Genetic Structure, Diversity, and Connectivity of Alabama Black Bear (*Ursus americanus*) Populations

by

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Abstract

Black bear (*Ursus americanus*) abundance and distribution has declined drastically across the southeastern United States, where a range reduction of 80% has occurred. In this study, the structure, diversity, and connectivity of two Alabama bear populations was examined from a genetic perspective. Microsatellite markers were employed to assess heterozygosity, allelic trends across populations, descriptive F-statistics, and connectivity with neighboring populations. It was confirmed that two separate subspecies occur within Alabama borders: *U. a. floridanus* in the Mobile River Basin (MRB) region and *U. a. americanus* subspecies in Northeastern Alabama (NAL) region. The MRB exemplifies major genetic concerns associated with fragmentation and isolation. Measures of genetic diversity demonstrated extreme low variability (polymorphism at 60% of loci; A = 1.58; Na = 2.000 ± 0.447 alleles; He = 0.246 ± 0.128; Ho = 0.256 ± 0.133). NAL population was confirmed as a re-colonizing front dispersing from the Smokey Mtn National Park in Tennessee. Prior to this study, bears had been absent from NAL for >100 years. The source population for NAL showed substantial genetic variability (Ho = 0.698 ± 0.061; He = 0.735 ± 0.017; Na = 6.400 ± 0.400; A = 3.12). However, due to random sampling, NAL bears are at risk for genetic loss through founders' effect. We observed a disparate average number of alleles per loci between NAL and its source (Na = 4.000 ± 0.000 and Na = 6.400 ± 0.400 respectively). Continued genetic monitoring, research focused on habitat use, dispersal, and conservation management are imperative to ensure the long term viability of Alabama black bears.

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List of Abbreviations

MRB	Mobile River Basin
NAL	Northeastern Alabama
Na	Average number of alleles per locus
Ho	Observed heterozygosity
He	Expected heterozygosity
A	Allelic richness
PI	Probability of identity
HWE	Hardy Weinberg equilibrium
F	Inbreeding Coefficient
MM1	Microsatellite Multiplex 1
MM2	Microsatellite Multiplex 2
MM3	Microsatellite Multiplex 3
K	Number of genetically distinct populations
MCMC	Markov chain Monte Carlo
TNP	Tennessee population
MSP	Mississippi population
GAP	Georgia population
PCR	Polymerase chain reaction

Chapter 1: Genetic Diversity and Structure of Black Bear (*Ursus americanus)* Populations in the Mobile River Basin and Northeastern Alabama

1.0 Introduction

1.1 Introduction: Background

The decline of large, charismatic carnivores is a global dilemma. The largest of the ursids, canids, and felids are imperiled by a multitude of anthropogenic threats, placing them at risk for extinction. Black bears (*Ursus americanus*) of North America exemplify these concerns. The overall distribution of black bears has decreased by 62% compared to historical levels (Pelton and Van Manen, 1994; Figure 1). Widespread agricultural land clearing, hunting, and anthropogenic persecution are the primary causes of this reduction. The effects of these negative factors are apparent in the small, fragmented, and insular nature of remaining bear populations. Protection for remnant and dwindling populations is often necessary.

Black bears have been disproportionately affected in the southeastern United States where a more extensive range reduction of 80% has occurred (Pelton and Van Manen, 1997). Of the three subspecies occurring within the region, two are considered a conservation concern. The Louisiana subspecies (*U. a. luteolus*) is classified as Threatened by the federal government under the Endangered Species Act (United States Fish and Wildlife Service Southeast Region, 2014). The Florida subspecies (*U. a. floridanus*) has been considered for federal listing on several

occasions (Bentzien, 1998). Many obstacles still complicate the conservation of threatened populations. For example, the Florida subspecies experiences significant variation in levels of lawful protection across its range due to disparate classifications. Once classified as threatened by the state of Florida, they are now considered game animals with strict hunting regulations. In Georgia, black bears have historically been considered game animals with fewer hunting regulations. Alabama also lists bears as game animals, but they lack an open season. Since responsibilities fall under state and provincial jurisdictions rather than a cohesive strategy at the federal level, conservation attempts are muddled by inconsistent management techniques (Williamson, 2002).

Alabama plays host to one of the most imperiled black bear populations of the southeastern United States; a population of the Florida subspecies occupying the Mobile River Basin (MRB). Located in the extreme southwest portion of Alabama, the population resides in portions of Baldwin, Clarke, Choctaw, Mobile, and Washington counties (Bentzien, 1998; Edwards, 2002; Silvano et al., 2007; Figure 2). The population is characterized by low numbers and appears to be highly isolated. The most recent estimate of population size was generated 15 years ago and concluded that fewer than 50 individuals remained (Edwards, 2002). At that time, the United States Fish and Wildlife Service did not consider the MRB population to be viable due to multiple threats from shrinking habitat availability, low population numbers, and the accompanying genetic losses (Bentzien, 1998; Edwards, 2002).

Reports of black bear extirpation from all but the southwest corner of Alabama have existed for nearly a century (Howell, 1921). However, scientific interest has recently been piqued by anecdotal reports of a population returning without anthropogenic assistance to the northeastern counties of DeKalb and Cherokee. This reappearance coincides with anecdotal

reports of expanding populations in the Appalachian range; therefore, investigators believe the bears of northeastern Alabama (NAL) would most likely be of the American subspecies. However, this novel and re-colonizing population has neither been confirmed nor previously studied. A lack of baseline information provides little direction for effective management action of a potentially small and precarious population.

Alabama black bears have received minimal scientific attention. Three cursory studies have transpired since the 1980's, during which 22 individuals were radio-tagged, a brief review of dietary preferences was generated, and 10 individuals were captured for tissue samples and morphological measurements (Kasbohm et al., 1994; Dusi and King, 1990; Edwards, 1998). Further research is necessary to assess the current status of black bears. In this study, we focused on genetic diversity and structure to assess the current conditions and potential viability of black bear populations in Alamaba. Genetic assessments included calculations of heterozygosity, allelic richness, allelic frequencies, and descriptive F-statistics. Results are essential for identifying populations in genetic distress and the necessity of genetic rescue.

1.2 Introduction: Study Area

Black bears once roamed across the majority of southeastern eco-regions, however they are now restricted to small, geographically isolated regions. Stressors from agricultural transformation and anthropogenic persecution resulted in a multitude of local extinctions. Early settlers of the 1700's rapidly extirpated bears from the 'Piedmont' and 'Ridge and Valley' regions due to favorable agricultural conditions (Edwards, 2002). These areas remain void of black bear populations to the present day. For the past 100 years, populations have been relegated to the 'Coastal Plain' and 'Mountain' physiographic regions (Pelton and Van Manen,

1997; Figure 3). These regions presented major barriers to early farmers and still provide asylum to bears in the form of swamps, grueling topography, and remoteness.

The MRB population resides in the 'Coastal Plain' region located in the extreme southwestern corner of Alabama and approximately centered at 30°55'38" N and 88°6'41" W. The region covers > 4,500 square kilometers, however the MRB black bear population is believed to inhabit a substantially smaller area adjacent to the Mobile-Tensaw River delta. Natural habitats available to MRB bears vary from hardwood bottomlands, bays, drainages, and pocosin swamps to pine flatwoods (Edwards, 2002; Pelton and Van Manen, 1997). Subtropical temperatures contribute to a lengthy growing season for mast-bearing plants. Sluggish streams, lakes and marshes are common due to a flat topography of < 90 meters. Vegetation associated with riparian corridors support a multitude of berry and nut producing species and often provides the sole, tenuous connection between bear habitat patches. Previous researchers have suggested that riparian corridors may require immediate conservation attention in order to maintain black bear population integrity (Pelton and Van Manen, 1997).

Anthropogenic presence in the MRB is significant. Black bears of the region share land with ~590,000 people (Mobile Area Chamber of Commerce, 2012). Habitats that are frequented by bears intermingle with and lay adjacent to large cities and towns, including: Mobile, Saraland, and Wagarville (Bentzien, 1998). The majority of forested lands are under industrial or private ownership, with only a small portion in the care of state or federal authorities. Management of private estates is dominated by timber, hunting, or agricultural concerns. Crop production in the area has a substantial economic impact, since Alabama ranks third in the nation for peanut production and tenth for cotton yields (Alabama Department of Agriculture, 2011).

Black bears of NAL inhabit the 'Mountain' physiographic region and reside on the Cumberland Plateau (southernmost section of the Appalachian Plateau; Figure 3). The study area was centered at 34°25'6" N and 85°35'48" W. Available habitat is dominated by eastern broadleaf forests, which are supported by a temperate climate yielding mild winters. The region is characterized by steep, dissecting slopes on either side of the plateau and topography ranging from 60 to 500 meters (Hersey et al., 2005).

In comparison to the MRB region, anthropogenic impacts in NAL are substantially less intense. Human populations of DeKalb and Cherokee counties amount to ~97,000 (United States Census Bureau Population Division, 2014) and potential bear habitat remains largely more contiguous than those of the Coastal Plain. Greater than 50% of the study area occurs on state or federal lands including: Talladega National Forest, Little River Canyon National Preserve, De Soto State Park, and Little River Wildlife Management Area (Figure 4). Sanctuaries such as these are strongly associated with stable bear populations throughout the southeast (Pelton and Van Manen, 1997). However, human presence is still identifiable and most evident at the research area boundaries in the form of large roadways, including: I-20 in the south and I-59 in the North. Remaining portions of the study area are under private ownership and maintained primarily for hunting purposes.

2.0 Methods

For this study, nuclear DNA was used to assess genetic diversity and differentiation between Alabama black bear populations. Non-invasive sampling was employed using hair snares and scat-detection dogs. Hair snares are low-cost and proven effective for study of elusive

carnivores (Gardner et al., 2010; Gompper et al., 2006; Mowat and Stobeck, 2000). However, use of hair snares often requires considerable effort to attain sufficient sample sizes. Therefore, scat detection canines were implemented in areas with known concentrations of black bears. Several tissue samples were opportunistically collected from bears killed by vehicle collisions.

2.1 Methods: Hair Snare Sampling

During the 2013 autumn season, hair snares were utilized for surveying the MRB region. Due to the capability of black bears to roam widely, we surveyed extensively throughout Mobile County. A systematic survey was conducted via a grid with each cell extending 8x8 kilometers (the approximate size of the average male bear home range in Alabama (Edwards, 2002); Figure 5). We attempted to place one hair snare in each cell. Not all landowners were cooperative; therefore, some grid cells could not be filled.

Hair snares were used to samples for bears in NAL during 2012 and 2013. In an attempt to confirm bear re-colonization, the 2012 surveying period was characterized as intense sampling in the vicinity of Little River National Preserve near Fort Payne, Alabama (Figure 6). This area is where the vast majority of anecdotal bear sightings had occurred during the years leading up to our study. In the fall of 2013, we spatially expanded efforts in NAL using a systematic survey (Figure 4). We attempted to place one hair snare within each grid cell of 8x8 kilometers. To maximize bear captures, exact snare locations within the cell were based on assumed habitat preferences.

Stations were constructed using 4-barbed, 2-strand wire at a height of 45 - 50 centimeters and encircling ≥3 trees (Long et al., 2008; Figure 7). Irregularities in the terrain were filled with woody debris to ensure consistent wire height. Precise location was determined using GPS. Six

trail cameras were placed at random stations to confirm snare effectiveness. If cub presence was suspected (via photos, tracks, scat, or anecdotal reports), then a second barbed wire was placed at a height of 20 - 25 centimeters.

A scent pile, consisting of logs and leaf litter, was arranged in the center of each enclosure and doused with a 50:50 mixture of mineral oil and vanilla or raspberry extract. The enclosure was additionally baited with cans of tuna that were suspended (> 2 meters) over the snare center. In order to appeal to the wide dietary preferences of black bears, commercial bear lure, road-kill carcasses, honey buns, Caven's Hiawatha Valley Predator Bait, jam / preservatives, and cat food were used intermittently as attractants when signs indicated a bear(s) was in the immediate area, but had not entered the enclosure. Stations were checked every 1 to 2 weeks and lure / bait refreshed. Hair snares were stationary throughout the study period.

Samples from individual barbs were collected via heat-sterilized tweezers and immersed in 100% ethanol or stored in coin envelopes with desiccant. Any barbs from which all hair could not be removed, were flame-sterilized to avoid contamination. Each sample was labeled with date, time, location, and cardinal directions of the wire strand on which it was collected, and an estimate of follicle number.

2.2 Methods: Scat-Detection Dog Sampling

Scat-detection dogs are canines trained to find scat from one or more target species of interest and have been used in natural resource management and conservation. Dogs are able to quickly cover large tracts of land with little additional effort from researchers (Wasser et al., 1997). Comparative studies suggest scat-detection dogs may increase sample sizes considerably over traditional techniques (Harrison, 2006; Long et al., 2007; Reindl-Thompson et al., 2006).

For instance, one study revealed that detector dogs located 56 confirmed bobcat scats while only 5 bobcats were detected using cameras and just one was identified with the use of hair snares (Harrison, 2006).

The application of scat-detection dogs was limited to the MRB region and permitted more intensive sampling, but had the drawback of limiting total area surveyed. During the autumn of 2011 and 2012, scat-detection dogs were used to survey portions of Baldwin, Mobile, Clarke, Washington, and Escambia counties. Detector dogs were active for 4 weeks during the autumn of each year. Teams of one scat-detection dog, a canine handler, and a biologist sampled a triangle-shaped transect of 1.5 kilometers in total length. The field biologist ensured the team remained on track with transect lines and collected samples. Handlers observed and directed the canines, which worked off-lead while moving back and forth across transects. This technique was adopted to ensure the greatest possible survey area (MacKay et al., 2008). Once scat was located, coordinates were recorded using GPS and two samples of 0.4 mL were removed from the driest portion of the scat (where DNA would have experienced minimum hydrolytic damage). Samples were stored in separate vials with 1.4 mL of 100% ethanol or a DMSO / EDTA / Tris / Salt solution (DETs). The remainder of scat was frozen (-20 degrees Celsius) and placed into storage (Wultsch et al., 2015).

2.3 Methods: Genotyping and Genetic Tagging

DNA was extracted from hair and scat using the appropriate Qiagen® QIAmp blood and tissue (hair) kit and stool (scat) kit (Qiagen Inc., Valencia, CA). Extractions were performed in a room dedicated to low quantity DNA samples and using one negative control per extraction. First, a species identification test was performed on scat samples using mitochondrial DNA

(mtDNA) as described in DeBarba et al 2014. This preliminary step had a two-fold purpose: 1) it determined whether DNA was of amplifiable quality and warranted further analysis; 2) confirmed species identification to ensure efforts were not wasted on non-target specimens. Mitochondrial DNA of hair and tissue samples was not assessed for species identification. Rather, hair and tissue samples were reliably identified as appropriate species by visual appearance and quality judged on the basis of hair follicle quantity. Tissue samples were considered positively identified as bear due to collection methods (e.g. from road side carcasses) and of superior quality in terms of nuclear DNA available for extraction.

Individual genotype was evaluated via microsatellite assessment. During the initial evaluation, a microsatellite multiplex consisting of 9 loci (MM1) was used, including: G10C, G10H, G10M, G10P, G10X, G1D, Mu15, Mu23, and a sex identification marker (SE 47+48; Appendix 1). Loci were selected according to previous research that revealed adequate variability and that loci were independent and unlinked within surrounding bear populations of Tennessee, Florida, and North Carolina (Boersen et al., 2003; Csiki et al., 2003; DeBarba and Waits, 2009; Miller et al., 1998; Paetkau et al., 1998a; Taberlet et al., 1997).

Amplification by polymerase chain reaction (PCR) for MM1 and MM2 had an initial denaturation period of 15 minutes at 95º Celsius. The initial denaturation was followed by 40 repetitions of a denaturing, annealing, and elongation cycle: 30 seconds at 95º Celsius, 1.5 minutes at 57-57º Celsius, and 1 minute at 72º Celsius respectively. The final elongation period lasted 30 minutes at 60º Celsuis. After amplification, samples were combined with 10 microliters of Formamide and 0.15 microliters of LIZ 500 dye. Assessment of markers was conducted on an Applied Biosystems 3130xL genetic analyzer. GeneMapper v3.1 was used to assess gels and identify microsatellite alleles of each sample (Applied Biosystems, Foster City, California). To

reduce occurrence of allelic drop-out, homozygous loci were confirmed with 3 PCR replicates. Heterozygous loci required 2 repeated assessments before they were accepted. Samples with accurate matching alleles at 7 or more loci were kept and used for additional analyses.

When chosen microsatellites lack the variability necessary to generate unique genotypes for each individual, then the number of sampled individuals is underestimated (Paetkau, 2003; Woods et al., 1999). To reduce occurrence of this error, probability of identity (PI) and the more rigorous P_{sib} equation, described in Waits et al 2001, was used to determine power of individual markers and genotypes. The P_{sib} equation calculates the probability that an individual shares the same genotype with a sibling bear and is the most conservative tool for describing power. Calculations were performed using GenAlEx v6.5 (Peakall and Smouse, 2012). The markers of MM1 yielded sufficient power for NAL population assessments; however, they were insufficient for accurate fingerprinting of MRB bears. A second multiplex (MM2) of six additional markers was required, including: G10B, D1A, G10L, Mu50, G10U, and GIA (Appendix 1). The application of MM1 and MM2 resulted in a total of 15 markers used to assess the MRB populations. Despite showing variability throughout testing in NAL, four markers (G10L, G10H, G10P, and D1A) were later discovered to be homozygotic in MRB bears (Appendix 2).

Another source of error generated by molecular techniques occurs when processing errors lead to multiple genotypes for a single individual. The effect of such errors is that abundance will be overestimated (Miller et al., 1998; Taberlet et al., 1996; Woods et al., 1999). Error rates of 5% per locus may bias genetic tagging results by > 200% (Waits and Leburg 2000, Roon et al., 2005). Stringent quality control protocols were used to avoid this inaccuracy and included: negatives for each PCR run, reanalyzing suspect genotypes (those of only 1 or 2 mismatched pairs), and manually assessing similar genotypes. Finally, the reliability of single capture

samples was also assessed using the maximum likelihood method as per Miller et al. (2002) in the program GenAlEx v6.5 (Peakall and Smouse, 2012). Samples with a reliability of < 0.95 were eliminated.

A population in Hardy Weinberg equilibrium (HWE) has allelic and genotypic frequencies remaining stable through time. HWE is violated when conditions such as mutation, migration, genetic drift, or non-random mating occurs (Gillespie, 1998). HWE of MRB and NAL groups was investigated using the HWE probability test with Markov Chain tecnhniques in the GenePop v4.2 program (Guo and Thompson, 1992; Raymond and Rousset, 1995).

2.4 Methods: Genetic Diversity of Alabama Populations

The most commonly used measure of genetic diversity is heterozygosity. This statistic describes the proportion of heterozygous, polymorphic loci within each population level, as well as the proportion of heterozygotes per locus. There are two measures of heterozygosity: 1) observed heterozygosity (Ho), which is the realized measure; and 2) expected heterozygosity (He), which is the expected proportions based on allelic frequencies (Allendorf and Luikart, 2007). When there is random mating, expected heterozygosity is a better standard for comparing relative amounts of variation among populations (Nei, 1977). Another measure of genetic diversity is the number of alleles (Na). Relative to heterozygosity, the number of alleles is sensitive to bottlenecks and may provide insight into a potentially re-colonizing front or severely isolated population. However, the number of alleles is strongly dependent on sample size, therefore, comparison of heterozygosity will be more meaningful in regards to comparisons of genetic diversity. Allelic frequencies were taken into consideration since measures of extreme frequency are a common effect of small population size and may indicate impact on overall

fitness (Frankham et. al, 2002).

Measures of diversity including heterozygosity, allelic frequencies, and average number of alleles per locus were assessed via the GenAlEx v6.5 program (Peakall and Smouse, 2012). Polymorphism was calculated and defined as a marker "for which the most common allele has a frequency of less than 0.95" (Hartl, 1988). Allelic richness (A) was also assessed since it allows for us to control for differences in sample size while making between population comparisons (Kalinowski, 2004). Allelic richness was determined using the statistical method of rarefaction as developed by Hurlbert (1971) in the HP-Rare program (Kalinowski, 2005).

The descriptive F-statistic is the most widely used metric to quantify genetic differentiation. This method uses a series of inbreeding coefficients (Fis, Fst, and Fit) to evaluate departures from HWE proportions. Fis expresses the degree of divergence between individuals of a subpopulation. Fst is the measure of divergence between populations (e.g. between MRB and NAL populations). Finally, Fit is global fit of HWE proportions within the entire surveyed sample (e.g. among all black bears of Alabama). Wright's F-statistics were utilized to investigate genetic differentiation at all hierarchical levels using the allele identity method in program GenePop v4.2 (Raymond and Rousset, 1995; Wright 1931). When subpopulation divergence is present due to differences in allele frequency proportions, then Fst is expected to be > 0. Single locus estimates follow standard ANOVA and multilocus estimates computed as in Weir and Cockerham (1984).

The number of private alleles is also an indicator of differentiation. A linear relationship exists between the number of unique alleles and gene flow such that: "The time during which a new allele remains private depends only on migrations rates, such that the proportion of alleles that are private decreases as migration rate increases" (Allendorf and Luikart, 2007). Private

allelic richness was calculated in the program HP-Rare (Kalinowski, 2005).

