research and Management*. 9:283-293.

Storer, T.I. and L.P. Tevis Jr. 1955. California grizzly. University of California Press. Berkeley, CA, USA.

Suckley, G. and G. Gibbs. 1860. Zoological Report, Chapter III. Pages. 107–139. *In* Reports of Explorations and Surveys, to Ascertain the Most Practicable and Economical Route for a Railroad from the Mississippi River to the Pacific Ocean, Volume 12, Book II. U.S. War Department, Washington, D.C. (36th Congress, 1stSession, House of Representatives Executive Document No. 56).

Taberlet, P. and J. Bouvet. 1992. Bear conservation genetics. *Nature*. 358:197.

Toonen, R.J. and S. Hughes 2001. Increased Throughput for Fragment Analysis on ABI Prism 377 Automated Sequencer Using a Membrane Comb and STRand Software. *Biotechniques*. 31:1320-1324.

U.S. Census Bureau. 2010. Urban and rural classification. U.S. Census Bureau. Washington D.C., USA

U.S. Census Bureau. 2014. Annual Estimates of the Resident Population. U.S. Census Bureau, Washington D.C., USA.

Waits, L.P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*. 10(1):249-256.

Waits, L.P. and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*. 69(4):1419-1433.

Waples, R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci*. *Conservation Genetics*. 7(2):167-184.

Waples, R.S. and O. Gaggiotti. 2006. Invited Review: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*. 15(6):1419-1439.

Waples, R.S. and C. Do. 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications*. 3(3):244-262.

Wasser, S.K., C.S. Houston, G.M. Koehler, G.G. Cadd, and S.R. Fain, S. R. 1997. Techniques for application of faecal DNA methods to field studies of Ursids. *Molecular Ecology*. *6*(11):1091-1097.

Wasser, S.K., B. Davenport, E.R. Ramage, K.E. Hunt, M.E. Parker, C. Clarke, and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Canadian Journal of Zoology*. 82(3):475–492.

White, G.C., D.R. Anderson, K.P. Burnham, and D.L. Otis. 1982. Capture-Recapture and Removal Methods for Sampling Closed Populations. Report LA-8787-NERP. Los Alamos National Laboratory, Los Alamos, NM, USA.

White, G.C. and K.P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. *Bird study*. 46(S1):S120-S139.

White, G. C. and R.A. Garrott. 2012. Analysis of wildlife radio-tracking data. Academic Press Inc. San Diego, California, USA.

Woods, J.G., D. Paetkau, D. Lewis, B.N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin.* 27:616–627.

Ypema, R. and J. Garcia. 2015. 2013 California Black Bear Take Report. State of California Natural Resources Agency, Department of Fish and Wildlife. Available at: https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=89695&inline

Xu, X., L. Lin, Z. Zhang, F. Shen, L. Zhang and B. Yue. 2008. A reliable, non-invasive PCR method for giant panda (*Ailuropoda melanoleuca*) sex identification. *Conservation Genetics*. 9:739-741.

Appendices

Appendix 1. Annual Report for Year-1 (July 2012 – July 2013)

Annual Report for Year-1 (July 2012 – July 2013)
Population Genetics Study of California's Black Bears
August 2013:

Please do not disseminate beyond California Department of Fish Wildlife; and do not upload any of this information to the internet (this is a preliminary report; might harm Jonathan's publication abilities). Thanks.

Agreement #: P1280004 00
Date: August 1, 2013
Project Title: Population Genetics Study of California's Black Bears

Principal Investigator: Holly Ernest
Title: Associate Professor in Residence
Graduate Student Investigator: Jamie Sherman
Department: University of California Davis, Veterinary Genetics Laboratory
Address: 1009 VM3B, 1089 Veterinary Medicine Drive, School of Veterinary Medicine, Davis, CA 95616
Contact: email: hbernest@ucdavis.edu phone: 530-754-8245

Project Background and Goals

Scientifically informed management of black bears requires information concerning population demographics, abundance and density. This is particularly important for considerations of adjustments to CDFW's hunting program and management actions in regions where increased risks for human interaction with bears are likely to occur, given that black bear populations are continuing to increase. Less-invasive methods involving hair snags and DNA analysis provide multiple sources of key individual bear and population data to support best practices management. This research project will produce analysis of genetics data and statistical analyses for black bear hair collected less-invasively in Mono (2012), San Luis Obispo (2013 and Monterey (2014) counties of California. The goal of this study is to individual identify black bears and their sex through DNA analysis and provide statistical analyses for abundance and density estimates in the study area.

