Genetic Diversity and Connectivity of Black Bears (Ursus americanus) in Alabama.

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 6, 2017

Keywords: Black Bear, *Ursus americanus*, Genetic Diversity, Genetic Structure, Population Modeling,

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Abstract

One of the major concerns in conservation today is the loss of genetic diversity which is a frequent consequence of population isolation and small population sizes. Fragmentation of populations and persecution of carnivores has posed a substantial threat to the persistence of free ranging carnivores in North America since the arrival of European settlers. Black bears have seen significant reductions in range size from their historic extent, which is most pronounced in the southeastern United States and even more starkly in Alabama where until recently bears were reduced to a single geographically isolated population in the Mobile River Basin. Recently a second population has naturally re-established itself in northeastern Alabama. We sought to determine size, genetic diversity and genetic connectivity for these two populations.

Both populations of black bears in Alabama had small population sizes and had moderate to low genetic diversity, but showed different levels of connectivity to surrounding populations of bears. The Mobile River Basin population had a small population size at only 86 individuals (76-124, 95% C.I.), the lowest genetic diversity of compared populations (richness =2.33, H_o and H_e =0.33), and showed near complete genetic isolation from surrounding populations across multiple tests. The newly recolonizing population in northeastern Alabama had a small but growing population doubling in 3 years (34 individuals 26-43, 95% C.I.), relatively moderate genetic diversity compared to surrounding populations (richness = 3.32, H_o =0.53, H_e =0.65), and showed a high level of genetic connectivity with surrounding populations.

Acknowledgments

I would like to first and foremost thank the Alabama Department of Conservation and Natural resources for funding my research. Additionally ADCNR provided continual logistical support, and a genuine interest in the work that we were doing which always helped keep me motivated. I would like to thank my advisor and entire committee; Dr. Steury, Dr. Waits, Dr. Grand and Dr. Santos, all of you have imbued me with invaluable skills and knowledge, both through coursework and personal tutelage. Additionally I would like to thank Dr. Jen Adams for showing me the ropes in genetics lab work and analysis, I would have been lost without her help.

This thesis would not have come to be had it not been for the numerous people who helped to collect the data in the field. Most notably Mary Shew with the National Park Service at Little River Canyon National Preserve and her staff provided an incredible amount of data collected from inside of the park at the core of the northern black bear population in Alabama. Far too many hardworking individuals to list made up the field crews for both study areas over the years, but I would like to specifically thank Sam Johnston for both his multiple stellar seasons on the project, but also for acting as this Californian's early guide to Alabama. Logistical support was also provided by Jacksonville State University, through use of their facilities at the Little River Canyon Center. Finally and probably most notably this research would not have been possible without the generous cooperation of numerous private land owners who allowed us to sample on their property, and often bent over backwards to help us, by lending us equipment, clearing roads, or otherwise going above and beyond to help this project be a success.

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List of Abbreviations

AIC_C	Akaike's information Criterion corrected for small sample size
CGA	Central Georgia
CMR	Capture mark recapture
DAPC	Discriminant analysis of principle components
DETs	Dimethyl sulfoxide Ethylenediaminetetraacetic Tris
DNA	deoxyribonucleic acid
ECM	even capture model
FL	Florida
Fst	Fixation index
G"st	Fixation index corrected for hypervariable loci and small number of populations
H_e	Heterozygosity expected
H_o	Heterozygosity observed
HWE	Hardy Weinberg Equilibrium
K	Number of genetically distinct populations
LE	Linkage equilibrium
LIRI	Little River Canyon National Preserve
LRT	Likelihood ratio test
MRB	Mobile River Basin
MS	Mississippi

$\hat{N}$	Estimate of population size
NAL	North Alabama
NCC	Costal North Carolina
NCM	Montane North Carolina
NGA	North Georgia
PCR	Polymerase chain reaction
PID_{sibs}	Probability of identity between siblings
TIRM	Two innate rates model
TN	Tennessee
WV	West Virginia