3.0 Results

3.1 Results: Collection of Samples

In the MRB region, the 2011 scat-detection dog surveying efforts yielded 95 possible bear scats. During the 2012 field season, 138 scats were collected. The majority of samples were found in high density slightly north of Saraland, Alabama (Figure 8). The remaining samples were located west of Wagarville and within the boundaries of Perdido River Wildlife Management area (Figure 8). In 2013, we erected and maintained 30 hair snare stations throughout Mobile County and were able to survey 1,920 square kilometers. A total of 30 samples were collected from 5 different cells of the systematic survey grid (Figure 5). Fifteen scat samples were opportunistically collected while erecting and maintaining hair sampling stations.

In the NAL area, a total of 47 hair snare stations were erected in the 2012 field season. Hair snare efforts yielded a total of 202 putative bear samples, collected from 15 separate stations. Images of bears were obtained from 8 snare stations (Figure 6). Forty-four separate photo events resulted in a total of 1,464 black bear pictures (single event defined as > 30 minute interval between bear photo captures). Most significantly, the photographic data yielded definitive evidence that black bears have repatriated NAL without anthropogenic assistance. Photographic evidence also confirmed reproduction among multiple breeding females. Through systematic surveys of the NAL area in 2013, we acquired 77 separate hair samples (Figure 4). Eight putative scat samples were opportunistically collected. A total of 565 genetic samples were

collected across Alabama, reflecting a survey effort that extended 4,416 square kilometers.

3.2 Results: MRB Genetic Analysis

Of the 271 samples collected from the MRB population, 120 were successfully genotyped (overall success rate of 44%) and represent 45 different individuals (Appendix 2). The ratio of female to male bears was 30:14. One individual was of unknown sex. Twenty-four genotypes were repeat-captures, averaging 4.125 repeats per individual. Two samples were collected from individuals killed in vehicle collisions. The remaining 19 genotypes were single-captures. The ratio of repeated versus single-captured individuals is not surprising due to the method of scat collection with detection dogs surveying large areas. Only one of the single-capture individuals (sample D101) required elimination due to poor reliability of 0.75.

To ensure adequate power of chosen loci, the PI and P_{sib} were assessed. When P_{sib} for all alleles combined was < 0.015, microsatellites were considered acceptable for genetic tagging purposes. P_{sib} was first considered utilizing the original 8 loci of MM1 (G10C, G10H, G10M, G10P, G10X, G1D, Mu15, Mu23, and excluding the sex marker; Appendix 2). However, the resulting PI = 2.9E-03 and P_{sib} = 0.062 were considered inadequate (Table 1). The MRB population was re-evaluated utilizing MM2 for a total of 15 loci. Sufficient power of MM1 and MM2 markers was confirmed with the new values of PI = 8.3E-05 and P_{sib} = 0.011 (Table 2).

Only 75% of markers were polymorphic in MRB individuals. The most powerful markers within MRB multiplexes were Mu15 and Mu50, each with P_{sib} = 0.50 (Table 2). The MRB population had no significant departures from HWE. Allele frequencies varied greatly (Figure 9) and were further analyzed across the MM1 loci to allow comparisons between MRB and NAL regions (excluding the sex locus SE47-48; Figure 10). The greatest number of alleles (Na) within

a single, polymorphic locus was 3 (observed at the G10C, G10M, and Mu15 loci; Table 3). The total number of alleles across MM1 loci was 17 in the MRB population. Observed heterozygosity at individual loci ranged from Ho = 0 at the G10P and G10H loci to Ho = 0.659 for the G10M marker (Table 3).

3.3 Results: NAL Genetic Analysis

Of the 294 samples collected from the NAL population, 161 were successfully genotyped (overall success rate of 55%) and represent 15 different individuals (Appendix 2). The ratio of female to male bears was 2:1 in the NAL region. All 15 genotypes were repeat-captures and averaged 10.73 repeats per individual. No samples required elimination due to poor reliability.

All 15 repeat-capture individuals were employed for assessment of marker power. Microsatellite power was adequate with the 8 loci of MM1 (PI = 6.5E-05 and P_{sib} = 0.013; Table 5). One hundred percent of microsatellites were polymorphic and ranged in power from P_{sib} = 0.93 (Mu23) as the weakest to P_{sib} = 0.43 (G10M) as the most powerful marker (Table 5). Within NAL, the G1D marker had the largest number of alleles with a total of 6. The total number of alleles across 8 MM1 loci in the NAL population was 32. Heterozygosity of loci ranged from Ho = 0.071 at Mu23 to Ho = 0.867 at both the G10M and Mu15 markers (Table 3). When assessed for HWE, four loci (G10C, G10H, G1D, and Mu15) showed significant departure ($P < 0.05$; Table 6). As a result, the NAL population, as a whole, departed from HWE (P = 0.0327).

3.4 Results: MRB and NAL Genetic Comparisons

In comparison to NAL, all measures of genetic diversity were extremely low for the

MRB individuals (Table 4). The average number of alleles per locus in the MRB population was Na = 2.125 ± 0.295 compared to Na = 4.000 ± 0.463 in NAL. When effects of sample size were taken into consideration through allelic richness, the MRB population continued to reflect low genetic diversity. The allelic richness of the MRB population averaged 1.89 across all loci compared to NAL, which averaged 2.69 across all loci. The expected heterozygosity and observed heterozygosity were substantially lower in MRB (He = 0.320 ± 0.089; Ho = 0.355 ± 0.098) than NAL (He = 0.484 ± 0.070; Ho = 0.569 ± 0.092). Additional analyses of MRB samples were made that included all 15 loci across MM1 and MM2. Observed global heterozygosity resulting from the more extensive assessment revealed even less variability with He = 0.278 ± 0.061 and Ho = 0.289 ± 0.064 (Table 7). Allelic patterns across regions strongly demonstrate a disparity in genetic diversity (Figure 11).

Wright's F-statistics were used to evaluate structure of black bear populations. The overall Fit within MRB and NAL groups was Fit = 0.5257. Strong divergence was present between populations and ranged by locus from Fst = 0.2259 at Mu15 to Fst = 0.8422 at G10P. The overall Fst between MRB and NAL populations was high at Fst = 0.5750. Little divergence was present at the individual level and Fis was equivalent to 0 across all loci. The global averaged Fis = -0.1159 (Table 8). The number of private alleles in the MRB population is 9 while NAL has 24 unique ones (mean frequency of 0.294). When controlled for sample size, the private allelic richness averaged 1.26 across all MRB loci and 2.06 across all NAL loci.

4.0 Discussion

Results support the historical information and founding hypothesis of low genetic

variability among MRB black bears. Low diversity was evident as early as the genotyping phase of methodology in which the MRB region lacked sufficient power for genetic tagging with an eight marker multiplex (MM1). Then, despite showing variability through testing with NAL samples, four markers from MM2 (G10L, G10H, G10P, and D1A) were homozygotic across MRB individuals. Consequently, only 75% of loci were polymorphic in our analyses.

The expected and observed measures of MRB heterozygosity (He = 0.278; Ho = 0.289) are the second lowest for studied bear species and populations in the literature (Appendix 4). The only population exhibiting lower genetic diversity is negligible in its difference (when assessed with 12 markers, the Chassahowitzka population located in Florida exhibited Ho = 0.287 and He = 0.217; Dixon, 2004). Furthermore, amongst all bear species and populations in literature, MRB displayed the lowest average number of alleles per locus (Na = 2.125). This markedly low MRB diversity was confirmed by controlling for sample size with the measurement of allelic richness (1.89). This suggests the population is extremely isolated with little to no gene flow. Seclusion from other populations and the small number of individuals may force mating to occur between closely related bears, placing the entire population at risk for inbreeding and an extinction vortex. Inbreeding occurs when closely related individuals mate and is a consequence of small population size leading to degradation of genetic variation. Symptoms such as prolapsed rectums, undescended testes, and lack of a tail have previously been documented amongst MRB bears and are indicative of severe inbreeding (Kasbohm et al., 1994).

Given the low genetic diversity estimated in this study, the long term viability of the MRB population is questionable. Management should focus on maintaining and creating corridors; thereby encouraging inter-population connectivity to maintain and increase genetic diversity. The population centered at Eglin Air Force Base (~95 km direct distance) is the nearest

from the Florida subspecies. Since black bears have been known to traverse distances of 800+ kilometers, a migration of this size is not insurmountable. However, considerations should be made for both natural and anthropogenic barriers (e.g. highways, farm lands, and major population centers) in the MRB region. Maintaining waterway vegetation for riparian corridors, wildlife crossings installed at major roadways, and legislation for lawful protection would be beneficial. If future monitoring reveals continued decline of genetic diversity, more intense management (i.e. translocation of individuals from neighboring populations) might require consideration as well.

The most substantial finding in the NAL region was confirmation of a self-perpetuating group of adult males and females, adolescents, and juveniles. The NAL population, confirmed with camera trap and genetic information, represents a re-colonizing front. This study is the first time that black bears have been documented in the area in > 100 years (Howell, 1921). The genetic diversity is greater for NAL as observed by heterozygosity (Ho = 0.569) and average number of alleles per locus (approximately twice as large as that of MRB (Na = 4.000 versus Na = 2.125). Evidence of such high relative genetic diversity suggests a high number of recent migrants. However, there is a possibility of founder effects occurring in future generations due to the non-random sampling from the original population and the extremely small number of individuals. Small population means genetic drift is extremely likely to occur, but inbreeding is not yet apparent with such high degrees of heterozygosity and number of alleles, therefore NAL population is most likely extremely young.

Departure from Hardy Weinberg equilibrium for the NAL population is also symptomatic of a re-colonizing front. The five assumptions of the Hardy Weinberg principle include: random mating of individuals and a lack of all mutations, genetic migrations, genetic drift, and natural

selection. Since the NAL population departs from equilibrium, one or more of these assumptions are being violated. A re-colonizing front would be expected to have both substantial migration from the original population and a high chance of genetic drift. Thus, we are not surprised to discover that half of the loci were not in Hardy Weinberg proportions. Since the effects of genetic drift are inversely proportionate to population size, the NAL population is at great risk for inbreeding in the future should gene flow from the source population cease. Continued monitoring of the population is imperative.

Divergence between subpopulations (or any predefined structure level) refers to a limited amount of dispersal and gene flow, leading to genetic drift and changes to allelic frequencies. The MRB and NAL populations have strong divergence with Fst = 0.5750. The Fst result reinforces our hypothesis of highly distinct populations, most likely consisting of separate subspecies (*Ursus americanus americanus* in NAL and *Ursus americanus floridanus* in MRB).

In conclusion, the greatest concern for black bear populations of Alabama is loss of genetic variability. This loss is already apparent within the MRB region there is evidence of potential inbreeding in genetic data as well as morphological deformities. In order to avoid local extinction, substantial management is needed to monitor, maintain, and increase the levels of current genetic diversity. While there is ample diversity among NAL individuals, they remain at high risk of inbreeding due to suspected low population numbers and genetic drift resulting from founders' effect. Continued observation of these tentative populations is vital.

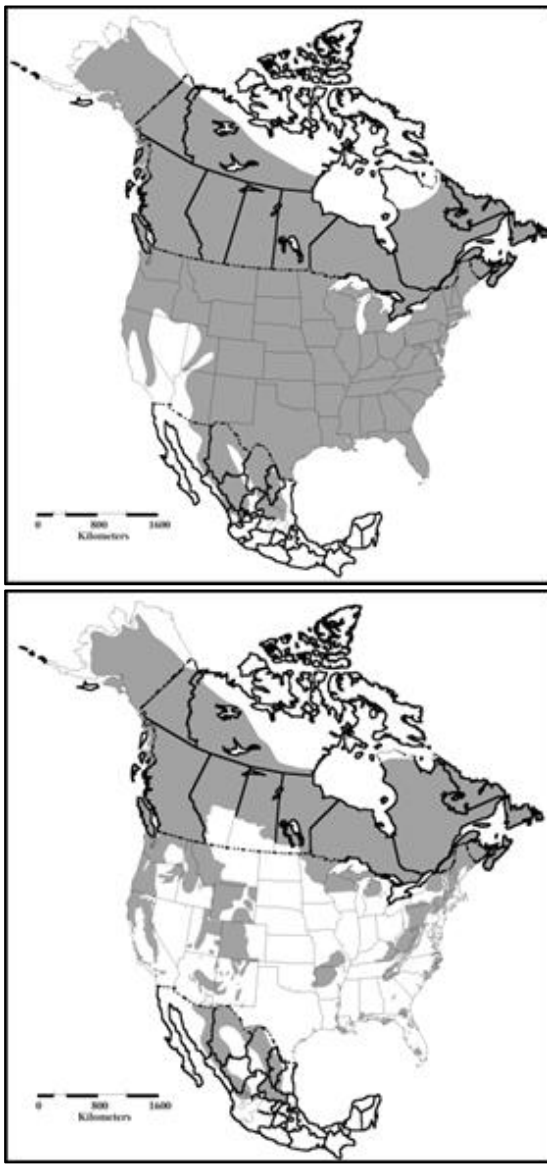

Figure 1: Historic (top) and the current (bottom) distribution of black bears in North America (Pelton and van Manen, 1997).

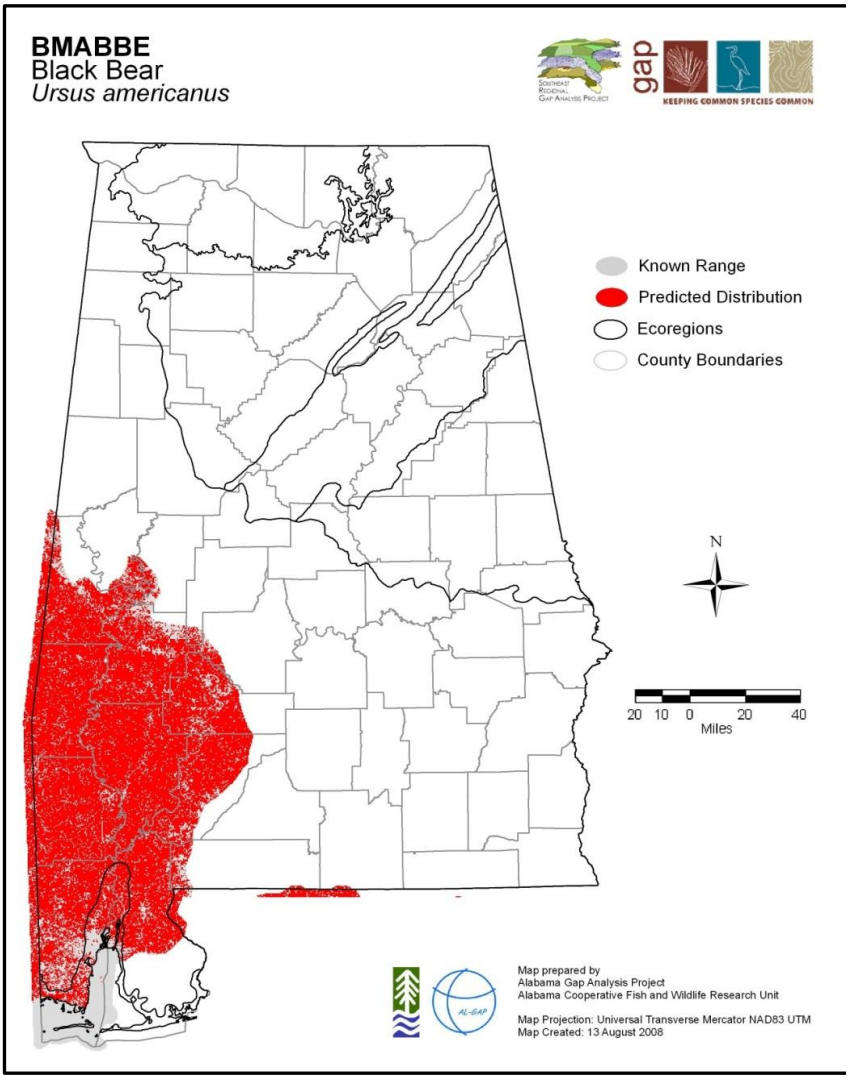

Figure 2: Known Alabama black bear range at the start of research in 2011 (Silvano et al., 2007)

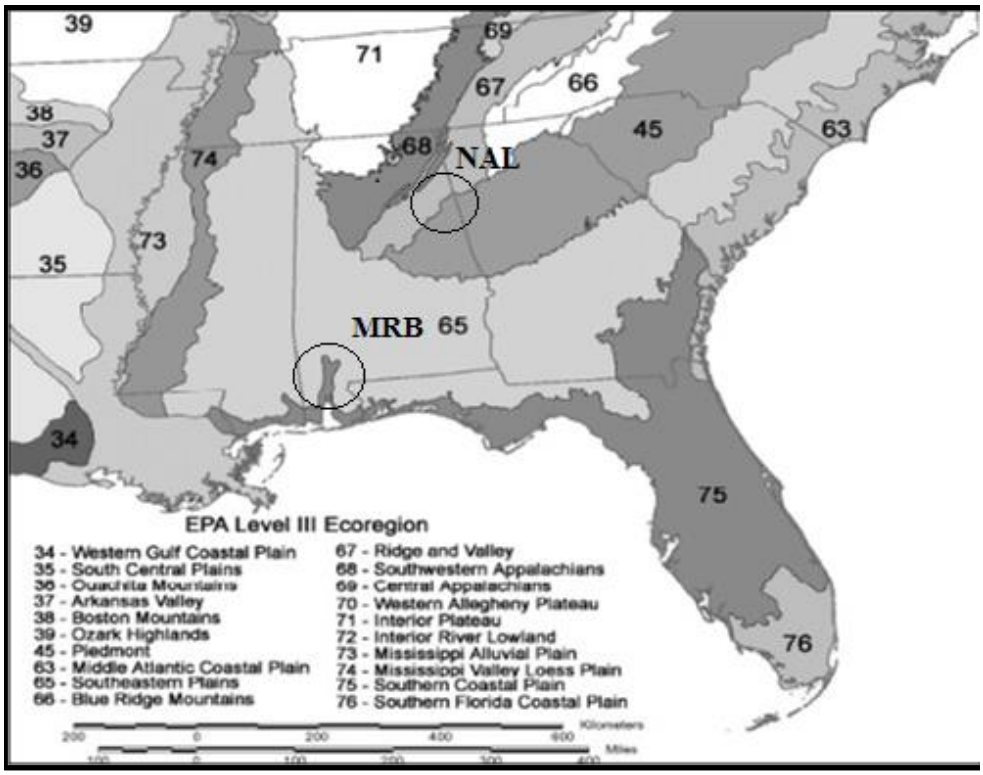

Figure 3: Ecoregions of the southeastern United States (Sohl and Sayler, 2008).

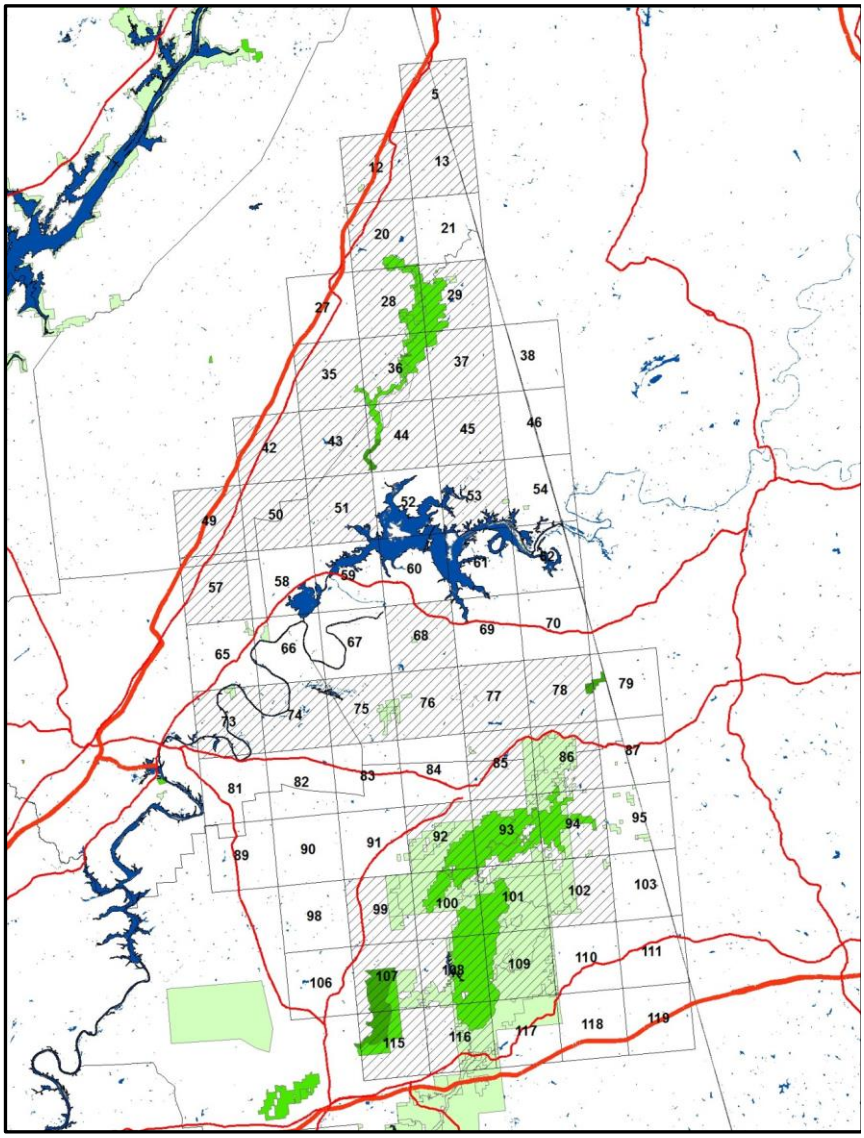

Figure 4: Expanded hair snare study area for NAL (2013) and associated systematic surveying grid (each cell is 8x8 km). Areas highlighted in green are state and federal properties. Grid cells with hash marks indicate where stations were erected and maintained (39 cells, surveying a total of 2,496 square kilometers). Samples were collected from cells 13, 20, 28, 29, 57, 68, 93, 94, 101, and 109.