Objectives

- Identify individual black bears by DNA analyses of sample tissue, blood or hair. (Mono, San Luis Obispo, and Monterey counties)
- Describe black bear populations' genetic diversity. (San Luis Obispo and Monterey counties)
- Enumerate selected black bear population parameters. (San Luis Obispo and Monterey counties)

- Determine population sources of individual black bears. (San Luis Obispo and Monterey counties)

Preliminary Report Overview

This document summarizes much of the preliminary work completed to date toward the fulfillment of the project goals and objectives.

Table 1 summarizes sample sets that UC Davis has received and the current progress of each sample set. All samples received are archived at the University of California Wildlife Genetics and Population Health Laboratory (Ernest Lab).

Table 1. Summary of samples received and current progress. CMR = Capture-Mark-Recapture and represent hairs collected from hair snags.

Sample Type	Location	Year	# of Samples Received	Expected Completion Date
Known Dead bears	Mono	2011-2012	31	Completed
Hair Snag	SVWA	2011	62	Completed
Hair Snag	TML	2011	71	Completed
Opportunistic	Mono	2010-2012	100	October 30, 2013
Hair Snag	SVWA	2010	57	October 30, 2013
Hair Snag	SVWA	2012	100	September 30, 2013
Hair Snag	TML	2012	104	September 30, 2013
Hair Snag	Monterrey	2013	71	June 30, 2013
Known Dead Bears	Monterrey	2013	1	June 30, 2013
Opportunistic	Monterrey	2013	1	June 30, 2013

Results

The preliminary data presented in this report consists of unique individual bear identifications for 2011 SVWA, 2011 TML and 2010-2012 Known Dead Bears (**Table 4**). Detailed results can be found in the accompanying Excel worksheet.

Table 4. Summary of Unique Individuals Identified in Mono County for 2011.

Sample Type	Location	Year(s)	# of Unique Individual Bears Identified
Known Dead Bears	Mono	2010-2012	28
CMR	SVWA	2011	31
CMR	TML	2011	14

There were no significant departures from Hardy Weinberg Equilibrium and Linkage Disequilibrium expectations. All assumptions were met to identify unique individuals with corresponding probabilities of identity.

Discussion

This work is preliminary and on-going, and thus is subject to change with the inclusion of additional samples/data sets.

There was a high rate of non-amplification for the 2011 TML samples. In the lab, all samples were stored and processed in an identical manner. Additionally, any samples that did not amplify a bear genotype after the first DNA extraction were independently extracted and tested a second time, if sufficient sample remained. Given these factors, it is likely that there were a great number of poor quality samples from TML in 2011. This could be due to differential environmental degradation (i.e. excess sunlight, rain, etc), a greater number of non-target species (i.e. small mammals), or some other unforeseen circumstance. The inclusion of the 2012 TML samples (and success rate vs. 2012 SVWA) might shed light on this matter.

Appendix 2. Interim Report – Mono County

Interim Report – Mono County
Population Genetics Study of California's Black Bears: Mono County
October 2013:

Principal Investigator: Holly Ernest
Title: Associate Professor in Residence
Graduate Student Investigator: Jamie Sherman
Department: University of California Davis, Veterinary Genetics Laboratory
Address: 1009 VM3B, 1089 Veterinary Medicine Drive, School of Veterinary Medicine, Davis, CA 95616
Contact: email: hbernest@ucdavis.edu phone: 530-754-8245

Preliminary Report (Mono County) Overview

This document summarizes the work completed to date toward the fulfillment of the project goals and objectives. Specifically data from the Opportunistic and 2010 Mono County CMR study are presented. This report completes the Mono County data set.

Table 1 summarizes sample sets that UC Davis has received and the current progress of each sample set. All samples received are archived at the University of California Wildlife Genetics and Population Health Laboratory (Ernest Lab).

Table 1. Summary of samples received and current progress. CMR = Capture-Mark-Recapture and represent hairs collected from hair snags.

Sample Type	Location	Year	# of Samples Received	Expected Completion Date
Known Dead bears	Mono	2011-2012	31	Completed
CMR	SVWA	2011	62	Completed
CMR	TML	2011	71	Completed
Opportunistic	Mono	2010-2012	92	Completed
CMR	SVWA	2010	47	Completed
CMR	SVWA	2012	100	Completed
CMR	TML	2012	104	Completed

CMR	SLO	2013	178	June 30, 2013
Known Dead Bears	SLO	2013	5	June 30, 2013
Opportunistic	SLO	2013	1	June 30, 2013

Results

The data presented in this report consists of unique individual bear identifications for Opportunistic and 2010 SVWA samples (**Table 4**). In addition, **Table 5**, presents a summary of all the unique individuals identified in Mono County (across all data sets) from 2010-2012. Detailed results can be found in the accompanying Excel worksheets.