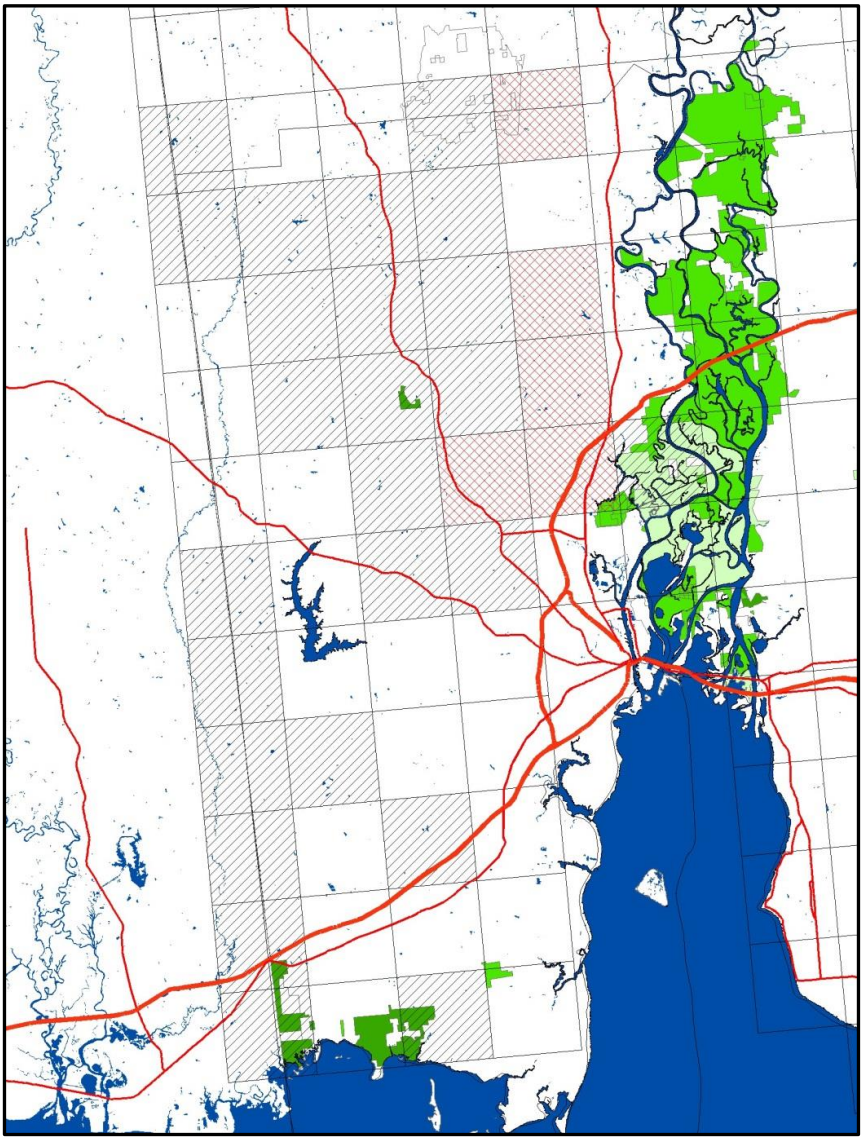

Figure 5: Locations of MRB hair snares and collected samples (both hair and scat) of 2013. Single hash (black) cells indicate that a hair snare station was set-up and maintained. Double hash marks (red) indicate samples were collected from grid cell.

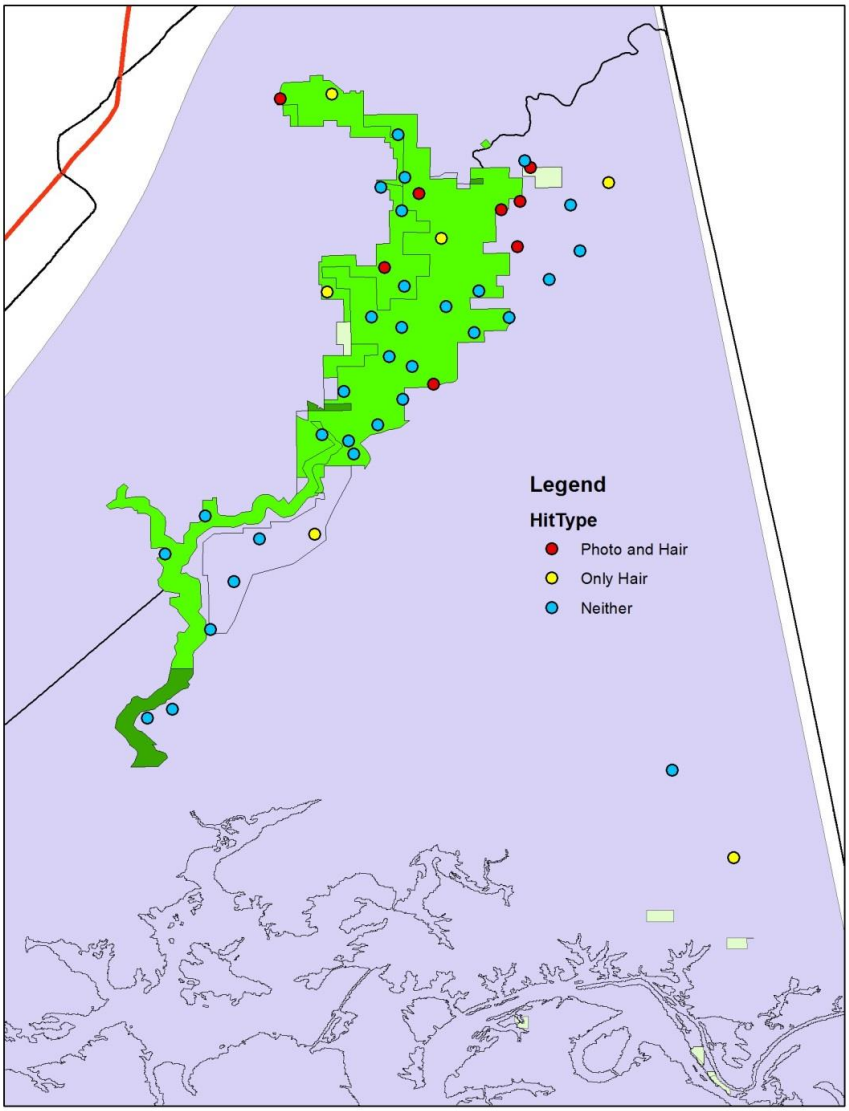

Figure 6: Locations of the 2012 hair snare stations in NAL. Red dots indicate that black bear photos and hair was collected from the station. Yellow dots indicate only hair was collected from the station. Blue dots indicate a hair snare was erected and maintained, but no hair or pictures were collected.

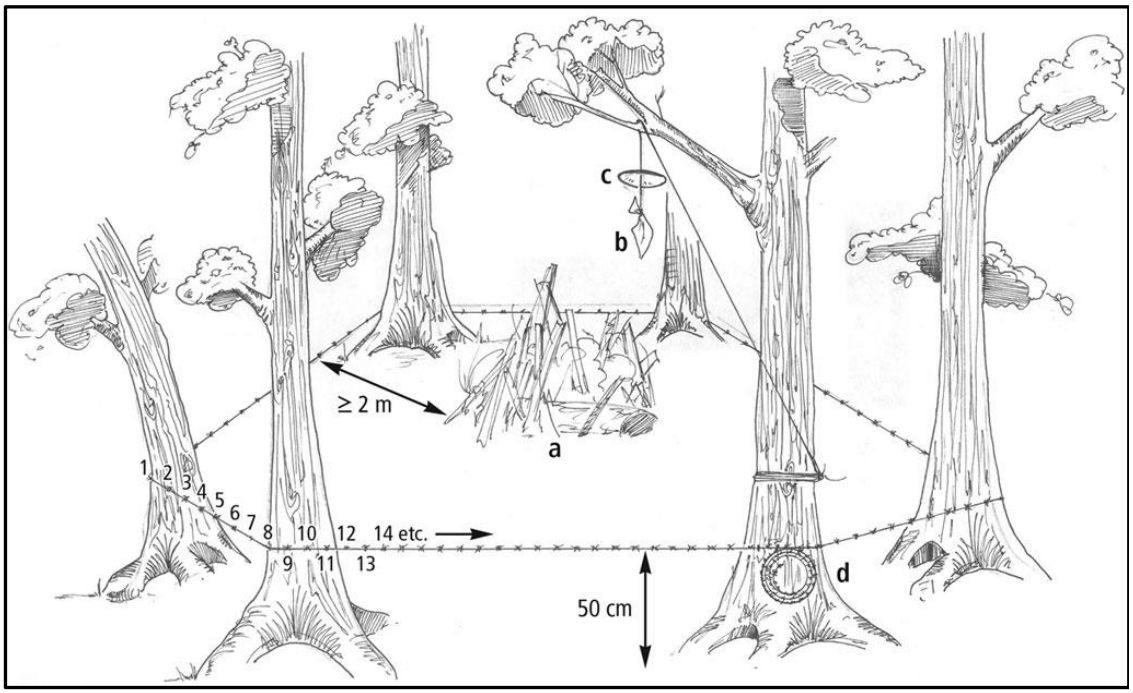

Figure 7: Hair snare station set-up for northeastern Alabama: (a) centrally located scent pile, (b) suspended lure, (c) optional rain protection / visual lure, (d) double-stranded, four-barbed wire at a height of 45 – 50 cm (Long et al., 2008).

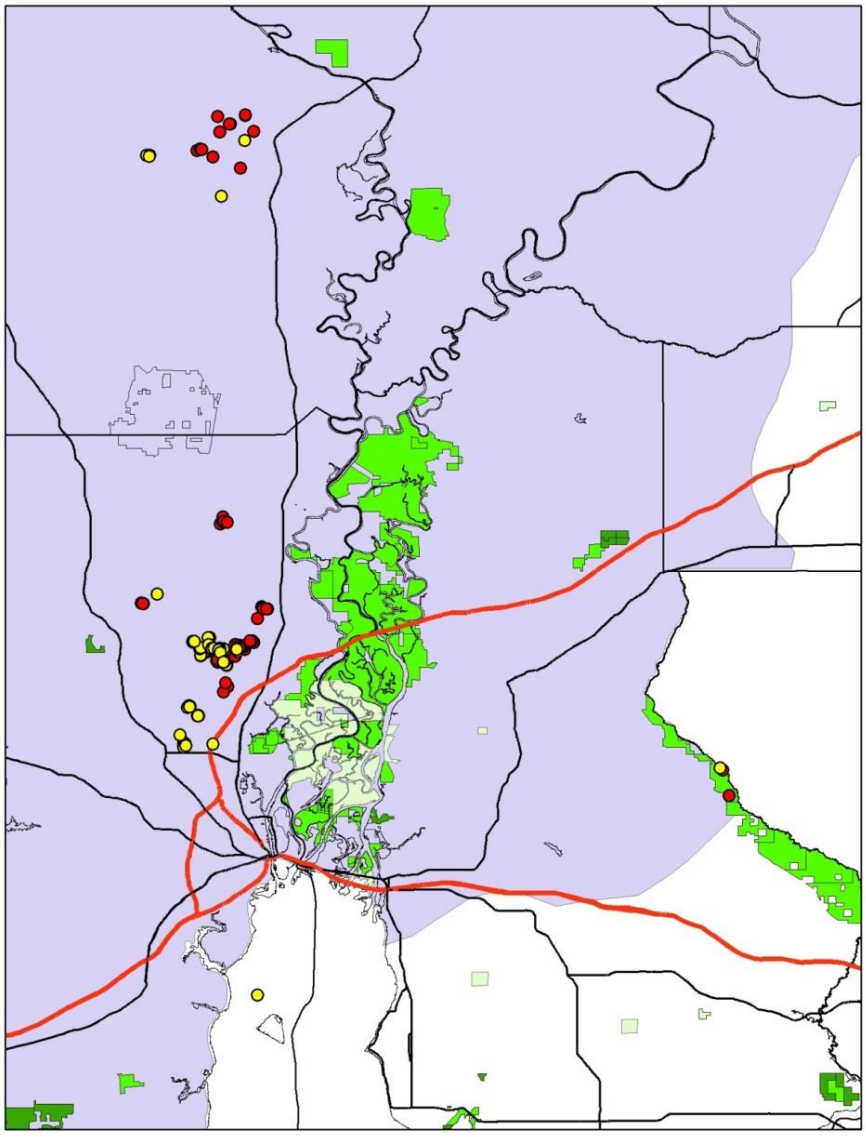

Figure 8: Locations of scat collected during the 2011 (indicated by red dots) and 2012 (indicated by yellow dots) field seasons in the MRB region. Portions of Mobile, Bladwin, Clarke, Washington and Escambia counties were surveyed.

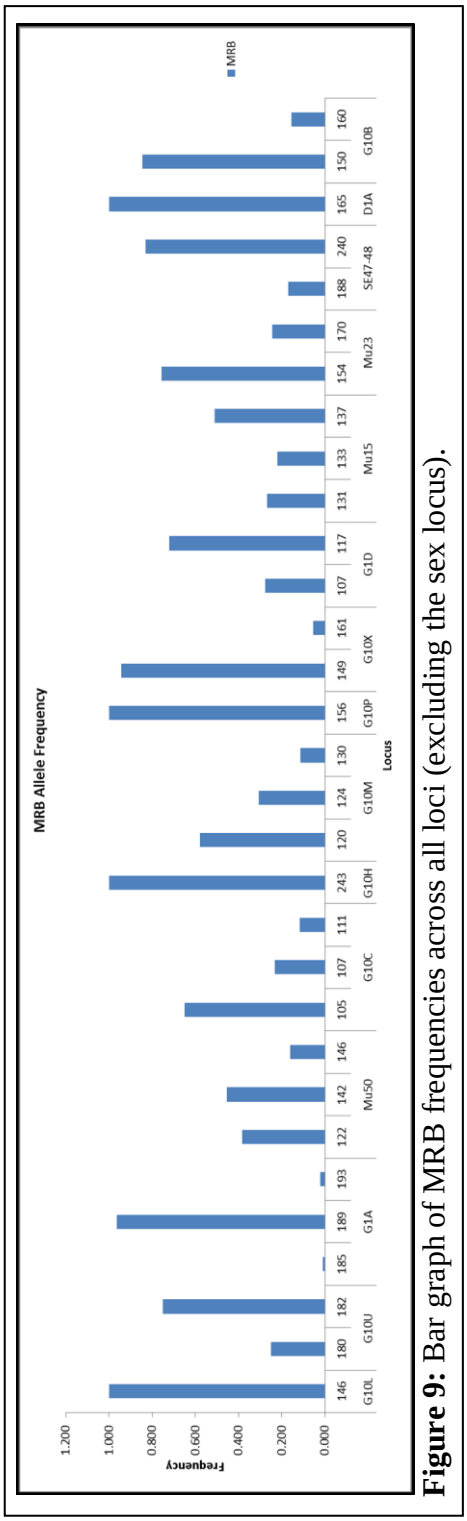

Figure 9: Bar graph of MRB frequencies across all loci (excluding the sex locus).

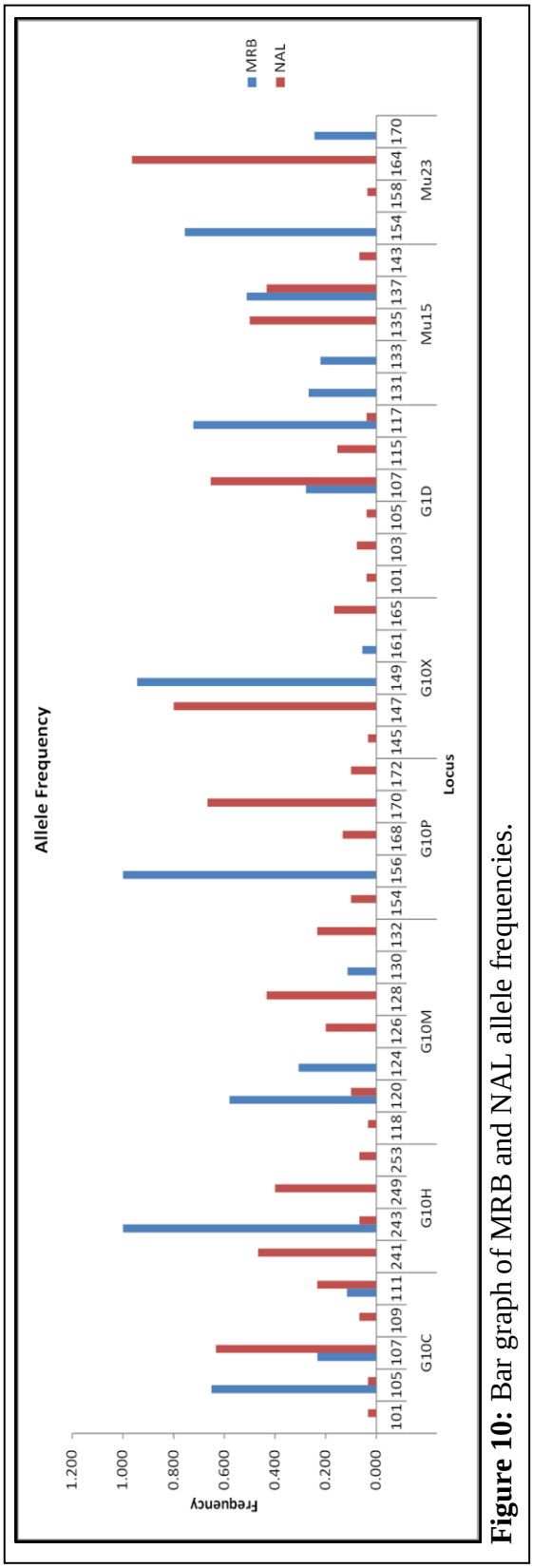

Figure 10: Bar graph of MRB and NAL allele frequencies.

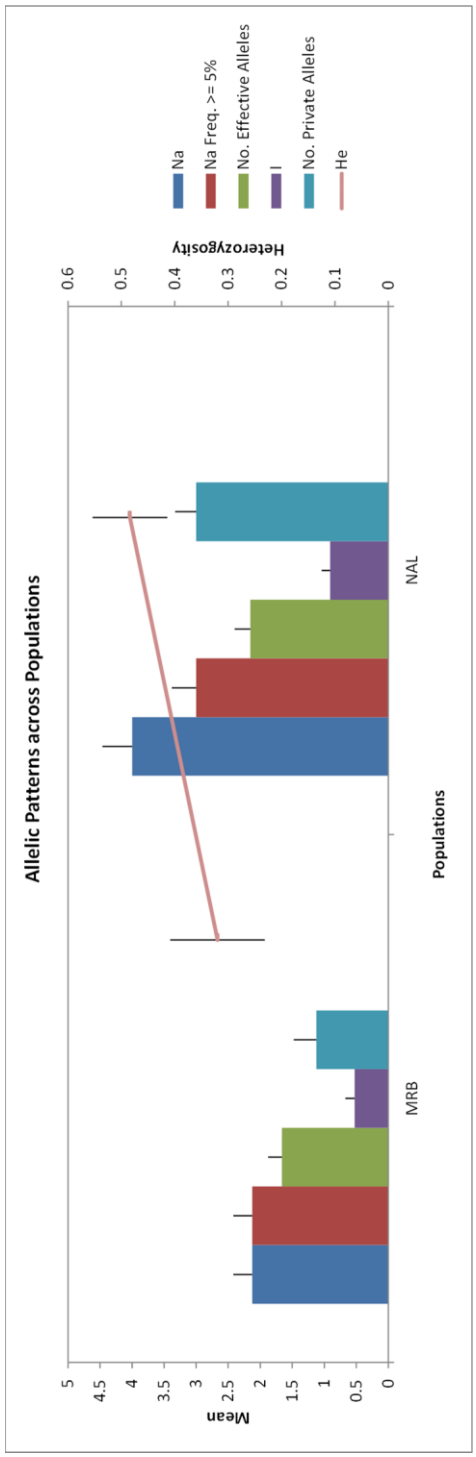

Figure 11: Allelic patterns across MRB and NAL (based off the values of table 9).

PI by Locus									
Pop	**N**	**G10C**	**G10H**	**G10M**	**G10P**	**G10X**	**G1D**	**Mu15**	**Mu23**
MRB	45	0.300	1.000	0.271	1.000	0.807	0.439	0.216	0.466
Psib by Locus									
MRB	45	0.571	1.000	0.539	1.000	0.899	0.659	0.495	0.682
PI MRB	0.003								
Psib MRB	0.062								

Table 1: MRB probability of identification (PI) and probability of differentiation between sibling individuals (Psib) per each locus of MM1.

PI by Locus																
Pop	**N**	**G10L**	**G10U**	**G1A**	**Mu50**	**G10C**	**G10H**	**G10M**	**G10P**	**G10X**	**G1D**	**Mu15**	**Mu23**	**SE47-48**	**D1A**	**G10B**
MRB	45	1.000	0.461	0.870	0.223	0.300	1.000	0.271	1.000	0.807	0.439	0.216	0.466	0.554	1.000	0.578
Psib by Locus																
MRB	45	1.000	0.678	0.934	0.495	0.571	1.000	0.539	1.000	0.899	0.659	0.495	0.682	0.747	1.000	0.763
PI MRB	8.314E-05															
Psib MRB	1.085E-02															

Table 2: MRB probability of identification (PI) and probability of differentiation between sibling individuals (Psib) with per each locus of MM1 and MM2.

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
MRB	**G10C**	43	3.000	2.034	0.869	0.558	0.508	0.514	-0.098
	G10H	42	1.000	1.000	0.000	0.000	0.000	0.000	N/A
	G10M	44	3.000	2.258	0.926	0.659	0.557	0.563	-0.183
	G10P	42	1.000	1.000	0.000	0.000	0.000	0.000	N/A
	G10X	45	2.000	1.117	0.215	0.111	0.105	0.106	-0.059
	G1D	36	2.000	1.670	0.591	0.444	0.401	0.407	-0.108
	Mu15	43	3.000	2.617	1.029	0.628	0.618	0.625	-0.016
	Mu23	43	2.000	1.585	0.556	0.442	0.369	0.373	-0.197
NAL	**G10C**	15	5.000	2.163	1.036	0.467	0.538	0.556	0.132
	G10H	15	4.000	2.586	1.083	0.667	0.613	0.634	-0.087
	G10M	15	5.000	3.409	1.367	0.867	0.707	0.731	-0.226
	G10P	15	4.000	2.074	0.999	0.600	0.518	0.536	-0.159
	G10X	15	3.000	1.495	0.591	0.400	0.331	0.343	-0.208
	G1D	13	6.000	2.167	1.139	0.615	0.538	0.560	-0.143
	Mu15	15	3.000	2.261	0.889	0.867	0.558	0.577	-0.554
	Mu23	14	2.000	1.074	0.154	0.071	0.069	0.071	-0.037

Table 3: MRB and NAL quantitative description of genetic diversity by locus (N = number of samples, Na = number of alleles, Ne = number effective alleles, I = Shannon's diversity index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased heterozygosity, and F = inbreeding coefficient).

		N	Na	Ne	I	Ho	He	uHe	F	A
MRB	**Mean**	42.250	2.125	1.660	0.523	0.355	0.320	0.324	-0.110	1.890
	SE	0.959	0.295	0.215	0.146	0.098	0.089	0.090	0.025	
NAL	**Mean**	14.625	4.000	2.154	0.907	0.569	0.484	0.501	-0.160	2.690
	SE	0.263	0.463	0.246	0.133	0.092	0.070	0.072	0.069	

Table 4: MRB and NAL quantitative description of genetic diversity by population (N = number of samples, Na = number of alleles, Ne = number effective alleles, I = Shannon's diversity index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased heterozygosity, and F = inbreeding coefficient), A = allelic richness.

PI by Locus									
Pop	**N**	**G10C**	**G10H**	**G10M**	**G10P**	**G10X**	**G1D**	**Mu15**	**Mu23**
NAL	15	2.6E-01	2.3E-01	1.3E-01	2.7E-01	4.8E-01	2.4E-01	2.9E-01	8.7E-01
Psib by Locus									
NAL	15	5.5E-01	5.0E-01	4.3E-01	5.6E-01	7.1E-01	5.4E-01	5.4E-01	9.3E-01
PI NAL	6.3E-05								
Psib NAL	1.3E-02								

Table 5: NAL probability of identification (PI) and probability of differentiation between sibling individuals (Psib) with 8 locus microsatellite multiplex.

Locus	P Value	SE
G10C	0.017	0.0029
G10H	0.029	0.0026
G10M	0.431	0.0101
G10P	1.000	0
G10X	1.000	0
G1D	0.410	0.0189
Mu15	0.038	0.0019
Mu23	NA	NA

Table 6: NAL Hardy Weinberg equilibrium by locus.

Pop		N	Na	Ne	I	Ho	He	uHe	F
MRB	Mean	42.867	2.067	1.556	0.455	0.289	0.278	0.281	0.237
	SE	0.576	0.206	0.151	0.099	0.064	0.061	0.062	0.035

Table 7: MRB measures of genetic diversity when assessed with 15 markers of multiplex 1 and multiplex two (N = number of samples, Na = number of alleles, Ne = number effective alleles, I = Shannon's diversity index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased heterozygosity, and F = inbreeding coefficient; Appendix 3).

Locus	Fis	Fst	Fit
G10C	-0.0123	0.3477	0.3397
G10H	-0.0695	0.7945	0.7802
G10M	-0.1829	0.3292	0.2064
G10P	-0.1416	0.8446	0.8225
G10X	-0.1182	0.8287	0.8084
G1D	-0.0986	0.4103	0.3522
Mu15	-0.1368	0.2265	0.1206
Mu23	-0.1706	0.7211	0.6736
All	-0.1177	0.5764	0.5265

Table 8: MRB and NAL locus and global Wright's F-statistics.

5.0 References

Alabama Department of Agriculture. 2011. "Alabama Agricultural Statistics." *United States Department of Agriculture National Agricultural Statistics Service*.

Allendorf, F. W. and G. Luikart. 2007. *Conservation and the Genetics of Populations*. Malden, Maine: Blackwell Publishing.

Bentzien, Michael M. 1998. "Endangered and Threatened Wildlife and Plants: New 12-Month Finding for a Petition to List the Florida Black Bear." *United States Fish and Wildlife Service* 63.

Boersen, M. R., J. D. Clark, and T. L. King. 2003. "Estimating Black Bear Population Density and Genetic Diversity at Tensas River, Louisiana Using Microsatellite DNA Markers." *Wildlife Society Bulletin* 31 (1): 197–207.

Csiki, Ildiki, Cynthia Lam, Audie Key, Erica Coulter, Joseph D. Clark, Richard M. Pace III, Kimberly G. Smith and Douglas D. Rhoads. 2003. "Genetic Variation in Black Bears in Arkansas and Louisiana Using Microsatellite DNA Markers." *Journal of Mammalogy* 84 (2): 691–701.

De Barba, M., and L.P Waits. 2009. Multiplex pre-amplification for noninvasive genetic sampling: is the extra effort worth it? Molecular Ecology Resources, 10 (4): 659-665. doi:10.1111/j.1755-0998.2009.02818..x.

Dixon, J. D. 2004. "Thesis: Conservation Genetics of the Florida Black Bear. "Master's Thesis, Gainesville, Florida: University of Florida.

Dobey, S.T. 2002. "Abundance and Density of Florida Black Bears in the Okefenokee National Wildlife Refuge and Osceola National Forest." Master's Thesis, Knoxville, Tennessee: University of Tennessee.

Edwards, A. S. 2002. "Status of the Black Bear in Southwestern Alabama." Knoxville, Tennessee: University of Tennessee.

Frankham, R., D.A. Briscoe, and J.D. Ballou. 2002. *Introduction to Conservation Genetics*. New York, New York, USA: Cambridge University Press.

Gardner, Beth, J. Andrew Royle, Michael T. Wegan, Raymond E. Rainbolt, and Paul D. Curtis. 2010. "Estimating Black Bear Density Using DNA Data From Hair Snares." *Journal of Wildlife Management* 74 (2): 318–25. doi:10.2193/2009-101.

Glaubitz, J.C. 2004. "Convert: A User-Friendly Program to Reformat Diploid Genotypic Data for Commonly Used Population Genetic Software Packages." *Molecular Ecology Notes*, no. 4: 309–210.

Gillespie, J.H. 1998. *Population Genetics: A Concise Guide*. Baltimore, MD: The John Hopkins University Press.

Gompper, Matthew E., Roland W. Kays, Justina C. Ray, Scott D. LaPoint, Daniel A. Bogan, and Jason R. Cryan. 2006. "A Comparison of Noninvasive Techniques to Survey Carnivore Communities in Northeastern North America." *Wildlife Society Bulletin* 34 (4): 1142–51. doi:10.2193/0091-7648(2006)34[1142:ACONTT]2.0.CO;2.

Guo, S. W. & Thompson, E. A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361-372.

Harrison, Tobert L. 2006. "A Comparison of Survey Methods for Detecting Bobcats." *Wildlife Society Bulletin* 34 (2): 548–52. doi:10.2193/00917648(2006)34[548:ACOSMF]2.0.CO2.

Hartl, Daniel, L. 1988. *A Primer of Population Genetics*. 2nd ed. Sinauer Associates, Incorporated.

Hersey, K.R., A.S. Edwards, and J.D. Clark. 2005. "Assessing American Black Bear Habitat in the Mobile-Tensaw Delta of Southwestern Alabama." *Ursus* 16 (2): 245–54. doi:10.2192/1537-6176(2005)016[0245:AABBHI]2.0.CO;2.

Howell, Arthur Holmes. 1921. *A Biological Survey of Alabama*. Washington Government Printing Office.

Hurlbert, S. H. 1971. "The nonconcept of species diversity: a critique and alternative parameters." *Ecology* 52: 577-586.

Kalinowski, S.T. 2004. "Counting alleles with rarefaction: private alleles and hierarchical sampling designs." *Conservation Genetics* 5:539-543.

Kalinowski, S.T. 2005. "HP-Rare: a computer program for performing rarefaction on measures of allelic diversity." *Molecular Ecology Notes* 5:187-189.

Kasbohm, J.W., D.A. Miller, and M.R. Vaughan. 1994. *Taxonomy of Black Bears in the Southeastern United States*. Second Annual Report to the U.S. Fish and Wildlife Service. Blacksburg, Virginia, USA: Virginia Polytechnical and State University.

Long, R.A., P. MacKay, William J. Zielinski, and Justina C. Ray. 2008. *Noninvasive Survey Methods for Carnivores*. 1st ed. Washington, DC.: Island Press.

——, Robert A., Therese M. Donovan, Paula Mackay, William J. Zielinski, and Jeffrey S.

Buzas. 2007. “Comparing Scat Detection Dogs, Cameras, and Hair Snares for Surveying Carnivores.” *Journal of Wildlife Management* 71 (6): 2018–25. doi:10.2193/2006-292.

Lu, Z., W.E. Johnson, M. Menotti-Raymond, N. Yuhki, J.S. Martenson, S. Mainka, H. Shi-Qiang, et al. 2001. “Patterns of Genetic Diversity in Remaining Giant Panda Populations.” *Conservation Biology*, no. 15: 1596–1607.

MacKay, P., D.A. Smith, R.A. Long, and M. Parker. 2008. *Noninvasive Survey Methods for Carnivores*. Washington, DC: Island Press.

Marshall, H. Dawn, and K. Ritland. 2002. “Genetic Diversity and Differentiation of Kermode Bear Populations.” *Molecular Ecology*, no. 11: 685–97.

Miller, C.R., Joyce, P., and Waits, J.L. 2002. “Assessing Allelic Dropout and Genotype Reliability Using Maximum Likelihood.” *Genetics* 160: 357–66.

Miller, Daniel A., Eric M. Hallerman, Michael R. Vaughan, and Kasbohm, John W. 1998. “Genetic Variation in the Black Bear Populations from Louisiana and Arkansas; Examining the Potential Influence of Reintroductions from Minnesota.” *Ursus* 10: 335–41.

Mobile Area Chamber of Commerce. 2012. “An Economic Overview of the Mobile Bay Region.” http://mobilechamber.com/regionaloverview.pdf.

Mowat, Garth, and Strobeck, Curtis. 2000. “Estimating Population Size of Grizzly Bears Using Hair Capture, DNA Profiling, and Mark-Recapture Analysis.” *The Journal of Wildlife Management* 64 (1): 183–93.

Nei, M. 1977. “F-statistics and analysis of gene diversity in subdivided populations.” *Annals of Human Genetics* 41: 225–233.

Paetkau, D. 2003. “An Empirical Exploration of Data Quality in DNA-Based Population Inventories.” *Molecular Ecology* 12 (6): 1375–87.

———, D., E.W. Born Amstrup, W. Calvert, A.E. Derocher, F. Messier Garner, I. Stirling, O. Wigg Taylor, and C. Strobeck. 1999. “Genetic Structure of the World’s Polar Bear Populations.” *Molecular Ecology* 8: 1571–84.

———, D., and C. Strobeck. 1994. “Microsatellite Analysis of Genetic Variation in Black Bear Populations.” *Molecular Ecology*, no. 3: 489–95.

———, David, and Strobeck, Curtis. 1998a. “Ecological Genetic Studies of Bears Using Microsatellite Analysis.” *Ursus (International Association for Bear Research and Management)* 10: 299–306.

———, D., G. F. Shields, and C. Strobeck. 1998b. "Gene Flow between Insular, Coastal and Interior Populations of Brown Bears in Alaska." *Molecular Ecology* 7 (10): 1283–92. doi:10.1046/j.1365-294x.1998.00440.x.

Peakall, R., and Smouse, P.E. 2012. "GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research - an Update." *Bioinformatics* 28: 2537–39.

Pelton, Micheal R., and Frank T. van Manen. 1997. "Status of Black Bears in the Southeastern United States." In *Proceedings of the Second International Symposium on the Trade of Bear Parts.*

Raymond, and Rousset. 1995. "GENEPOP (version 4.2): Population Genetics Software for Exact Tests and Ecumenicism." *Journal of Heredity*, no. 86: 248–49.

Reindl-Thompson, Sara A., John A. Shivik, Alice Whitelaw, Aimee Hurt, Kenneth F. Huggins, and McCleery. 2006. "Efficacy of Scent Dogs in Detecting Black-Footed Ferrets at a Reintroduction Site in South Dakota." *Wildlife Society Bulletin* 34 (5): 1435–39. doi:10.2193/0091-7648(2006)34[1435:EOSDID]2.0.CO;2.

Roon, D.A., L.P. Waits, and K.C. Kendall. 2005. "A Simulation Test of the Effectiveness of Several Methods for Error-Checking Non-Invasive Genetic Data." *Animal Conservation* 8: 203–2015.

Ruiz-Garcia, M. 2003. "Molecular Population Genetic Analysis of the Spectacled Bear (Tremarctos Ornatus) in the Northern Andean Area." *Hereditas*, no. 138: 81–93.

Saitoh, T.Y., H. Kanamori Ishibashi, and E. Kitahara. 2001. "Genetic Status of Fragmented Populations of the Asian Black Bear (Ursus Thibetanus) in Western Japan." *Population Ecology* 43 (3): 221–27.

Silvano, A. L., J. B. Grand, E. R. Irwin, K. J. Kleiner, M. D. Mackenzie, M. S. Mitchell, K. Cook, M. J. Elliot, E. A. Kramer, A. J. McKerrow, M. J. Rubino, S. Smith, and S. G. Williams. 2007. Black Bear Provisional Predicted Habitat Distribution Map of Alabama. Alabama Gap Analysis Project. Accessed January 1, 2013 from www.auburn.edu\gap.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L.P. Waits, and J. Bouvet. 1996. "Reliable Genotyping of Samples with Very Low DNA Quantities Using PCR." *Nucleic Acids Research* 24 (16): 3189–94. doi:10.1093/nar/24.16.3189.

——— , P., J.J. Camarra, S. Griffen, E. Uhres, O. Hanotte, L.P. Waits, C. Dubois-Paganon, T. Burke, and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology. 6: 869–876.

Thompson, L.M. 2003. "Abundance and Genetic Structure of Two Black Bear Populations prior to Highway Construction in Eastern North Carolina." Master's Thesis, Knoxville, Tennessee: University of Tennessee.

United States Fish and Wildlife Service Southeast Region. 2014. "Louisiana Black Bear (*Ursus americanus lutelos*) 5-Year Review: Summary and Evaluation." Louisiana Ecological Services Field Office. Accessed January 2, 2016 from http://ecos.fws.gov/docs/five_ year_ review/doc4348.pdf.

United States Census Bureau, Population Division. 2014. "Annual Estimates of the Resident Population: April 1, 2010 to July 1, 2014". Accessed January 2, 2016 from http://factfinder.census.gov/faces/tableservices/jsf/pages/productview.xhtml?src =bkmk.

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: 249-256.

——, L., P. Taberlet, J.E. Swenson, F. Sandergen, and R. Franzen. 2000. "Nuclear DNA Microsatellite Analysis of Genetic Diversity and Gene Flow in the Scandinavian Brown Bear (Ursus Arctos)." *Molecular Ecology* 9: 421–31.

——, L. and P.L. Leburg. 2000. "Biases Associated with Population Estimation Using Molecular Tagging." *Animal Conservation* 3: 191–99.

Warrilow, J., M. Culver, and M. Vaughan. 2001. "Subspecific Affinity of Black Bears in the White River National Wildlife River." *Journal of Heredity* 92 (3): 226–33.

Wasser, S. K., C. S. Houston, G. M. Koehler, G. G. Cadd, and S. R. Fain. 1997. "Techniques for Application of Faecal DNA Methods to Field Studies of Ursids." *Molecular Ecology* 6 (11): 1091–97. doi:10.1046/j.1365-294X.1997.00281.x.

Weir, B. S. & Cockerham, C. C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

Williamson, Douglas F. 2002. "In the Black: Status, Management, and Trade of the American Black Bear." *TRAFFIC North America*.

Woods, John, David Paetkau, David Lewis, Bruce N. McLellan, Mike Proctor, and Strobeck, Curtis. 1999. "Genetic Tagging of Free-Ranging Black and Brown Bears." *Wildlife Society Bulletin* 27 (3): 616–27.

Wright, S. 1931. "Evolution in Mendelian Populations." *Genetics*, no. 16: 97–159.

Wultsch, C., L. P. Waits, E. M. Hallerman, and M. J. Kelly. 2015. "Optimizing collection methods for noninvasive genetic sampling of neotropical felids." Wildlife Society Bulletin, 39: 403-412. Doi:10.1002/wsb.540.

Chapter 2: Genetic Structure and Connectivity of Alabama Black Bear (*Ursus americanus*) Populations with Neighboring States

1.0 Introduction

The erosion of genetic variation is a global threat to the persistence of wildlife populations. Compounding effects of habitat loss, global climate change, and anthropogenic activities have resulted in diminished and geographically isolated populations. Mutational meltdown, genetic drift, and inbreeding are experienced at elevated rates among these smaller, segregated populations (Amos and Balmford, 2001). The consequences from these genetic threats have been widely acknowledged and include reduced survival, diminished fecundity, and the potential to contribute to an extinction vortex, which Amos and Balmford (2001) definded as a situation in which a loss of abundance "… reduces fitness, which in turn hastens the decline, increasing both inbreeding depression and vulnerability to stochastic events in a destructive feedback loop" (Amos and Balmford, 2001; and references therein). Consequently, genetically impoverished populations are less capable of adaptation and more prone to extinction (Amos and Balmford, 2001; Frankham et al., 2002; e.g. Jimenez et al., 1994).

Large carnivores are at the greatest risk of extinction. Characteristics typical of these species, including long generation span, relatively low fecundity rates, need for vast expanses of contiguous habitat, and poor track record of co-habitation alongside human populaces, has led to worldwide declines. Many large carnivore populations are being driven to the edge of extinction

and left isolated for generations, resulting in an inevitable loss of genetic variability. Black bears (*Ursus americanus*) of the southeastern United States exemplify the threat of genetic degradation to large carnivore populations. Habitat loss due to agriculture, hunting pressures, and anthropogenic presence have led to an extensive range reduction of ~80%; frequently resulting in isolated populations of diminutive size (Pelton and Van Manen, 1997).

The purpose of this study was to identify the severity of genetic threats, connectivity, and genetic structure among black bears of Alabama and neighboring states. Measures of genetic diversity, differentiation, and gene flow were considered across bear populations from Alabama, Georgia, Mississippi, and Tennessee. Genetic assessments included calculations of heterozygosity, allelic richness, allelic frequencies, and descriptive F-statistics. Results are essential for identifying populations in genetic distress and the necessity of genetic rescue.

2.0 Methods

2.1 Methods: Alabama Sample Collection

The two populations surveyed in Alabama included individuals of the Mobile River Basin (MRB) and Northern Alabama (NAL) regions. The MRB population resides in the 'Coastal Plain' region located in the extreme southwestern corner of Alabama and approximately centered at 30°55'38" N and 88°6'41" W. The region covers > 4,500 square kilometers, however the MRB black bear population is believed to inhabit a substantially smaller area adjacent to the Mobile-Tensaw River delta. Black bears of NAL inhabit the 'Mountain' physiographic region and reside on the Cumberland Plateau (southernmost section of the Appalachian Plateau; Figure 3). The study area was centered at 34°25'6" N and 85°35'48" W.

Nuclear DNA was used to assess genetic diversity and differentiation among black bear populations. Non-invasive sampling techniques were employed using hair snares and scat-detection dogs. Hair snares are low-cost and proven effective for study of elusive carnivores (Gardner et al., 2010; Gompper et al., 2006; Mowat and Stobeck, 2000). However, hair snares require considerable effort to attain sufficient sample sizes. Therefore, detection canines were implemented in areas with known, high concentrations of black bears. Two tissue samples were opportunistically collected from bears killed by vehicle collisions.

2.2 Methods: Alabama Hair Snare Sampling

During the 2013 autumn season, hair snares were utilized for surveying the MRB region. Due to the capability of black bears to roam widely, we surveyed extensively throughout Mobile County. A systematic survey was conducted via a grid (Chapter 1, Figure 5). Each grid cell extended 8x8 kilometers (approximate size of the average home range of Alabama male bears; Edwards, 2002). We attempted to place one hair snare in each cell. Not all landowners were cooperative; therefore, some grid cells could not be filled.

Hair snares were used to samples for bears in NAL in 2012 and 2013. In an attempt to confirm bear re-colonization of the NAL area, the 2012 surveying period was characterized as intense sampling in the vicinity of Little River National Preserve near Fort Payne, Alabama (Chapter 1, Figure 6). This area is where the vast majority of anecdotal bear sightings had occurred during the years leading up to our study. In the fall of 2013, we spatially expanded efforts in NAL using a systematic survey (Chapter 1, Figure 4). We attempted to place one hair snare within each grid cell of 8x8 kilometers. To maximize bear captures, exact snare locations within the cell were based on assumed habitat preferences.