Table 4. Summary of Unique Individuals Identified in Mono County from Opportunistic and 2010 SVWA Samples.

Sample Type	Location	Year(s)	# of Unique Individual Bears Identified	# of 2012 Individuals Previously Identified
Opportunistic	TML & SVWA	2010-2012	42	20
Hair Snag	SVWA	2010	27	15

Table 5. Summary of Unique Individuals Identified in Mono County from 2010-2012.

Sample Type	Location	Year(s)	# of Samples Genotyped	# of Unique Individual Bears Identified
Hair Snag	SVWA	2010	43	27
Hair Snag	SVWA	2011	56	29
Hair Snag	TML	2011	31	14
Hair Snag	SVWA	2012	84	40
Hair Snag	TML	2012	72	32
Opportunistic	Mono	2010-2012	78	42
Known Dead	Mono	2010-2012	28	28

There were no significant departures from Hardy Weinberg Equilibrium and Linkage Disequilibrium expectations. All assumptions were met to identify unique individuals with corresponding probabilities of identity.

Discussion

The rate of non-amplification for the Opportunistic samples was approximately 7%. The rate of non-amplification for the 2010 SVWA samples was approximately 2%. Any samples that did not amplify a bear genotype after the first DNA extraction were independently extracted and tested a second time, if sufficient sample remained.

Twenty of the individuals identified opportunistically were captured in one of the previous data sets. Two of these individuals (JF ID: 1028 and 167/ 937) were originally identified as known dead bears. The sample collection dates indicate that these bears were sampled opportunistically before death. Fifteen of the individuals identified in SVWA in 2010 were captured in one of the previous data sets. There were no samples collected in TML in 2010.

In summary, a total of **507** samples were collected from Mono County between the years of 2010-2012. Of those, successful genotypes were obtained from **392** samples, and **163 unique individuals** were identified.

Appendix 3. Annual Report for Year-2 (July 2013-June 2014)

Annual Report for Year-2 (July 2013-June 2014)
Population Genetics Study of California's Black Bears
June 2014:

Please do not disseminate beyond California Department of Fish Wildlife; and do not upload any of this information to the internet (this is a preliminary report; might harm peer-review publication abilities for this work). Thanks.

Agreement #: P128000400
Date: June 27, 2014
Project Title: Population Genetics Study of California's Black Bears

Principal Investigator: Holly Ernest DVM, PhD
Title: Professor in Residence
Graduate Student Investigator: Jamie Sherman
Department: University of California Davis, Veterinary Genetics Laboratory
Address: 1009 VM3B, 1089 Veterinary Medicine Drive, School of Veterinary Medicine, Davis, CA 95616
Contact: email: hbernest@ucdavis.edu phone: 530-754-8245

Project Background and Goals

Scientifically informed management of black bears requires information concerning population demographics, abundance and density. This is particularly important for considerations of adjustments to CDFW's hunting program and management actions in regions where increased risks for human interaction with bears are likely to occur, given that black bear populations are continuing to increase. Less-invasive methods involving hair snags and DNA analysis provide multiple sources of key individual bear and population data to support best practices management. This research project will produce analysis of genetics data and statistical analyses for black bear hair collected less-invasively in Mono (2012), San Luis Obispo (2013) and Monterey (2014) counties of California. The goal of this study is to individual identify black bears and their sex through DNA analysis and provide statistical analyses for abundance and density estimates in the study area.

Objectives

- Identify individual black bears by DNA analyses of sample tissue, blood or hair. (Mono, San Luis Obispo, and Monterey counties)
- Describe black bear populations' genetic diversity. (San Luis Obispo and Monterey counties)
- Enumerate selected black bear population parameters. (San Luis Obispo and Monterey counties)
- Determine population sources of individual black bears. (San Luis Obispo and Monterey counties)

Year-2 Annual Report Overview

This document summarizes much of the preliminary work completed to date toward the fulfillment of the project goals and objectives.

Table 1 summarizes sample sets that UC Davis has received and the current progress of each sample set. All samples received are archived at the University of California Wildlife Genetics and Population Health Laboratory (Ernest Lab). Results from year two are presented in this report. Results from year one can be found in the 2013 preliminary and annual reports.

Table 1. Summary of samples received and current progress. CMR = Capture-Mark-Recapture and represent hairs collected from hair snags. Opportunistic = samples collected outside of CMR study (i.e. natural rubs, live handling, etc.).