Stations were constructed using 4-barbed, 2-strand wire at a height of 45 - 50 centimeters and encircling ≥3 trees (Long et al., 2008; Chapter 1, Figure 7). Irregularities in the terrain were filled with woody debris to ensure consistent wire height. Precise location was determined using GPS. Trail cameras were placed at random stations to confirm snare effectiveness. If cub presence was suspected (via photos, tracks, scat, or anecdotal reports), then a second barbed wire was placed at a height of 20 - 25 centimeters.

A scent pile, consisting of logs and leaf litter, was arranged in the center of each enclosure and doused with a 50:50 mixture of mineral oil and vanilla or raspberry extract. The enclosure was additionally baited with cans of rotten tuna that were suspended (> 2 meters) over the snare center. In order to appeal to the wide dietary preferences of black bears, commercial bear lure, road-kill carcasses, honey buns, Caven's Hiawatha Valley Predator Bait, jam / preservatives, and cat food were used intermittently as attractants when signs indicated a bear(s) was in the immediate area, but had not entered the enclosure. Stations were checked every 1 to 2 weeks and lure / bait refreshed. Hair snares were stationary throughout the study period.

Samples from individual barbs were collected via heat-sterilized tweezers and immersed in 100% ethanol or stored in coin envelopes with desiccant. Any barbs, from which all hair could not be removed, were flame-sterilized to avoid contamination. Each sample was labeled with date, time, location, and cardinal directions of the wire strand on which it was collected, and an estimate of follicle number.

2.3 Methods: Alabama Scat-Detection Dog Sampling

Scat-detection dogs are canines trained to find scat from one or more target species of interest and have been used in natural resource management and conservation. Dogs are able to

quickly cover large tracts of land with little additional effort from researchers (Wasser et al., 1997). Comparative studies suggest scat-detection dogs may increase sample sizes considerably over traditional techniques (Harrison, 2006; Long et al., 2007; Reindl-Thompson et al., 2006). For instance, one study revealed that detector dogs located 56 confirmed bobcat scats while only 5 bobcats were detected using cameras and just one with the use of hair snares (Harrison, 2006).

The application of scat-detection dogs was limited to the MRB region and allowed more intensive sampling, but had the drawback of limiting the total area surveyed. During the autumn of 2011 and 2012, scat-detection dogs were used to survey portions of Baldwin, Mobile, Clarke, Washington, and Escambia counties. Detector dogs were active for 4 weeks during the autumn of each year. Teams of one scat-detection dog, a canine handler, and a biologist sampled a triangle-shaped transect of 1.5 kilometers in total length. The field biologist ensured the team remained on track with transect lines and collected samples. Handlers observed and directed the canines, which worked off-lead while moving back and forth across transects. This technique was adopted to ensure the greatest possible survey area (MacKay et al., 2008). Once scat was located, coordinates were recorded using GPS and two samples of 0.4 mL were removed from the driest portion of the scat (where DNA would have experienced minimum hydrolytic damage). Samples were stored in separate vials with 1.4 mL of 100% ethanol or DMSO / EDTA / Tris / Salt solution (Wultsch et al., 2015). The remainder of scat was frozen (-20 degrees Celsius) and placed into storage.

2.4 Methods: Out-of-State Sample Collection

Samples from Mississippi, Georgia, and Tennessee were collected via the cooperative efforts of multiple entities. All Mississippi samples were obtained by hair snares and provided by

Dr. Jerrold L. Belant of Mississippi State University. Mississippi samples ranged across the entirety of the state. Dr. Michael Chamberlain of the University of Georgia's Warnell School of Forestry and Natural Resources provided Georgia samples in the form of tissue collected via hole-punched ears of captured individuals. The sampled region was located in central Georgia near Warner Robins. Tennessee samples were provided as extracted DNA. Originally, they were collected via hair snares from northwestern Great Smokey Mountains National Park with DNA extractions performed as per Settlage et al. 2005 (Figure 12).

2.5 Methods: Genotyping

DNA was extracted from hair and scat using the appropriate Qiagen® QIAmp blood and tissue (hair) kit and stool (scat) kit (Qiagen Inc., Valencia, CA). Extractions were performed in a room dedicated to low quantity DNA samples and using one negative control per extraction. First, mitochondrial DNA (mDNA) was analyzed from scat to positively identify species. This preliminary step had a two-fold purpose: 1) it determined whether DNA was of amplifiable quality and warranted further analysis; 2) confirmed species identification to ensure efforts were not wasted on non-target specimens.

Mitochondrial DNA of hair and tissue samples was not extracted and assessed. Rather, hair samples were reliably identified as appropriate species by visual appearance and quality judged on the basis of hair follicle quantity. Tissue samples were considered positively identified as bear due to collection methods (e.g. bear capture and road side carcasses) and of superior quality in terms of nuclear DNA available for extraction.

Individual genotype was evaluated via microsatellite assessment. A multiplex consisting of 5 nuclear DNA microsatellite loci: G10C, G10H, G10P, G10X, Mu15 and a sex identification

marker (SE 47-48). Chosen markers had previously demonstrated adequate allele variability as well as being independent and unlinked within the Alabama region (Chapter 1 of this study). The sex identification marker was excluded for purposes of genotyping, assessment of structure, and calculations of genetic diversity.

Amplification by polymerase chain reaction (PCR) for MM1 and MM2 had an initial denaturation period of 15 minutes at 95º Celsius. The initial denaturation was followed by 40 repetitions of a denaturing, annealing, and elongation cycle: 30 seconds at 95º Celsius, 1.5 minutes at 57-57º Celsius, and 1 minute at 72º Celsius respectively. The final elongation period lasted 30 minutes at 60º Celsuis. After amplification, samples were combined with 10 microliters of Formamide and 0.15 microliters of LIZ 500 dye. Assessment of markers was conducted on an Applied Biosystems 3130xL genetic analyzer. GeneMapper v3.1 was used to assess gels and identify microsatellite alleles of each sample (Applied Biosystems, Foster City, California). To reduce occurrence of allelic drop-out, homozygous loci were confirmed with 3 PCR replicates. Heterozygous loci required 2 repeated assessments before they were accepted.

Samples had previously been confirmed as separate individuals via collection methods (i.e. hole punch of captured and tagged bears) or genetic analysis from other studies (Settlage et al., 2005; Chapter 1 of this thesis).

2.6 Methods: Genetic Structure

Numerous definitions are provided in the literature to describe a population (Waples and Gaggiotti, 2006). To avoid confusion, populations will henceforth be referred to as genetically distinct groups of individuals that demonstrate significantly divergent allelic frequencies. Determining the optimum number of populations was necessary before diversity, structure, and

potential connectivity could be assessed. To determine groupings of genetically distinct populations amongst our samples, aspatial modeling was performed using the program Structure v2.3.4 (Pritchard et al., 2000). The program Convert v1.31 was used to convert input file from GenePop to the appropriate Structure format (Glaubitz, 2004). This method is useful for determining the optimum number of genetically distinct populations (K) and assigning individuals to each cluster; it is a Bayesian approach that does not require prior insight into population structure. The most likely K was determined by minimizing Hardy-Weinberg disequilibrium and linkage disequilibrium within hypothesized populations. Markov chain Monte Carlo (MCMC) simulation was used to estimate the posterior probability of fitting the assumption of K populations: P(X/K) (Pritchard et al., 2000; Falush et al., 2003). Values of K ranging from 1 to 10 were assessed. The most likely partition of the dataset was selected using 20 independent runs with a 10,000 repetition burn-in period and 50,000 MCMC randomizations for each hypothetical K value. Best supported K value was determined according to maximum likelihood and plotted in the program Structure Harvester v0.6.94 (Earl and vonHoldt, 2012). The program Clumpak v1.1 was used to confirm K findings via a hierarchical Evanno method (Evanno, 2005; Kopelman et al.,2015). The delta K was calculated across all samples, then repeated for each inferred population cluster until additional substructure could not be discerned (Coulon et al., 2008). This hierarchical approach allows detection of substructure that is otherwise not perceived among larger data sets Balkenhol et al., 2014).

The Q-value is a descriptive statistic that was used to describe the proportion of an individual's genotypic ancestry that can be attributed to each identified genetic group. Each individual was assigned to the group in which their ancestry (Q) was greatest using the admixture model with correlated allele frequencies in the Structure v2.3.4 program (Pritchard et

al, 2000). If the Q-value was < 0.75, then individuals were considered mixed ancestry (providing evidence of migration). The arbitrary cut-off of < 0.75 was applied because it represents the amount of ancestry equivalent to descending from a minimum of one grandparent from outside the assigned group (Pritchard et al, 2000). If Q-values indicated admixture between populations, then individuals were grouped with their population of greatest ancestry for all further analyses of differentiation and diversity.

A population in Hardy Weinberg equilibrium (HWE) has allelic and genotypic frequencies that remain stable through time. HWE can be violated when conditions such as mutation, migration, genetic drift, or non-random mating occurs (Gillespie, 1998). HWE of structured populations was investigated using the HWE probability test with Markov Chain method as described in Guo and Thompson, 1992 in the GenePop v4.2 program (Raymond and Rousset, 1995).

2.8 Methods: Genetic Diversity

The most commonly used measure of genetic diversity is heterozygosity. This statistic describes the proportion of heterozygous, polymorphic loci within each population level, as well as the proportion of heterozygotes per locus. There are two measures of heterozygosity: 1) observed heterozygosity (Ho), which is the realized measure; and 2) expected heterozygosity (He), which is the expected proportions based on allelic frequencies (Allendorf and Luikart, 2007). When there is random mating, expected heterozygosity is a better standard for comparing relative amounts of variation among populations (Nei, 1977). Another measure of genetic diversity is the number of alleles (Na). Relative to heterozygosity, the number of alleles is sensitive to bottlenecks and may provide insight into a potentially re-colonizing front or severely

isolated population. However, the number of alleles is strongly dependent on sample size. Allelic richness (A) was also assessed since it allows for us to control for differences in sample size while making between population comparisons (Kalinowski, 2004). Allelic richness was determined using the statistical method of rarefaction as developed by Hurlbert (1971) in the HP-Rare program (Kalinowski, 2005).

Inbreeding occurs when closely related individuals mate. It is a consequence of small population size that leads to degradation of genetic variation. The inbreeding coefficient (F) provides insight by describing the probability that two alleles at a given locus are identical by descent (Allendorf and Luikart, 2007). Measures of diversity including heterozygosity, inbreeding coefficients, and number of alleles were assessed via the GenAlEx v6.5 program (Peakall and Smouse, 2012).

2.9 Methods: Genetic Differentiation

The descriptive F-statistic is the most widely used metric to quantify genetic differentiation. This method uses a series of inbreeding coefficients (Fis, Fst, and Fit) to evaluate departures from HWE proportions. Fis expresses the degree of divergence between individuals of a subpopulation. Fst is the measure of divergence between populations (e.g. between MRB and NAL populations). Finally, Fit is global fit of HWE proportions within the entire surveyed sample (e.g. among all black bears of Alabama). Wright's F-statistics were utilized to investigate genetic differentiation at all hierarchical levels using the allele identity method in program GenePop v4.2 (Raymond and Rousset, 1995; Wright 1931). When subpopulation divergence is present due to differences in allele frequency proportions, then Fst is expected to be > 0. Single locus estimates follow standard ANOVA and multilocus estimates computed as in Weir and

Cockerham (1984).

Though F-statistics are the most popular measurement of genetic structure, they are limited since there is an assumption of infinite alleles. Therefore, R-statistics were also calculated to confirm results. R-statistics do not make the assumption of infinite alleles, but rather operate off the concept that alleles are generated in a step-wise fashion. R-statistics were calculated using GenePop v4.2 (Raymond and Rousset, 1995.)

Additionally, the number of unique alleles may also be an important indicator of differentiation since a linear relationship exists between the number of unique alleles resulting from mutation and gene Qflow. "The time during which a new allele remains private depends only on migrations rates, such that the proportion of alleles that are private decreases as migration rate increases" (Allendorf and Luikart, 2007). Private allelic richness was provided as a quantitative assessment of differentiation and calculated using the statistical method of rarefaction in the HP-Rare program (Kalinowski, 2005).

3.0 Results

3.1 Results: Genotyping

A total of 120 MRB samples were successfully genotyped and represent 45 individuals. The ratio of female to male bears was 30:14. One individual was of unknown sex. A total of 161 NAL samples were successfully genotyped and represent 15 individuals. The ratio of female to male bears was 2:1. Thirty samples of extracted DNA were received and successfully processed from the Great Smokey Mountain National Park in Tennessee. The ratio of female to male bears

was 3:7. Fifteen samples were successfully processed from Mississippi and included a female to male ratio of 1:2. The samples from Georgia included 20 successfully genotyped individuals of a female to male ratio of 11:9. A total of 125 individuals were identified across all five regions and implemented in diversity and differentiation assessments.

3.2 Results: Genetic Structure of Populations

All samples were used for the structure assignment test. The maximum likelihood (L(K)) values plateaued and, therefore, are best supported at five populations (Figure 13; Table 9). The K=5 finding was confirmed by hierarchical assessment via the Evanno method in the program Clumpak v1.1 (Kopelman et al., 2015). Individuals were assigned to the population with which they shared the highest degree of ancestry based on Q-values.

All MRB and 6 of the 15 samples from Mississippi (MS-II28, MS-JJ30, MS-K515, MS-N528, MS-P16, and MS-RCB9) clustered into a single population (Table 9). This structured MRB population totaled 51 individuals and extended from the southwest corner of Alabama into the southeast quarter of Mississippi. The structured NAL population included 14 sampled from the NAL region and 2 from the Tennessee region (BR10-012 and BR10-077; Table 9). Twenty-six Tennessee samples and one NAL sample (NAL02) clustered into a structured Tennessee population (TNP) located in the Great Smokey Mountains National Park (Table 9). The remaining 9 individuals of Mississippi region and one of the Tennessee region samples (BR10-142; Table 9) clustered into a structured Mississippi population (MSP) that extends throughout the majority of Mississippi, but excluding the southeastern most corner. The final cluster was the structured Georgia population (GAP) and consisted of the 20 samples received from the central

Georgia region and one sample from Tennessee (BR10-026; Table 9). Figure 14 provides a geographic map of the five structured populations.

Inferred ancestry from Q-plot values provided evidence of recent admixture between several populations (Figure 15; Table 9). Three individuals clustered with NAL showed evidence of recent genetic admixture with bears of the TNP (samples BR10-012, BR10-077, and H-1G1; Table 9). Two individuals of the TNP cluster appear to be descendants of recent migrants; one of which (BR10-059) showed admixture with GAP and the other (BR10-163) showed admixture with the NAL population (Table 9). One of the MSP clustered samples (BR10-142) displayed admixture with TNP (Table 9). A single individual (BR10-026) clustered with GAP had shared inferred ancestry with the TNP. Finally, a first generation migrant was also present in the NAL region; sample H-1G1 had an inferred ancestry of 0.919 with TNP, but was collected in the NAL region (Table 9). Surprisingly, evidence of admixture was not noted in the MRB population despite geographical overlapping between MRB and MSP populations at the Alabama / Mississippi border (Figure 14).

3.3 Results: Genetic Diversity

When testing for HWE among the five genetically distinct populations, the MSP and NAL population departed significantly from equilibrium ($P = 0.032$ and $P < 0.001$ respectively). The MSP departed significantly at one locus (G10P; $P = 0.040$). The NAL population departed significantly at a total of two loci: the G10H ($P = 0.017$) and Mu15 ($P = 0.005$). Polymorphism across loci was 100% for all populations with the exception of MRB, which exhibited polymorphism at 60% of markers. Expected heterozygosity for MRB, NAL, TNP, MSP, and GAP varied substantially across the populations and was $He = 0.246$, $He = 0.513$, $He = 0.735$,

He = 0.515, and He = 0.401 respectively (Table 10). Observed heterozygosity of each population reflected a similar trend of genetic diversity across MRB, NAL, TNP, MSP, and GAP (Ho = 0.256, Ho = 0.588, Ho = 0.698, Ho = 0.440, and Ho = 0.362 respectively; Table 10).

The average number of alleles per locus and allelic richness of populations were supportive of diversity levels suggested by measurements of heterozygosity. The MRB, NAL, TNP, MSP, and GAP were calculated to have Na = 2.000, Na = 4.000, Na = 6.400, Na = 3.200, and Na = 3.200 respectively (Table 10). When assessed for allelic richness, MRB, NAL, TNP, MSP, and GAP averaged A = 1.58, A = 2.29, A = 3.12, A = 2.28, and A = 1.95 respectively.

3.4 Results: Genetic Differentiation

Differentiation was assessed globally, between populations, and between individuals. Across all populations the global Fit = 0.46080 across all loci, the Fst = 0.4403, and the Fis = 0.0365 (Table 11). When assessed between populations, the MRB population showed very strong divergence across all other pairwise comparisons (Fst ranging from Fst = 0.5318 to Fst = 0.8987; Table 12). A high degree of divergence was also noted between the GAP and MSP (Fst = 0.7556 and Rst = 0.4726; Table 12). The lowest levels of genetic differentiation were noted between the TNP and NAL population (Fst = 0.1059 and Rst = 0.1333; Table 12). The number of unique alleles is an important indicator of genetic distinctiveness, therefore, private allelic richness was also calculated. The MRB, NAL, TNP, MSP, and GAP had average private allelic richness of A = 0.48, A = 0.37, A = 0.87, A = 0.60, and A = 0.30 respectively.

4.0 Discussion

4.1 Discussion: Structure of Populations

There are five structured populations (MRB, NAL, TNP, MSP, and GAP) from 5 different geographical locations spanning 4 states (Figure 15). The MRB and NAL populations both exist within Alabama borders. Samples from the southeastern corner of the Mississippi region expanded the scientifically confirmed range of the MRB population (chapter 1 of this study; Figure 14). Known range of the recolonizing NAL front was also spatially expanded into Tennessee (chapter 1 of this study; Figure 14). Two individuals (BR10-012 and BR10-077) were geographically located in the Great Smokey Mountains National Park of Tennessee, but genetically assigned to the NAL population through clustering by means of Q-values (Table 9).

Despite existing within Alabama borders, MRB and NAL populations are strongly differentiated (Fst = 0.6803; Table 12). Q-values did not indicate recent admixture between MRB and NAL populations; this is congruent with our initial assumption of two separate subspecies occurring within Alabama borders (*U. a. floridanus* in the MRB region and the *U. a. americanus* subspecies in NAL; Bentzien, 1998 and chapter 1 of this study). Strong divergence between MRB and all other populations (TNP, MSP, and GAP) suggests the MRB population is the only cluster consisting of the *U. a. floridanus* subspecies (Fst = 0.6722, Fst = 0.5318, and Fst = 0.8987 respectively; Table 12). The different subspecies accounts for a lack of gene flow despite geographical overlap between MRB and MSP. The MRB population appears highly isolated and not genetically impacted by other populations of this study. Small, isolated population are at risk for inbreeding and the MRB individuals should be monitored closely to avoid genetic decline.

Prior to this study, reports of black bear extirpation from the NAL region had existed for nearly a century (Howell, 1921). However, this study confirmed a black bear population of NAL; this represents a re-colonizing front returning without anthropogenic assistance (Chapter 1 of this study). The NAL population is not as strongly differentiated from neighboring populations (Fst = 0.1059, Fst = 0.275, and Fst = 0.2107 for TNP, MSP and GAP respectively; Table 12). This leads us to the conclusion that the re-colonizing NAL front consists of the American subspecies (*U. a. americanus*). The comparatively low Fst of 0.1059 suggests the TNP cluster is the source population for NAL and was further confirmed by evidence of admixture. Recent genetic admixture ($Q < 0.75$) was evident between four NAL and TNP individuals (BR10-012, BR10-077, BR10-163, and H-1G1; Table 9). Q-values indicate gene flow between NAL and TNP occurs with frequency. This finding was strengthened by a first generation migrant (NAL02) that was geographically located in the north Alabama region, but clustered with TNP due to an inferred ancestry of Q = 0.919 (Table 9).

Divergence between MSP and NAL was moderate (Fst = 0.275; Table 12). The MSP cluster did not have evidence of direct impact on NAL through recent admixture. However, there was recent admixture with TNP that would impact NAL indirectly and account for the observed low divergence (BR10-142 with an MSP inferred ancestry of Q = 0.527 and TNP inferred ancestry of Q = 0.285; Table 9).

The GAP population exists only in Georgia and is moderately diverged from the American subspecies populations of NAL and TNP (Fst = 0.2107 and Fst = 0.2137 respectively; Table 12). There was evidence of recent migrants from the TNP into the GAP cluster (BR10-026 with a GAP inferred ancestry of Q = 0.685 and a TNP inferred ancestry of Q = 0.188; Table 9).

However, the GAP is strongly divergent from the MSP (Fst = 0.7556; Table 12). One possible cause of this stronger divergence is the phenomenon of isolation by distance.

In conclusion, Fst values indicate that geographically adjacent populations of the *U. a. americanus* subspecies have high levels of connectivity. Unfortunately, the Fst value between the geographically separated GAP and MSP clusters indicates genetic flow across the landscape is insufficient for the prevention drift. The MRB population is already extremely isolated and in a position that risks inbreeding. Future research should be directed towards identifying corridors, determining barriers to genetic flow, and assessment of successful dispersal behavior. The GAP cluster offers an excellent opportunity to study these movement behaviors; especially how they pertain to anthropogenic barriers. Available habitat of the Georgia region appears limited and the population is dispersing into the vast and much more contiguous habitat provided by the Tennessee region. However, the geographical distance between the Georgia region and Tennesee region is considerable (~450 kilometers). Migrating GAP individuals are crossing multiple interstates (e.g. I-20, I-85, and possibly I-75) and major human populaces (e.g. metropolitan Atlanta and surrounding suburbs). Results of such studies would provide valuable insight into the management and creation of wildlife corridors for populations in genetic distress and isolation.

4.2 Discussion: Genetic Diversity of Populations

Genetic diversity of MRB, NAL, TNP, MSP, and GAP is approximately evenly divided between global Fit and population level Fst and indicates a high degree of diversity between populations, but not within them (Fit = 0.4608 and Fst = 0.4430; Table 11).

Measures of diversity demonstrated particularly low variability amongst MRB individuals (polymorphism at 60% of loci; A = 1.58; Na = 2.000 ± 0.447 alleles; He = 0.246 ±

0.128; Ho = 0.256 ± 0.133) and is consistent with previous findings (chapter 1 of this study; Figure 16). MRB is one of the smallest, most fragmented and insular bear populations in the world. In the known literature, only one bear population (an Asiatic black bear species (*Ursus tibetanus*) located in Western Japan) had a lower observed heterozygosity of Ho = 0.243 across 6 loci (Appendix 4; Saitoh et al., 2001). This extreme lack of genetic variability is a cause of utmost concern for long term viability of MRB.

The NAL population is a novel and re-colonizing front that has arisen over the past couple years and likely consists of few individuals. NAL should be closely monitored for signs of genetic drift. The extreme difference in average number of alleles per locus for NAL versus TNP (source population), suggests that genetic drift via founders' effect is of great concern (Na = 3.800 ± 0.374 and Na = 7.400 ± 0.510 respectively). The average number of alleles per locus measurement is very sensitive to recent bottlenecking and is a good indicator of founders' effect. Genetic monitoring is imperative to ensure the long term success of this returning population.

Measures of genetic diversity revealed much greater variability in TNP, MSP, and GAP that compare favorably with other black bear populations of literature (Figure 16; Appendix 4). Unlike MRB, these populations are neither insular nor isolated. The relatively high number of migrants suggested by the degree of admixture may even be the cause of HWE violation among the MSP and NAL population.

In conclusion, low diversity and strong isolation as determined by measurements of divergence suggest there is little to no genetic connectivity between MRB and other bear populations. Seclusion from other populations and the small number of individuals (< 50 individuals at last population estimate; Edwards, 2002) may force mating to occur between closely related bears, placing the entire population at risk for inbreeding leading to an extinction

vortex (Amos and Balmford, 2001). Potential symptoms of inbreeding depression have already been documented among SMP bears and include morphological abnormalities such as cryptorchidism, prolapsed rectums, and kinked or absent tails (Kasbohm et al. 1994). Strong conservation management in the form of genetic rescue should be considered for the viability of MRB. Efforts should include the construction of wildlife corridors, translocation, and continued genetic monitoring.

The NAL population is similarly at risk of inbreeding. It is a young, likely small population experiencing a bottleneck via founders' effect. However, NAL has not yet showed signs of genetic loss (He = 0.513 ± 0.045 and Ho = 0.588 ± 0.102; Table 10). Further research should be aimed at understanding black bear habitat preferences, use, and movement patterns. Results would help biologists identify and preserve habitat for species conservation and ensure NAL bears have access to the limited suitable habitat.

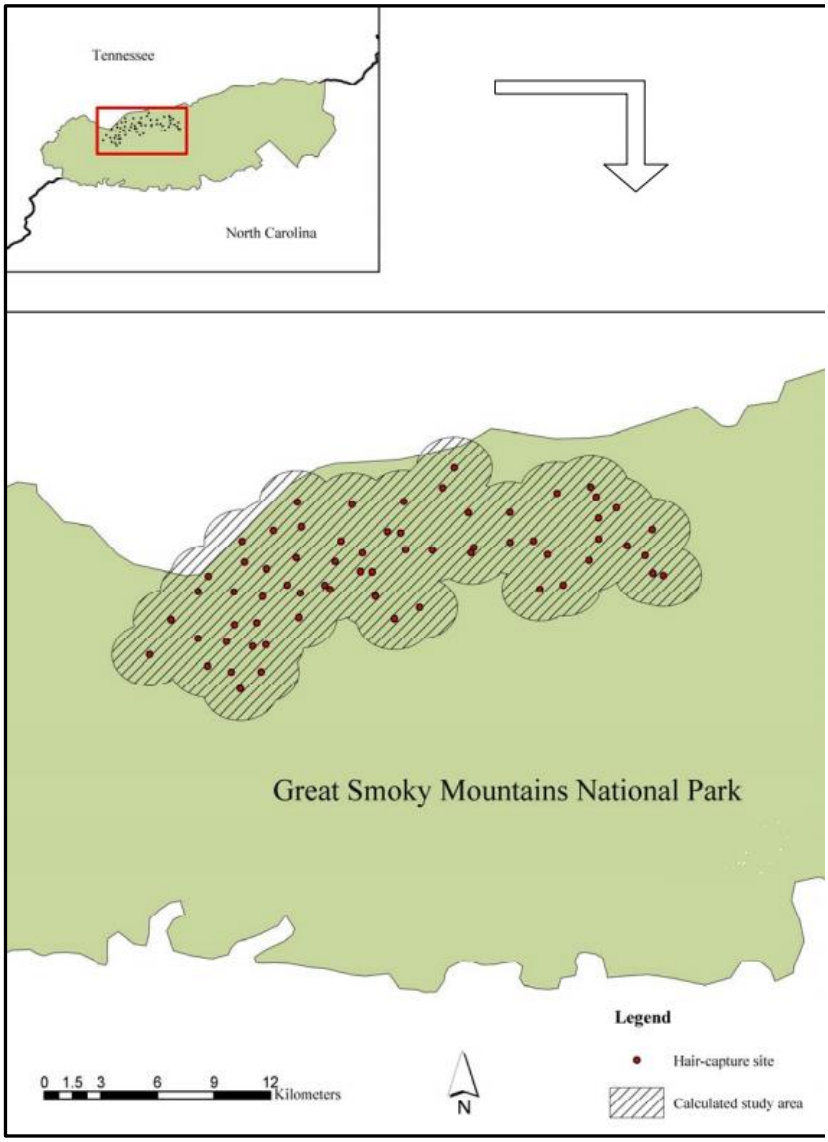

Figure 12: Map of Tennessee sampling area in Great Smoky Mountains National Park. Image generated by Settlage, 2005.

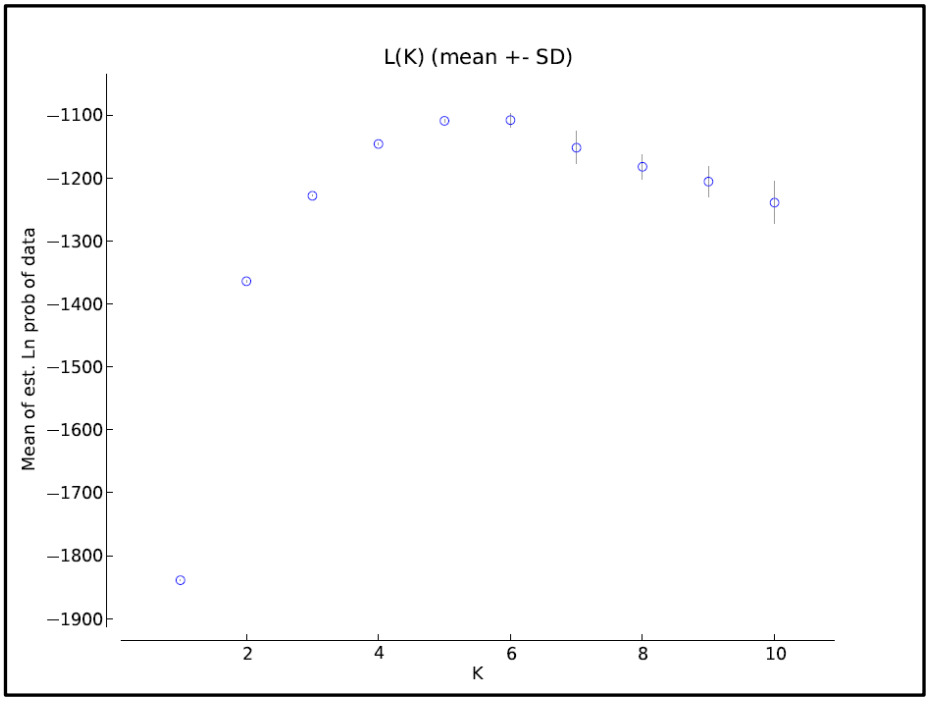

Figure 13: The best supported value of K = 5, according to the maximum likelihood, L(K), output provided by Structure v2.3.4 and plotted in the program Structure Harvester v0.6.94 (Pritchard et al., 2000; Earl and vonHoldt, 2012).

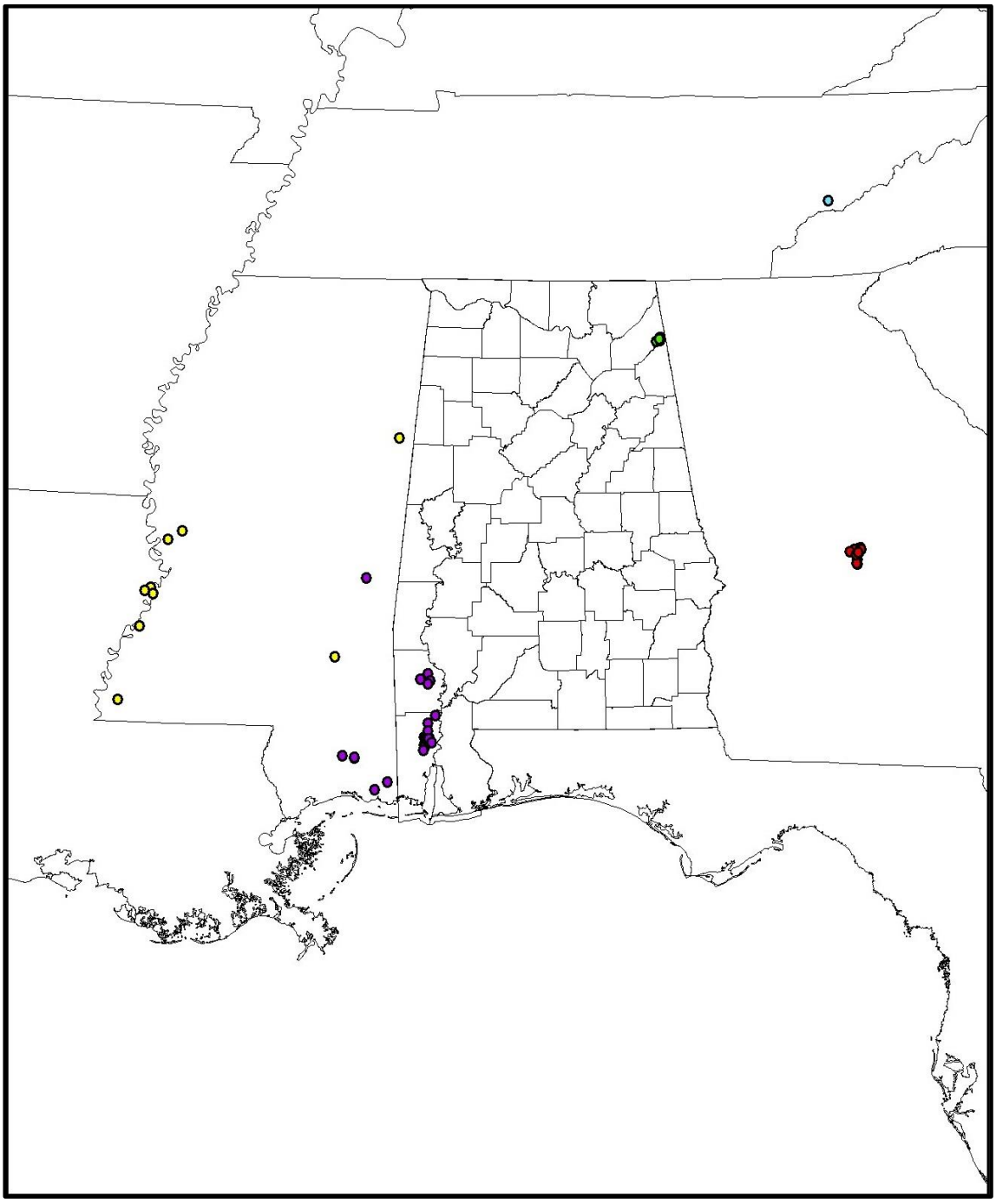

Figure 14: Five genetically distinct populations: MRB (purple), NAL (green), TNP (teal), MSP (yellow), and CGA (red).

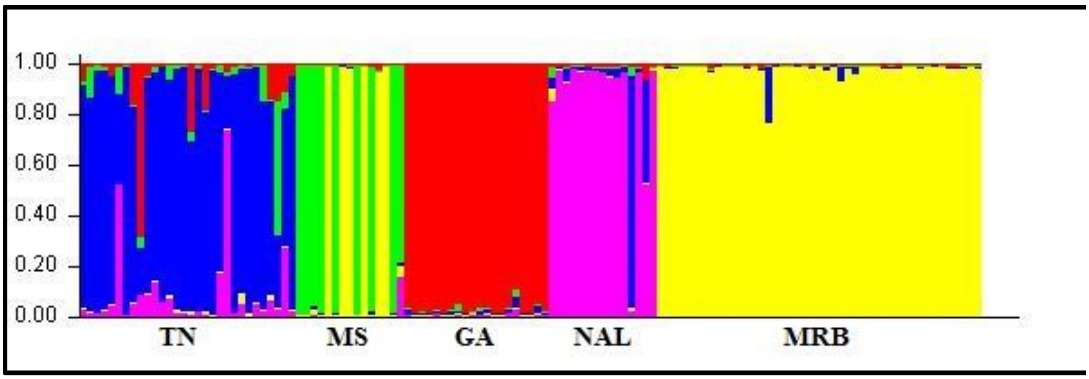

Figure 15: Inferred ancestry from the 5 regions of study as determined by Q plot ancestry values.

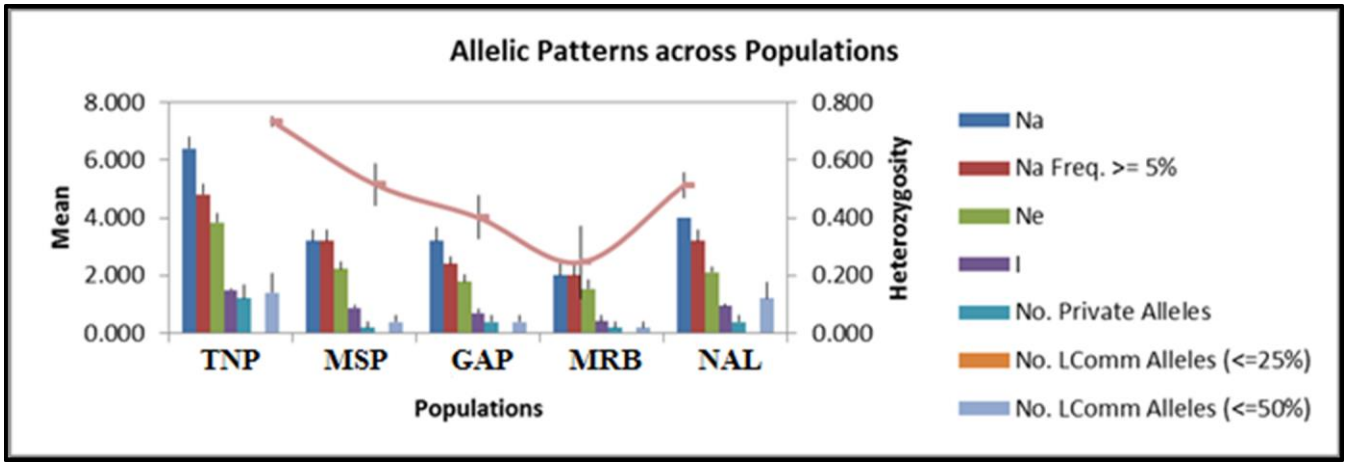

Figure 16: Allelic patterns across structured populations: Tennessee population (TNP), Mississippi population (MSP), Georgia population (GAP), Mobile River Basin population (MRB), and northern Alabama population (NAL).

	Region of Origin	Cluster 1 GAP	Cluster 2 MSP	Cluster 3 TNP	Cluster 4 MRB	Cluster 5 NAL
BR10-012**	TN	0.011	0.105	0.357	0.003	0.524
BR10-026**	TN	0.685	0.037	0.188	0.004	0.086
BR10-059**	TN	0.266	0.039	0.672	0.009	0.015
BR10-077**	TN	0.029	0.018	0.208	0.004	0.741
BR10-142**	TN	0.150	0.527	0.285	0.003	0.035
BR10-163**	TN	0.109	0.067	0.542	0.007	0.275
MS-II28	MS	0.005	0.005	0.004	0.983	0.004
MS-JJ30	MS	0.003	0.004	0.004	0.986	0.003
MS-K515	MS	0.007	0.004	0.005	0.980	0.004
MS-N528	MS	0.004	0.004	0.004	0.985	0.003
MS-P16	MS	0.024	0.005	0.006	0.958	0.007
MS-RCB9	MS	0.004	0.004	0.004	0.984	0.003
NAL02	NAL	0.009	0.034	0.919	0.013	0.024
H-1G1**	NAL	0.059	0.005	0.407	0.003	0.526

Table 9: Q-Values of samples clustering with a population outside of their region of origin and those displaying recent mixed ancestry (as indicated by ***).

Pop		N	Na	Ne	I	Ho	He	uHe	F	A
TNP	**Mean**	26.400	6.400	3.843	1.504	0.698	0.735	0.749	0.049	3.120
	SE	0.400	0.400	0.296	0.063	0.061	0.017	0.018	0.084	
MSP	**Mean**	10.000	3.200	2.220	0.876	0.440	0.515	0.542	0.208	2.280
	SE	0.000	0.374	0.276	0.123	0.108	0.071	0.075	0.136	
GAP	**Mean**	21.000	3.200	1.784	0.706	0.362	0.401	0.410	0.057	1.950
	SE	0.000	0.490	0.237	0.127	0.065	0.075	0.077	0.110	
MRB	**Mean**	49.000	2.000	1.533	0.422	0.256	0.246	0.248	-0.050	1.580
	SE	0.548	0.447	0.311	0.214	0.133	0.128	0.129	0.010	
NAL	**Mean**	16.000	4.000	2.124	0.936	0.588	0.513	0.529	-0.127	2.290
	SE	0.000	0.000	0.193	0.070	0.102	0.045	0.047	0.131	

Table 10: Quantitative descriptions of genetic diversity across structured populations (N = number of samples, Na = number of alleles, Ne = number effective alleles, I = Shannon's diversity index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased heterozygosity, and F = inbreeding coefficient), A = allelic richness.

		Fis	Fst	Fit
G10C		0.0667	0.4004	0.4404
G10H		0.0879	0.5333	0.5743
G10P		0.0883	0.5736	0.6112
G10X		0.0530	0.5272	0.5523
Mu15		-0.0540	0.1426	0.0963
	All	0.0365	0.4403	0.4608

Table 11: Average Fis, Fst, and Fit across five structured populations.

	TNP	MSP	GAP	NAL	MRB
TNP		0.3218	0.2137	0.1059	0.6722
MSP	0.2021		0.7556	0.2575	0.5318
GAP	0.2329	0.4726		0.2107	0.8987
NAL	0.1333	0.3494	0.3308		0.6803
MRB	0.4587	0.6032	0.5885	0.6274	

Table 12: Assessment of population divergence (Fst values on bottom of diagonal and Rst values on top).

5.0 References

Allendorf, F.W. and G. Luikart. 2007. *Conservation and the Genetics of Populations*. Malden, Maine: Blackwell Publishing.

Amos, W. and A. Balmford. 2001. "When Does Conservation Genetics Matter?" *Heredity* 87: 257–65. doi:10.1046/j.1365-2540.2001.00940.x.

Balkenhol, Niko, Joseph D. Holbrook, Dave Onorato, Peter Zager, Craig White, and Lisette P. Waits. 2014. "A multi-method approach for analyzing hierarchical genetic structures: a case study with cougars *Puma concolo*. *Ecography* 37: 001-012.

Bentzien, Michael M. 1998. "Endangered and Threatened Wildlife and Plants; New 12-Month Finding for a Petition to List the Florida Black Bear." *United States Fish and Wildlife Service*.

Boersen, M. R., J. D. Clark, and T. L. King. 2003. "Estimating Black Bear Population Density and Genetic Diversity at Tensas River, Louisiana Using Microsatellite DNA Markers." *Wildlife Society Bulletin* 31 (1): 197–207.

Bohonak, A.J. 2001. "Invasion Genetics of New World Medflies: Testing Alternative Colonization Scenarios." *Biological Invasions*, 3: 103–11.

Calinski, T., and J. Harabasz. 1974. "A Dendrite Method for Cluster Analysis." *Communications in Statistics*, 3: 1–27.

Csiki, Ildiki, Cynthia Lam, Audie Key, Erica Coulter, Joseph D. Clark, Richard M. Pace III, Kimberly G. Smith, and Douglas D. Rhoads. 2003. "Genetic Variation in Black Bears in Arkansas and Louisiana Using Microsatellite DNA Markers." *Journal of Mammalogy* 84 (2): 691–701.