Year	Sample Type	Location	Year	# of Samples Received	Expected Completion Date
1	Known Dead bears	Mono	2011-2012	31	Completed
	CMR	SVWA	2011	62	Completed
	CMR	TML	2011	71	Completed
	Opportunistic	Mono	2010-2012	82	Completed
	CMR	SVWA	2010	57	Completed
	CMR	SVWA	2012	100	Completed
	CMR	TML	2012	104	Completed
2	CMR	San Luis Obispo	2013	178	Completed
	Known Dead Bears	San Luis Obispo	2013	7	Completed

	Opportunistic	San Luis Obispo	2013	1	Completed
3	CMR	Monterey	2014	76	December 2014
	Known Dead Bears	Monterey	2014	1	December 2014
	Opportunistic	Monterey	2014	0	December 2014

Results

The preliminary data presented in this report represents a summary of the analysis of the 187 samples received from San Luis Obispo County in 2013 (**Table 4**). Of the 187 samples collected, 119 amplified a bear genotype. From those 119 genotypes, 64 unique individuals were identified. Twenty-nine of those unique individuals were captured by DNA more than once, however some of the recaptures occurred within the same sampling session. Forty-two of the samples received were identified as non-target species, primarily canines. An additional 26 samples could not be amplified due to poor quality. These samples typically contained fewer than 3 guard roots. It should be noted that species identifications (bear or non-bear) could not be determined for poor quality samples.

Table 4. Summary of sample sizes and genotyping success.

# of Samples Received	187
# of Samples Successfully Genotyped	119
# of Unique Genotypes (individuals) * from the successfully genotyped samples	64
# of Non-Target Species	42
# of Poor Quality Samples	26

*Non-target species were identified as any species other than bear (i.e. hairs might have come from dog, coyote, pig, cow, horse, etc.). Samples classified as non-target species either amplified a canine genotype, or, did not amplify any genotype and hairs collected did not resemble bear hair (determined by CDFW or UC Davis).
*Poor quality samples included samples that did not amplify a genotype and/or contained few or no roots in the envelope.

A total of 54 sampling sites were employed across San Luis Obispo County, however bears were only observed by DNA identification at 21 of those sites. The number of unique individuals that visited each of the 21 sites is presented in **Table 5**. Most notably, five of the sampling sites were visited by five or more different bears within the 8 week study period.

Table 5. Summary of bear activity as determined by DNA identification at sampling sites in San Luis Obispo. Sites where bear activity was not observed by DNA identification have been excluded from the table.

# of Unique Individuals that Visited	Cell	Approximate Location/Nearest Landmark	Latitude	Longitude
3	30-T	Santa Margarita Ranch	35.37255	-120.60715
7	30-V	Cuesta Pass	35.33535	-120.58752
3	30-Z	Righetti Dam	35.24731	-120.58469
5	31-S	Pozo Rd near Santa Margarita	35.39135	-120.59269
2	31-W	Rinconada Creek	35.31119	-120.55105
3	32-V	Pilitas Creek	35.34167	-120.51039
6	33-U	Las Pilitas Rd	35.35365	-120.46612
3	33-Y	Forest Rte. 30S11	35.27573	-120.46835
5	34-T	Parkhill Rd	35.36828	-120.41879
1	34-V	Blinn Ranch Trail/River Rd	35.32894	-120.42814
2	35-S	Forest Rte. 29S10	35.39675	-120.36391
1	35-U	Turkey Flats near Parkhill Rd	35.35265	-120.37429
1	36-DD	Forest Rte. 32S07 near Pine Creek	35.16204	-120.33089
1	36-HH	Dry Canyon	35.07159	-120.32314
1	36-V	Pozo Rd near Fraser Canyon	35.32547	-120.3139
3	37-W	Pine Mountain Rd near Pozo Stair Steps	35.32931	-120.26841
4	37-Y	American Canyon Rd	35.26658	-120.27902
4	38-BB	Agua Escondido Rd	35.20875	-120.23558
2	39-II	Cuyama Highway (CA-166) near Willow Spring	35.06231	-120.21709
5	40-Z	Avenales Ranch	35.24385	-120.15491
1	42-FF	Gifford Spring	35.12504	-120.04073

Discussion

This work is preliminary and on-going, and thus is subject to change with the inclusion of additional samples/data sets.

Samples from San Luis Obispo had a low non-amplification rate of 14%, indicating that both sampling and analysis techniques are producing valuable data. Approximately 22% of the samples collected were determined to be “non-bear” and thus classified as non-target species. This number is reasonable and expected, due to the proximity of many other animals, such as cattle, horses, dogs, etc. to many of the sampling sites, as indicated by the field crew communications.