Earl, Dent A., and Bridgett M. vonHoldt. 2012. "STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method." *Conservation Genetics Resources* 4 (2): 359–61. doi:10.1007/s12686-011-9548-7.

Dixon, J. D. 2004. "Thesis: Conservation Genetics of the Florida Black Bear." Master's Thesis Gainesville, Florida: University of Florida.

Dobey, S.T. 2002. “Abundance and Density of Florida Black Bears in the Okefenokee National Wildlife Refuge and Osceola National Forest.” Master’s Thesis, Knoxville, Tennessee: University of Tennessee.

Edwards, A. S. 2002. “Thesis: Status of the Black Bear in Southwestern Alabama.” Master’s Thesis, Knoxville, Tennessee: University of Tennessee.

Evanno, G., S. Regnaut and J. Gould. 2005. “Detecting the number of clusters of individuals using the software structure: A simulation study.” Molecular Ecology 14: 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: 564–67.

————, L., P.E. Smouse, and J.M. Quattro. 1992. “Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data.” *Genetics*, 8: 479–91.

Frankham, R., D.A. Briscoe, and J.D. Ballou. 2002. *Introduction to Conservation Genetics*. New York, New York, USA: Cambridge University Press.

Gardner, Beth, J. Andrew Royle, Michael T. Wegan, Raymond E. Rainbolt, and Paul D. Curtis. 2010. “Estimating Black Bear Density Using DNA Data From Hair Snares.” *Journal of Wildlife Management* 74 (2): 318–25. doi:10.2193/2009-101.

Glaubitz, J.C. 2004. “Convert: A User-Friendly Program to Reformat Diploid Genotypic Data for Commonly Used Population Genetic Software Packages.” *Molecular Ecology Notes*, no. 4: 309–210.

Gompper, Matthew E., Roland W. Kays, Justina C. Ray, Scott D. LaPoint, Daniel A. Bogan, and Jason R. Cryan. 2006. “A Comparison of Noninvasive Techniques to Survey Carnivore Communities in Northeastern North America.” *Wildlife Society Bulletin* 34 (4): 1142–51. doi:10.2193/0091-7648(2006)34[1142:ACONTT]2.0.CO;2.

Guo, S. W. & Thompson, E. A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361-372.

Harrison, Tobert L. 2006. “A Comparison of Survey Methods for Detecting Bobcats.” *Wildlife Society Bulletin* 34 (2): 548–52. doi:10.2193/0091-7648(2006)34[548:ACOSMF]2.0.CO;2.

Hurlbert, S. H. 1971. “The nonconcept of species diversity: a critique and alternative parameters.” *Ecology* 52: 577-586.

Jimenez, Ja, Ka Hughes, G. Alaks, L. Graham, and R.C. Lacy. 1994. “An Experimental-Study of Inbreeding Depression in a Natural Habitat.” *Science* 266 (5183): 271–73. doi:10.1126/science.7939661.

Kalinowski, S.T. 2004. "Counting alleles with rarefaction: private alleles and hierarchical sampling designs." *Conservation Genetics* 5:539-543.

Kalinowski, S.T. 2005. "HP-Rare: a computer program for performing rarefaction on measures of allelic diversity." *Molecular Ecology Notes* 5:187-189.

Kasbohm, J.W., D.A. Miller, and M.R. Vaughan. 1994. *Taxonomy of Black Bears in the Southeastern United States*. Second Annual Report to the U.S. Fish and Wildlife Service. Blacksburg, Virginia, USA: Virginia Polytechnical and State University.

Kopelman, N.M., J. Mayzel, M. Jaksobsson, N.A. Rosenberg, and I. Mayrose. 2015. "Clumpak: a program for identifying clustering modes and packaging population structure inferences across K." Molecular Ecology Resources (5):1179-91.

Long, R.A., P. MacKay, William J. Zielinski, and Justina C. Ray. 2008. *Noninvasive Survey Methods for Carnivores*. 1st ed. Washington, DC.: Island Press.

——, Robert A., Therese M. Donovan, Paula Mackay, William J. Zielinski, and Jeffrey S. Buzas. 2007. "Comparing Scat Detection Dogs, Cameras, and Hair Snares for Surveying Carnivores." *Journal of Wildlife Management* 71 (6): 2018–25. doi:10.2193/2006-292.

Lu, Z., W.E. Johnson, M. Menotti-Raymond, N. Yuhki, J.S. Martenson, S. Mainka, H. Shi-Qiang, et al. 2001. "Patterns of Genetic Diversity in Remaining Giant Panda Populations." *Conservation Biology*, no. 15: 1596–1607.

Manly, B.F.J. 1994. *Multicariate Statistical Methods: A Primer*. London: Chapman and Hall.

Marshall, H. Dawn, and K. Ritland. 2002. "Genetic Diversity and Differentiation of Kermode Bear Populations." *Molecular Ecology*, no. 11: 685–97.

Michalakis, Y., and L. Excoffier. 1996. "A Generic Estimation of Population Subdivision Using Distances between Alleles with Special Reference for Microsatellite Loci." *Genetics* 3 (142): 1061–64.

Miller, Craig R., and L.P. Waits. 2003. "The History of Effective Population Size and Genetic Diversity in the Yellowstone Grizzly (Ursus Arctos): Implications for Conservation." In *Proceedings of the National Academy of the United States of America*, 100:4334–39.

Miller, Daniel A., Eric M. Hallerman, Michael R. Vaughan, and Kasbohm, John W. 1998. "Genetic Variation in the Black Bear Populations from Louisiana and Arkansas; Examining the Potential Influence of Reintroductions from Minnesota." *Ursus* 10: 335–41.

Mowat, Garth and Curtis Strobeck. 2000. "Estimating Population Size of Grizzly Bears Using

Hair Capture, DNA Profiling, and Mark-Recapture Analysis." *The Journal of Wildlife Management* 64 (1): 183–93.

Paetkau, D., E.W. Born Amstrup, W. Calvert, A.E. Derocher, F. Messier Garner, I. Stirling, O. Wigg Taylor, and C. Strobeck. 1999. "Genetic Structure of the World's Polar Bear Populations." *Molecular Ecology*, no. 8: 1571–84.

———, David, and Strobeck, Curtis. 1998a. "Ecological Genetic Studies of Bears Using Microsatellite Analysis." *Ursus (International Association for Bear Research and Management)* 10: 299–306.

———, D., G. F. Shields, and C. Strobeck. 1998b. "Gene Flow between Insular, Coastal and Interior Populations of Brown Bears in Alaska." *Molecular Ecology* 7 (10): 1283–92. doi:10.1046/j.1365-294x.1998.00440.x.

———, D., and C. Strobeck. 1994. "Microsatellite Analysis of Genetic Variation in Black Bear Populations." *Molecular Ecology*, no. 3: 489–95.

Peakall, R., and Smouse, P.E. 2012. "GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research - an Update." *Bioinformatics* 28: 2537–39.

Pelton, Micheal R., and Frank T. van Manen. 1997. "Status of Black Bears in the Southeastern United States." In *Proceedings of the Second International Symposium on the Trade of Bear Parts*.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. "Inference of Population Structure Using Multilocus Genotype Data." *Genetics* 155 (2): 945–59.

Raymond and Rousset. 1995. "GENEPOP (version 4.2): Population Genetics Software for Exact Tests and Ecumenicism." *Journal of Heredity*, 86: 248–49.

Reindl-Thompson, Sara A., John A. Shivik, Alice Whitelaw, Aimee Hurt, Kenneth F. Huggins, and McCleery. 2006. "Efficacy of Scent Dogs in Detecting Black-Footed Ferrets at a

Ruiz-Garcia, M. 2003. "Molecular Population Genetic Analysis of the Spectacled Bear (Tremarctos Ornatus) in the Northern Andean Area." *Hereditas*, no. 138: 81–93.

Saitoh, T.Y., H. Kanamori Ishibashi, and E. Kitahara. 2001. "Genetic Status of Fragmented Populations of the Asian Black Bear (Ursus Thibetanus) in Western Japan." *Population Ecology* 43 (3): 221–27.

Settlage, Katie E. 2005. "Thesis: Efficacy of DNA Sampling to Monitor Population Abundance of Black Bears in the Southern Appalachians." Master's Thesis, University of Tennessee.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L.P. Waits, and

J. Bouvet. 1996. “Reliable Genotyping of Samples with Very Low DNA Quantities Using PCR.” *Nucleic Acids Research* 24 (16): 3189–94. doi:10.1093/nar/24.16.3189.

Thompson, L.M. 2003. “Abundance and Genetic Structure of Two Black Bear Populations prior to Highway Construction in Eastern North Carolina.” Master’s Thesis, Knoxville, Tennessee: University of Tennessee.

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: 249-256.

—— , L., P. Taberlet, J.E. Swenson, F. Sandergen, and R. Franzen. 2000. “Nuclear DNA Microsatellite Analysis of Genetic Diversity and Gene Flow in the Scandinavian Brown Bear (Ursus Arctos).” *Molecular Ecology* 9: 421–31.

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

Warrilow, J., M. Culver, and M. Vaughan. 2001. “Subspecific Affinity of Black Bears in the White River National Wildlife River.” *Journal of Heredity* 92 (3): 226–33.

Wasser, S.K., C.S. Houston, G.M. Koehler, G.G. Cadd, and S.R. Fain. 1997. “Techniques for Application of Faecal DNA Methods to Field Studies of Ursids.” *Molecular Ecology* 6 (11): 1091–97. doi:10.1046/j.1365-294X.1997.00281.x.

Weir, B. S. & Cockerham, C. C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

Wright, S. 1931. “Evolution in Mendelian Populations.” *Genetics*, 16: 97–159.

———, S. 1943. “Isolation by Distance.” *Heredity*, no. 28: 114–38.

Wultsch, C., L. P. Waits, E. M. Hallerman, and M. J. Kelly. 2015. “Optimizing collection methods for noninvasive genetic sampling of neotropical felids.” Wildlife Society Bulletin, 39: 403-412. Doi:10.1002/wsb.540.

Appendix 1

Microsatellite description (* indicates use in interstate population analyses and comparisons).

Primer ID	Multiplex	Name	sequence
G10C*	1	G10C Fp	5'-AAAGCAGAAGGCCTTGATTTCCTG-3'
		G10C Rp	5'-GGGGACATAAACACCGAGACAGC-3'
G10H*	1	G10H F	5'-CAACAAGAAGACCACTGTAA-3'
		G10H R	5'-AGAGACCACCAAGTAGGATA-3'
G10M	1	G10M FIm	5'-GTTTGCCTCTTTGCTACTGGA-3'
		G10M Rm	5'-CAAATAATTTAAATGCATCCCAGGGG-3'
G10P*	1	G10P F	5'-ATCATAGTTTTACATAGGAGGAAGAAA-3'
		G10P Rp	5'-TCATGTGGGGAAATACTCTGAA-3'
G10X*	1	G10XF	5'-CCCTGGTAACCACAAATCTCT-3'
		G10XR	5'-TCAGTTATCTGTGAAATCAAAA-3'
G1D	1	G1D Flm	5'-CCATCTCTCTTTTCCTTTAGGG-3'
		G1D Rlm	5'-CTACTCTTCCTACTCTTTAAGAG-3'
Mu15*	1	Mu15 F	5'-GCCTGACCATCCAACATC-3'
		Mu15 R	5'-AAATAAGGGAGGCTTGGGT-3'
Mu23	1	Mu23 F	5'-GCCTGTGTGCTATTTTATCC-3'
		Mu23 RI	5'-AATGGGTTTCTTGTTTAATTAC-3'
G10B	2	G10BF	5'-GCCTTTTAATGTTCTGTTGAATTTG-3'
		G10BR	5'-GACAAATCACAGAAACCTCCATCC-3'
D1A	2	D1a F	5'-GCTCCAAATGCTACACCCTCCTC-3'
		D1a R	5'-TCTCCCGCCCCTGCTTCTG-3'
G10L	2	G10L R	5'-GAAGATACAGAAACCTACCCATGC-3'
		G10L F	5'-GTACTGATTTAATTCACATTTCCC-3'
Mu50	2	Mu50 Fb	5'-GGAGGCGTTCTTTCAGTTGGT-3'
		Mu50 Rib	5'-TGGAACAAAACTTAACACAAATG-3'
G10U	2	G10U F	5'-TGCAGTGTCAGTTGTTAGGAA-3'
		G10U R	5'-GTATTTCCAATGCCCTAAGTGAT-3'
G1A	2	G1AF	5'-GACCCTGCATACTCTCCTCTGATG-3'
		G1AR	5'-GCACTGTCCTGCGTAGAAGTGAC-3'
SE 47+48	Sex Marker	SE 47	5'-CAGCCAAACCTCCCTCTGC-3'
		SE 48	5'-CCCGCTTGGTCTTGTCTGTTGC-3'

Appendix 2

Flat genotypes of MRB and NAL regions (MM1).

Sample	Pop	G10C		G10H		G10M		G10P		G10X		G1D		Mu15		Mu23	
25	MRB	105	105	243	243	120	130	156	156	149	149	117	0	131	137	154	154
20	MRB	105	107	243	243	120	120	156	156	149	149	117	0	137	137	154	154
A109	MRB	105	107	243	243	120	124	156	156	149	149	117	117	131	137	154	154
D1	MRB	105	105	243	243	120	124	156	156	149	149	117	117	131	133	154	170
C9	MRB	105	105	243	243	124	130	156	156	149	149	107	117	131	133	154	154
B103	MRB	105	111	243	243	120	124	156	156	149	149	117	117	133	137	154	154
C5	MRB	105	111	243	243	120	120	156	156	149	149	107	117	131	133	154	154
I10	MRB	107	107	243	243	124	124	156	156	149	149	117	117	133	137	154	170
S-4A1	MRB	105	105	243	243	120	130	156	156	149	161	107	117	131	137	154	170
B105	MRB	105	111	243	243	120	124	156	156	149	149	107	117	133	137	154	170
B101	MRB	105	105	243	243	120	120	156	156	149	149	117	117	131	133	154	170
S-4C9	MRB	105	111	243	243	120	124	156	156	149	149	107	117	137	137	154	154
S-5F7	MRB	105	107	243	243	120	130	156	156	149	149	107	117	137	137	154	154
S-6A7	MRB	105	111	243	243	120	124	156	156	149	149	117	117	133	137	154	154
B5	MRB	107	107	243	243	124	0	156	156	149	149	117	117	133	137	154	154
G27T	MRB	105	0	243	243	120	124	156	0	149	161	107	117	137	0	154	154
C30	MRB	105	111	243	243	120	120	156	156	149	149	107	117	131	131	154	170
H4	MRB	105	105	243	243	124	124	156	156	149	149	107	117	131	137	154	170
G25T	MRB	105	105	243	243	120	120	156	156	149	149	117	117	131	131	154	170
H105	MRB	105	105	243	243	120	120	156	156	149	149	117	0	131	133	154	170
S-4D3	MRB	105	105	243	243	120	124	156	156	149	149	117	117	131	131	154	154
D22	MRB	105	107	243	243	120	124	156	156	149	149	117	117	137	137	154	170
S-4I9	MRB	105	111	243	243	120	120	156	156	149	149	107	107	131	137	154	154
S-4B1	MRB	105	107	243	243	120	124	156	0	149	161	107	117	137	137	154	154
23	MRB	105	105	243	243	120	130	156	156	149	149	0	0	133	137	154	154
11	MRB	105	107	243	243	120	120	156	156	149	149	117	117	137	0	154	154
S-5D9	MRB	105	111	0	0	120	124	156	0	149	149	107	117	133	137	154	170
A29T	MRB	105	0	243	243	120	124	156	156	149	149	107	117	137	137	170	170
E22	MRB	105	107	243	243	120	120	156	156	149	149	117	117	131	137	154	170

2	MRB	105	107	243	243	120	124	156	156	149	149	0	0	131	131	0	0
18	MRB	105	105	243	243	124	130	156	156	149	149	0	0	131	137	154	170
C28	MRB	105	107	243	243	120	124	156	156	149	149	117	117	137	137	154	170
7	MRB	105	107	243	243	120	124	156	156	149	149	0	0	137	137	154	170
S-4I1	MRB	105	107	243	243	120	124	156	156	149	149	107	117	133	137	154	154
S-6B5	MRB	105	105	243	243	120	120	156	156	149	149	107	117	133	137	154	154
S-4I7	MRB	105	105	243	243	120	120	156	156	149	149	117	117	133	137	154	154
S-5D5	MRB	105	107	0	0	120	124	156	156	149	161	117	117	133	137	154	170
24	MRB	105	105	243	243	120	120	156	156	149	149	0	0	137	137	154	170
G21T	MRB	105	111	243	243	124	130	156	156	149	149	117	117	131	137	154	154
S-4D9	MRB	105	105	243	243	120	130	156	156	149	149	107	117	137	137	154	170
S-5H9	MRB	105	107	243	243	120	124	156	156	149	149	107	117	133	133	154	154
S-6H3	MRB	107	107	243	243	120	120	156	156	149	161	117	117	131	137	154	154
S-6C7	MRB	105	111	243	243	124	130	156	156	149	149	117	117	133	137	154	154
S-4E7	MRB	105	105	0	0	120	124	156	156	149	149	107	107	137	137	154	170
28	MRB	105	107	243	243	120	130	156	156	149	149	0	0	131	137	0	0
H-1A10	NAL	105	109	241	249	118	128	170	170	147	147	101	105	135	137	164	164
H-1A5	NAL	107	111	241	249	120	128	170	170	147	147	103	107	135	137	164	164
H-1B10	NAL	107	107	241	249	126	132	168	170	147	165	107	115	135	137	164	164
H-1B3	NAL	111	111	241	241	128	128	170	170	147	147	103	107	135	143	164	164
H-1C4	NAL	107	111	249	253	128	132	154	170	147	147	107	107	135	137	164	164
H-1D7	NAL	107	107	241	241	126	132	170	170	147	165	107	115	135	137	164	164
H-1E3	NAL	107	107	241	249	120	128	170	170	147	147	107	107	135	137	164	164
H-1F10	NAL	107	111	241	249	128	132	170	172	147	147	107	107	135	143	164	164
H-1F9	NAL	107	111	241	241	126	128	168	170	145	147	107	117	135	137	164	164
H-2J10	NAL	107	107	241	249	126	132	168	170	147	165	107	115	135	137	164	164
H-2J3	NAL	107	111	249	253	128	132	154	170	147	147	107	107	135	137	164	164
NAL02	NAL	101	109	243	243	126	132	170	172	147	165	0	0	137	137	158	164
NAL17	NAL	107	107	241	249	128	128	154	170	147	147	107	0	135	137	164	0
H-1G1	NAL	107	107	249	249	126	128	168	172	147	165	107	115	135	135	164	164
H-1E4	NAL	107	107	241	249	120	128	170	170	147	147	107	107	135	137	164	164

Appendix 3

Flat genotypes of MRB (MM1 and MM2).

Sample	POP	G10L		G10U		G1A		Mu50		G10C		G10H		G10M	
25	MRB	146	146	180	182	189	189	122	142	105	105	243	243	120	130
20	MRB	146	146	182	182	189	189	122	122	105	107	243	243	120	120
A109	MRB	146	146	180	182	189	189	122	142	105	107	243	243	120	124
D1	MRB	146	146	182	182	189	189	122	142	105	105	243	243	120	124
C9	MRB	146	146	182	182	189	189	142	142	105	105	243	243	124	130
B103	MRB	146	146	182	182	189	189	146	146	105	111	243	243	120	124
C5	MRB	0	0	180	182	189	189	122	146	105	111	243	243	120	120
I10	MRB	146	146	180	182	189	189	142	146	107	107	243	243	124	124
S-4A1	MRB	146	146	180	180	189	189	142	142	105	105	243	243	120	130
B105	MRB	146	146	182	182	189	189	122	122	105	111	243	243	120	124
B101	MRB	146	146	182	182	185	189	122	122	105	105	243	243	120	120
S-4C9	MRB	146	146	182	182	189	189	122	142	105	111	243	243	120	124
S-5F7	MRB	146	146	180	182	189	189	142	142	105	107	243	243	120	130
S-6A7	MRB	146	146	182	182	189	189	142	146	105	111	243	243	120	124
B5	MRB	146	146	180	182	189	189	142	146	107	107	243	243	124	0
G27T	MRB	146	146	182	182	189	189	142	146	105	0	243	243	120	124
C30	MRB	0	0	180	182	189	189	142	146	105	111	243	243	120	120
H4	MRB	146	146	182	182	189	189	122	122	105	105	243	243	124	124
G25T	MRB	146	0	180	0	189	189	142	142	105	105	243	243	120	120

Sample	POP	G10P		G10X		G1D		Mu15		Mu23		SE47-48		D1A		G10B	
25	MRB	156	156	149	149	117	0	131	137	154	154	240	240	165	165	150	150
20	MRB	156	156	149	149	117	0	137	137	154	154	240	240	165	165	150	150
A109	MRB	156	156	149	149	117	117	131	137	154	154	240	240	165	165	150	150
D1	MRB	156	156	149	149	117	117	131	133	154	170	240	240	165	165	150	150
C9	MRB	156	156	149	149	107	117	131	133	154	154	188	240	165	165	150	150
B103	MRB	156	156	149	149	117	117	133	137	154	154	240	240	165	165	150	150
C5	MRB	156	156	149	149	107	117	131	133	154	154	240	240	165	165	150	160
I10	MRB	156	156	149	149	117	117	133	137	154	170	240	240	165	165	150	150
S-4A1	MRB	156	156	149	161	107	117	131	137	154	170	240	240	165	165	150	150
B105	MRB	156	156	149	149	107	117	133	137	154	170	240	240	165	165	150	160
B101	MRB	156	156	149	149	117	117	131	133	154	170	240	240	165	165	150	150
S-4C9	MRB	156	156	149	149	107	117	137	137	154	154	240	240	165	165	160	160
S-5F7	MRB	156	156	149	149	107	117	137	137	154	154	240	240	165	165	150	160
S-6A7	MRB	156	156	149	149	117	117	133	137	154	154	240	240	165	165	150	150
B5	MRB	156	156	149	149	117	117	133	137	154	154	188	240	165	165	150	150
G27T	MRB	156	0	149	161	107	117	137	0	154	154	188	240	165	165	150	150
C30	MRB	156	156	149	149	107	117	131	131	154	170	188	240	165	165	150	160
H4	MRB	156	156	149	149	107	117	131	137	154	170	240	240	165	165	150	150
G25T	MRB	156	156	149	149	117	117	131	131	154	170	188	240	165	165	150	150

Sample	POP	G10L		G10U		G1A		Mu50		G10C		G10H		G10M	
H105	MRB	146	146	182	182	189	189	142	142	105	105	243	243	120	120
S-4D3	MRB	146	146	182	182	189	189	122	142	105	105	243	243	120	124
D22	MRB	146	146	180	180	189	189	142	146	105	107	243	243	120	124
S-4I9	MRB	146	146	182	182	189	189	122	122	105	111	243	243	120	120
S-4B1	MRB	146	146	180	180	189	189	142	142	105	107	243	243	120	124
23	MRB	146	146	180	182	189	189	122	142	105	105	243	243	120	130
11	MRB	146	146	182	182	189	189	122	142	105	107	243	243	120	120
S-5D9	MRB	146	146	182	182	189	189	122	122	105	111	0	0	120	124
A29T	MRB	146	146	182	182	189	0	122	142	105	0	243	243	120	124
E22	MRB	146	146	180	182	189	189	122	142	105	107	243	243	120	120
2	MRB	146	146	180	182	189	193	142	146	105	107	243	243	120	124
18	MRB	146	146	182	182	189	189	122	122	105	105	243	243	124	130
C28	MRB	146	0	182	182	189	0	142	142	105	107	243	243	120	124
7	MRB	146	146	182	182	189	189	122	142	105	107	243	243	120	124
S-4I1	MRB	146	146	182	182	189	189	122	122	105	107	243	243	120	124
S-6B5	MRB	146	146	182	182	189	189	142	142	105	105	243	243	120	120
S-4I7	MRB	146	146	182	182	189	189	122	122	105	105	243	243	120	120
S-5D5	MRB	146	146	180	182	189	189	0	0	105	107	0	0	120	124

Sample	POP	G10P		G10X		G1D		Mu15		Mu23		SE47-48		D1A		G10B	
H105	MRB	156	156	149	149	117	0	131	133	154	170	240	240	165	165	150	160
S-4D3	MRB	156	156	149	149	117	117	131	131	154	154	240	240	165	165	150	150
D22	MRB	156	156	149	149	117	117	137	137	154	170	240	240	165	165	150	150
S-4I9	MRB	156	156	149	149	107	107	131	137	154	154	240	240	165	165	150	150
S-4B1	MRB	156	0	149	161	107	117	137	137	154	154	240	240	165	165	150	150
23	MRB	156	156	149	149	0	0	133	137	154	154	188	240	165	165	150	150
11	MRB	156	156	149	149	117	117	137	0	154	154	240	240	165	165	150	150
S-5D9	MRB	156	0	149	149	107	117	133	137	154	170	188	240	165	165	150	160
A29T	MRB	156	156	149	149	107	117	137	137	170	170	240	240	165	165	150	150
E22	MRB	156	156	149	149	117	117	131	137	154	170	240	0	165	165	150	150
2	MRB	156	156	149	149	0	0	131	131	0	0	240	240	165	165	150	160
18	MRB	156	156	149	149	0	0	131	137	154	170	188	240	165	165	150	150
C28	MRB	156	156	149	149	117	117	137	137	154	170	240	240	165	165	150	150
7	MRB	156	156	149	149	0	0	137	137	154	170	240	240	165	165	150	160
S-4I1	MRB	156	156	149	149	107	117	133	137	154	154	240	240	165	165	150	150
S-6B5	MRB	156	156	149	149	107	117	133	137	154	154	240	240	165	165	150	160
S-4I7	MRB	156	156	149	149	117	117	133	137	154	154	188	240	165	165	150	150
S-5D5	MRB	156	156	149	161	117	117	133	137	154	170	240	240	165	165	150	150

Sample	POP	G10L		G10U		G1A		Mu50		G10C		G10H		G10M	
24	MRB	146	146	180	180	189	189	122	142	105	105	243	243	120	120
G21T	MRB	146	146	180	182	189	189	122	142	105	111	243	243	124	130
S-4D9	MRB	146	146	180	182	189	189	142	0	105	105	243	243	120	130
S-5H9	MRB	146	146	180	182	189	189	146	146	105	107	243	243	120	124
S-6H3	MRB	146	146	182	182	189	189	122	142	107	107	243	243	120	120
S-6C7	MRB	146	146	182	182	189	189	122	146	105	111	243	243	124	130
S-4E7	MRB	146	146	182	182	189	189	142	142	105	105	0	0	120	124
28	MRB	146	146	182	182	189	193	142	146	105	107	243	243	120	130

Sample	POP	G10P		G10X		G1D		Mu15		Mu23		SE47-48		D1A		G10B	
24	MRB	156	156	149	149	0	0	137	137	154	170	240	240	165	165	150	150
G21T	MRB	156	156	149	149	117	117	131	137	154	154	188	240	165	165	150	150
S-4D9	MRB	156	156	149	149	107	117	137	137	154	170	188	240	165	165	160	160
S-5H9	MRB	156	156	149	149	107	117	133	133	154	154	240	240	165	165	150	150
S-6H3	MRB	156	156	149	161	117	117	131	137	154	154	188	240	165	165	150	160
S-6C7	MRB	156	156	149	149	117	117	133	137	154	154	188	240	165	165	150	150
S-4E7	MRB	156	156	149	149	107	107	137	137	154	170	188	240	165	165	150	150
28	MRB	156	156	149	149	0	0	131	137	0	0	188	240	165	165	150	150

Appendix 4

Flat genotypes of all individuals (microsatellite multiplex 3).

Sample	Pop	G10C		G10H		G10P		G10X		Mu15	
BR10-004	TN	103	107	249	249	170	176	147	153	133	139
BR10-006	TN	109	109	247	249	172	174	147	147	0	0
BR10-007	TN	107	107	243	253	168	168	147	165	139	141
BR10-008	TN	107	109	243	243	170	172	147	165	135	139
BR10-009	TN	115	115	249	249	154	174	149	165	135	137
BR10-012	TN	107	107	249	253	170	170	145	153	135	141
BR10-013	TN	109	113	249	257	170	174	145	165	135	139
BR10-024	TN	107	109	249	253	172	176	145	165	135	137
BR10-026	TN	107	109	241	249	176	176	145	145	137	137
BR10-033	TN	107	109	243	249	170	176	147	165	135	137
BR10-044	TN	107	109	241	253	168	174	147	165	135	137
BR10-048	TN	107	115	249	253	154	174	147	165	133	135
BR10-049	TN	109	111	243	243	168	168	145	147	135	137
BR10-055	TN	107	109	243	243	168	170	165	165	133	135
BR10-056	TN	101	107	243	243	168	168	147	165	135	135
BR10-059	TN	109	109	249	253	172	172	147	149	137	139
BR10-060	TN	101	109	249	253	0	0	147	147	135	135
BR10-063	TN	107	107	257	257	172	174	147	149	133	137
BR10-069	TN	103	107	243	249	174	176	145	165	139	139
BR10-070	TN	111	111	253	253	168	168	145	153	133	135
BR10-077	TN	107	111	263	263	172	172	147	147	135	137
BR10-100	TN	109	113	241	249	0	0	165	170	137	139
BR10-112	TN	103	111	243	243	168	168	147	161	135	137
BR10-126	TN	107	107	243	243	168	172	165	165	133	139
BR10-130	TN	109	115	249	253	170	174	147	165	135	137
BR10-132	TN	107	109	243	243	160	168	165	165	135	137
BR10-141	TN	107	109	249	249	168	170	149	165	133	137
BR10-142	TN	109	109	247	249	172	172	147	147	137	141
BR10-163	TN	107	107	243	249	170	176	147	153	135	135
BR10-246	TN	107	107	249	249	174	174	147	165	137	139
MS-AR1	MS	109	109	241	243	160	160	147	153	141	141
MS-F920	MS	109	109	241	243	160	160	147	153	141	141
MS-G470	MS	109	109	243	243	156	160	147	147	135	137
MS-I789	MS	109	109	241	241	156	172	153	153	137	141
MS-II28	MS	105	105	243	243	156	156	149	149	137	137
MS-J320	MS	109	113	241	243	160	160	153	153	135	141
MS-JJ30	MS	105	105	243	243	156	156	149	161	131	137
MS-K515	MS	105	107	243	243	156	156	149	149	131	137
MS-KK31	MS	109	109	241	241	160	160	153	153	137	141
MS-N528	MS	105	105	243	243	156	156	149	161	131	137
MS-O800	MS	109	109	241	243	166	172	153	153	137	137
MS-P16	MS	107	107	243	243	156	156	149	149	137	137
MS-RCB9	MS	105	105	243	243	156	156	149	149	133	137

MS-U755	MS	109	109	241	243	156	160	153	153	135	135
MS-W23	MS	111	111	241	243	156	160	147	153	135	137
GA-101	GA	107	107	245	249	176	176	149	149	133	135
GA-102	GA	107	107	249	249	172	176	149	149	135	137
GA-104	GA	107	109	249	249	176	176	149	149	135	137
GA-107	GA	107	107	249	249	172	176	149	149	137	137
GA-108	GA	107	107	241	249	176	176	155	155	135	135
GA-109	GA	107	107	249	249	172	172	149	149	137	137
GA-110	GA	107	107	241	249	172	172	149	149	137	139
GA-111	GA	107	109	241	249	172	176	149	149	135	135
GA-113	GA	107	107	249	249	176	176	149	149	137	137
GA-114	GA	107	107	241	249	172	176	149	155	135	137
GA-115	GA	107	109	241	249	172	176	149	149	137	137
GA-116	GA	107	107	249	249	172	172	147	149	133	133
GA-118	GA	107	107	249	249	172	176	149	149	137	137
GA-119	GA	107	107	249	249	172	176	149	149	133	135
GA-120	GA	107	107	249	249	176	176	147	155	135	137
GA-121	GA	107	107	247	249	172	172	147	149	137	137
GA-122	GA	107	107	249	249	172	176	149	149	135	137
GA-123	GA	107	107	249	249	172	176	149	149	133	137
GA-124	GA	107	107	249	249	172	176	147	149	137	139
GA-125	GA	107	107	249	249	172	176	149	149	135	135
25	MRB	105	105	243	243	156	156	149	149	131	137
20	MRB	105	107	243	243	156	156	149	149	137	137
A109	MRB	105	107	243	243	156	156	149	149	131	137
D1	MRB	105	105	243	243	156	156	149	149	131	133
C9	MRB	105	105	243	243	156	156	149	149	131	133
B103	MRB	105	111	243	243	156	156	149	149	133	137
C5	MRB	105	111	243	243	156	156	149	149	131	133
I10	MRB	107	107	243	243	156	156	149	149	133	137
S-4A1	MRB	105	105	243	243	156	156	149	161	131	137
B105	MRB	105	111	243	243	156	156	149	149	133	137
B101	MRB	105	105	243	243	156	156	149	149	131	133
S-4C9	MRB	105	111	243	243	156	156	149	149	137	137
S-5F7	MRB	105	107	243	243	156	156	149	149	137	137
S-6A7	MRB	105	111	243	243	156	156	149	149	133	137
B5	MRB	107	107	243	243	156	156	149	149	133	137
G27T	MRB	105	0	243	243	156	0	149	161	137	0
C30	MRB	105	111	243	243	156	156	149	149	131	131
H4	MRB	105	105	243	243	156	156	149	149	131	137
G25T	MRB	105	105	243	243	156	156	149	149	131	131
H105	MRB	105	105	243	243	156	156	149	149	131	133
S-4D3	MRB	105	105	243	243	156	156	149	149	131	131
D22	MRB	105	107	243	243	156	156	149	149	137	137

S-4I9	MRB	105	111	243	243	156	156	149	149	131	137
S-4B1	MRB	105	107	243	243	156	0	149	161	137	137
23	MRB	105	105	243	243	156	156	149	149	133	137
11	MRB	105	107	243	243	156	156	149	149	137	0
S-5D9	MRB	105	111	0	0	156	0	149	149	133	137
A29T	MRB	105	0	243	243	156	156	149	149	137	137
E22	MRB	105	107	243	243	156	156	149	149	131	137
2	MRB	105	107	243	243	156	156	149	149	131	131
18	MRB	105	105	243	243	156	156	149	149	131	137
C28	MRB	105	107	243	243	156	156	149	149	137	137
7	MRB	105	107	243	243	156	156	149	149	137	137
S-4I1	MRB	105	107	243	243	156	156	149	149	133	137
S-6B5	MRB	105	105	243	243	156	156	149	149	133	137
S-4I7	MRB	105	105	243	243	156	156	149	149	133	137
S-5D5	MRB	105	107	0	0	156	156	149	161	133	137
24	MRB	105	105	243	243	156	156	149	149	137	137
G21T	MRB	105	111	243	243	156	156	149	149	131	137
S-4D9	MRB	105	105	243	243	156	156	149	149	137	137
S-5H9	MRB	105	107	243	243	156	156	149	149	133	133
S-6H3	MRB	107	107	243	243	156	156	149	161	131	137
S-6C7	MRB	105	111	243	243	156	156	149	149	133	137
S-4E7	MRB	105	105	0	0	156	156	149	149	137	137
28	MRB	105	107	243	243	156	156	149	149	131	137
H-1A10	NAL	105	109	241	249	170	170	147	147	135	137
H-1A5	NAL	107	111	241	249	170	170	147	147	135	137
H-1B10	NAL	107	107	241	249	168	170	147	165	135	137
H-1B3	NAL	111	111	241	241	170	170	147	147	135	143
H-1C4	NAL	107	111	249	253	154	170	147	147	135	137
H-1D7	NAL	107	107	241	241	170	170	147	165	135	137
H-1E3	NAL	107	107	241	249	170	170	147	147	135	137
H-1F10	NAL	107	111	241	249	170	172	147	147	135	143
H-1F9	NAL	107	111	241	241	168	170	145	147	135	137
H-2J10	NAL	107	107	241	249	168	170	147	165	135	137
H-2J3	NAL	107	111	249	253	154	170	147	147	135	137
NAL02	NAL	101	109	243	243	170	172	147	165	137	137
NAL17	NAL	107	107	241	249	154	170	147	147	135	137
H-1G1	NAL	107	107	249	249	168	172	147	165	135	135
H-1E4	NAL	107	107	241	249	170	170	147	147	135	137

Appendix 5

Measures of bear genetic diversity throughout the literature.

Species	Population / Region	n	Ho	He	A	Loci	Citation
Florida Black Bear							
(Ursus americanus floridanus)	MRB	51	0.256	0.246	1.28	5	Chapter 2 of this study
	Chassahowitzka	29	0.287	0.271	2.25	12	Dixon, 2005
	Glades	28	0.327	0.384	2.75	12	Dixon, 2005
	Eglin	40	0.613	0.537	4.08	12	Dixon, 2005
	Ocala	40	0.579	0.61	4.75	12	Dixon, 2005
	Aucilla	9	0.556	0.616	3.83	12	Dixon, 2005
	Mobile River	13	0.39	0.629	2.88	7	Warrilow et al., 2001
	Big Cypress	41	0.642	0.65	5.5	12	Dixon, 2005
	Saint Johns	40	0.65	0.663	5.58	12	Dixon, 2005
	Apalachicola	40	0.69	0.708	5.92	12	Dixon, 2005
	Osceola	41	0.705	0.713	6.67	12	Dixon, 2005
	South Alabama	19	0.316	N/A	2.88	8	Edwards, 2002
	Okefenokee	39	0.663	N/A	6.13	8	Dobey, 2002
	Osceola	37	0.679	N/A	5.75	8	Dobey, 2003
Louisiana Black Bear	Coastal Louisiana	20	0.38	0.428	4.2	5	Csiki et al., 2003
(Ursus americanus luteolus)	Tensas River	14	0.52	0.48	3.57	7	Warrilow et al., 2001
	Lower Atchafalaya Basin	26	0.42	0.54	6.14	7	Warrilow et al., 2001
	Upper Atchafalaya Basin	20	0.55	0.66	6	7	Warrilow et al., 2001
	Pointe Coupee Parish	16	0.546	0.686	5.6	5	Csiki et al., 2003
	Ouchitas	6	0.733	0.754	4.6	5	Csiki et al., 2003
	Ozarks	13	0.723	0.761	5.8	5	Csiki et al., 2003
	Tensas River	36	0.576	N/A	3.8	12	Boersen et al., 2003
	North Carolina (treatment)	66	0.667	N/A	6	10	Thompson, 2003
	North Carolina (control)	115	0.664	N/A	6.9	10	Thompson, 2003

Species	Population / Region	n	Ho	He	A	Loci	Citation
American Black Bear	White River	18	0.447	0.317	1.8	5	Csiki et al., 2003
(Ursus americanus)	White River	22	0.38	0.33	2.43	7	Warrilow et al., 2001
	Central Georgia Population (GAP)	21	0.362	0.401	1.95	5	Chapter 2 of this study
	Terra Nova	23	0.36	0.36	2.25	4	Paetkau and Strobeck, 1994
	Newfoundland Islands	33	0.427	0.414	3	8	Paetkau and Strobeck, 1998
	Mississippi Population (MSP)	10	0.44	0.515	2.28	5	Chapter 2 of this study
	Northeastern Alabama (NAL)	16	0.588	0.513	2.29	5	Chapter 2 of this study
	Tennessee Population of Great Smokey Mtn. (TNP)	27	0.698	0.735	3.12	5	Chapter 2 of this study
	Nimpkish	19	N/A	0.621	4.4	10	Marshall et al., 2002
	Gribbel Island	16	N/A	0.664	5.4	10	Marshall et al., 2002
	Don Penninsula	23	N/A	0.667	5.9	10	Marshall et al., 2002
	Roderick Island	11	N/A	0.668	4.8	10	Marshall et al., 2002
	North of Roderick	13	N/A	0.673	6.6	10	Marshall et al., 2002
	Pooley Island	10	N/A	0.692	5	10	Marshall et al., 2002
	Hawkesbury Island	20	N/A	0.699	5.7	10	Marshall et al., 2002
	Princess Royal Island	50	N/A	0.707	6.5	10	Marshall et al., 2002
	West of Hawkesbury	6	N/A	0.724	4.2	10	Marshall et al., 2002
	Yeo Island	10	N/A	0.725	5.1	10	Marshall et al., 2002
	Ozarks	14	0.54	0.73	6	7	Warrilow et al., 2001
	Ouchitas	6	0.56	0.73	4.86	7	Warrilow et al., 2001
	East of Princess Royal Island	21	N/A	0.747	6.3	10	Marshall et al., 2002
	Cook County	36	0.54	0.77	8.71	7	Warrilow et al., 2001
	Minnesota	10	0.576	0.772	5.6	5	Csiki et al., 2003
	Terrace	17	N/A	0.793	7.5	10	Marshall et al., 2002
	La Mauricie	32	0.783	0.8	8.75	4	Paetkau and Strobeck, 1994
	Banff	31	0.801	0.8	8	4	Paetkau and Strobeck, 1994
	West Slope	116	0.8	0.806	9.5	8	Paetkau and Strobeck, 1998

Species	Population / Region	n	Ho	He	A	Loci	Citation
Grizzly Bear	NN	29	0.66	0.66	5.5	19	Waits et al., 2000
(Ursus arctos)	NS	108	0.66	0.66	6.2	19	Waits et al., 2000
	M	88	0.65	0.66	5.8	19	Waits et al., 2000
	Franz Josef	32	N/A	0.66	6.7	16	Paetkau et al. 1999
	South Beaufort Sea	30	N/A	0.69	6.4	16	Paetkau et al. 1999
	Svalbard	31	N/A	0.69	6.9	16	Paetkau et al. 1999
	East Greenland	31	N/A	0.69	6.8	16	Paetkau et al. 1999
	North Beaufort Sea	30	N/A	0.7	6.8	16	Paetkau et al. 1999
	Chukchi Sea	30	N/A	0.7	6.8	16	Paetkau et al. 1999
Spectacled Bear	Ecuador	42	N/A	0.245	3	4	Ruiz-Garcia, 2003
(Tremarctos oranatus)	Colombia	32	N/A	0.392	2.8	4	Ruiz-Garcia, 2003
	Venezuela	8	N/A	0.607	2	4	Ruiz-Garcia, 2003
Asian Black Bear	West Chugoku	52	0.272	0.3	2	6	Saitoh et al., 2001
(Ursus thibetanus)	East Chugoku	24	0.243	0.301	2.5	6	Saitoh et al., 2001
	West Kinki	66	0.311	0.324	3.33	6	Saitoh et al., 2001
	East Kinki	67	0.445	0.45	4.17	6	Saitoh et al., 2001
Giant Panda	Qinling	14	0.57	N/A	3.3	18	Lu et al., 2001
(Ailuropoda melanoleuca)	Minshan	7	0.58	N/A	3.5	18	Lu et al., 2001
	Qionlai	15	0.49	N/A	4.3	18	Lu et al., 2001