Fifty-four sampling sites were employed throughout San Luis Obispo County, providing a sample coverage across 1,080 km^2. According to GIS models, San Luis Obispo County has approximately 2905 km^2 of suitable bear habitat (37% of bear habitat was sampled). The main factor that limited sampling coverage was the lack of access of CDFW personnel to private lands in the northern half of the county. The limited coverage and geographic locations of sample sites will be taken into account when conducting further analysis, such as population estimation.

A few interesting trends came to light upon preliminary analysis of capture histories. One of the known dead bears collected on July 8, 2013, was also "captured" at a hair corral less than 15 km away approximately two weeks earlier, on June 24, 2013. Five of the unique bears identified visited more than one sampling site throughout the eight week sampling period. One of these bears visited two different sampling sites within the same week. None of the bears captured visited greater than two different sites. Lastly, one of the bears visited the same sampling site three weeks in a row.

Upcoming work for year 3

Year three is the final year of the contract agreement. In summary, the work remaining for year three includes: genetic typing of Monterey samples once received from CDFW, mark-recapture analysis for both San Luis Obispo and Monterey to generate estimates of census size and density, and development of candidate sampling schemes/methods for CDFW consideration in a long-term statewide monitoring plan as outlined in the UCD-CDFW contract amendment. A more detailed description of these tasks is listed below:

- Conduct population genetic statistical analysis including summary statistics for genetic diversity (allelic diversity, heterozygosity, etc.), probability of identity, estimates of null alleles, levels of inbreeding, etc.
- Assess bear census sizes (Nc), using minimum numbers of bears sampled and mark-recapture analysis, and estimate effective population (Ne) sizes.
- Determine population sources of individual black bears and determine the likelihood of each bear being a resident versus an immigrant.
- Assess feasibility of using noninvasive methods for bear monitoring at state level and compare with other population estimation methods (i.e. hunter harvest, sightings, etc).

All work is scheduled to be completed and a final report summarizing all findings will be submitted on June 15, 2015.

Appendix 4. Hair Lysis Buffer Protocol

Hair Lysis Buffer Protocol

83 µL water, 8.3 µL 10× PCR buffer, 8.3 µL 25 mM $MgCl_2$, and 0.4 µL Tween 20) (Maniatis, Fritsch and Sambrook 1989). Samples were incubated with 100 µL of hair lysis buffer and 0.5 µL of proteinase K for 45 min at 60 °C and then 45 min at 95 °C. The result DNA solution was centrifuged and excess hair material was removed.

Appendix 5. PCR Protocol

PCR Protocol

Polymerase chain reaction was carried out using 1.5 µL of DNA with a QIAGEN Multiplex PCR Kit (QIAGEN Inc., Valencia, CA, USA). Multiplex kit protocol was modified as follows: 6.25 µL of 2x QIAGEN multiplex PCR master mix (final concentration, 1x), 1.25uL of primer mix (final concentration of 0.02 to 0.08 µM for each primer), 1.25 µL of 5x Q-solution, 1.75 µL of distilled water, and 1.5 µL of DNA (~10-20 ng) for a total reaction volume of 12.5 µL.

Table 2. Summary of 14 Microsatellite Loci used for genotyping individual samples.

Multiplex	Loci	Reference
Bear 1 A	G1A G10B* G10C G10H G10o	Brown *et al.* 2009
Bear 1 B	G1D G10L	Brown *et al.* 2009
Bear F	A007 A002 B001 D103 D112 D116 D118	Meredith *et al.* 2009

* removed from analysis due to departure from Hardy-Weinberg equilibrium in multiple populations

Table 3. Summary of 2 Loci used to provide sex determination for individual samples.

Multiplex	Loci	Reference
Bear Sex	AME SRY/ZF	Xu *et al.* 2008 Pagès *et al.* 2009

PCR amplifications were carried out in a Bio-Rad MyCyler (Bio-Rad, Hercules, CA, USA) using the multiplex PCR protocol for amplification of microsatellite loci with Q solution (QIAGEN Multiplex PCR kit; QIAGEN): 15 min at 95°C (initial activation step), followed by 40 cycles consisting of 94°C for 30 s, 57°C or 55°C (for sexing markers) for 90 s, and 72°C for 90 s, followed by a final extension step of 72°C for 10 min. PCR products were separated with a 3730 DNA Analyzer (Applied Biosystems Inc.) with each capillary containing 1 µL of a 1:10 dilution of PCR product and deionized water, 0.05 µl GeneScan 500 Liz Size Standard and 9.95 µl of HiDi formamide (both products Applied Biosystems Inc.) that was denatured at 95 °C for 3 min.