Chapter 1: Black bear (Ursus americanus) population structure and genetic exchange in Alabama

Intro

Fragmentation and loss of connectivity of populations has plagued North American wildlife since the arrival and expansion of European settlers (Shaw and Lee 1997, Woodroffe 2000, Sargeant et al. 2012, Unger et al. 2012). Increases in urbanization, agriculture, and extractive industries have fragmented the physical landscape and destroyed habitat, while market hunting and persecution of perceived or real competitors and threats to human interests (livestock, agriculture, and personal safety) have directly caused separation of formerly contiguous populations (Noss et al. 1996, Suryawanshi et al. 2013). Isolated populations are more vulnerable to local extinction and loss of genetic diversity; connectivity among populations can facilitate the restocking of low population numbers and the maintenance or addition of genetic variability (Wilcox and Murphy 1985, Burel and Baudry 2003, Aurambout et al. 2005). Isolated populations are also at a higher risk for genetic drift – the fixation of alleles due to random mating events (Balkenhol et al. 2015) – and inbreeding depression, which is the negative effect on survival and reproduction of individuals within a population due to low genetic diversity (Charlesworth and Charlesworth 1987). Despite the dire consequences of inbreeding depression, genetic rescue – the introduction of beneficial genetic variation – can meaningfully improve both fitness and measures of genetic diversity with the introduction of a single individual (Tallmon et al. 2004, Hedrick and Garcia-Dorado 2016). Consequently, persistence of free ranging populations of wildlife often depends on connectivity among individual sub-populations.

Isolation is a common cause of inbreeding depression in wild carnivore populations (Liberg et al. 2005, Räikkönen et al. 2009, Norén et al. 2016), making it important to identify carnivore populations that have been affected by habitat loss and fragmentation. One such species of carnivore, black bear (*Ursus americanus*), has suffered fragmentation and a significant range reduction to 45% of its historic extent across North America (Servheen 1990, Pelton and Van Manen 1997, Unger et al. 2012, Scheick and McCown 2014). This trend is more extreme in the Southeastern United States, where the black bear range has been reduced to 20% of its historic extent (Pelton and Van Manen 1997) with substantial fragmentation among the remaining populations (Howell 1921, Triant et al. 2004, Dixon et al. 2007). For example, black bears in Alabama were reduced to a single isolated population in the Mobile River Basin (MRB; Howell 1921). This population was determined to have a low probability for long-term persistence by the U.S. Fish and Wildlife Service due to its small population size and isolation from other bear populations (U.S. Fish and Wildlife Service 1998). Recently, a second population has re-established itself in Northeastern Alabama (NAL), with an unknown identity of or level of connectivity with its source population. Understanding how these populations interact with each other and other regional black bear populations is important to managing their continued persistence.

In this study, we sought to evaluate the level of genetic exchange between black bear populations in Alabama and among those in nearby states. Our objectives were to: 1) determine the degree of genetic structure between the MRB, and NAL populations within Alabama, and between Alabama and populations in surrounding states, and 2) evaluate the level of genetic exchange between all pairs of populations. Such information is critical for evaluating the potential long-term viability of black bears in Alabama. We predict that the MRB population will

be highly isolated from surrounding populations with little to no current genetic exchange, while the NAL population will show minimal structure and continuing genetic exchange with surrounding populations.

Methods

Study Area

Bears within Alabama were sampled from two breeding populations. The MRB population samples were collected from Mobile and Washington counties in the south of the state between the Mississippi border and the Tombigbee River. The NAL samples were collected in the northeastern corner of the state in a small portion of Cherokee and DeKalb counties in and around Little River Canyon National Preserve. These two areas represent the entirety of breeding populations of black bears in Alabama (Figure 1).

Within the MRB study area, bears utilized both natural and human-dominated landscapes. Natural and near natural habitat available to bears in the MRB varied from woody wetlands to pine plantations (Homer et al. 2015). Bears also utilized areas in close proximity to suburban and exurban homes, often frequenting yards without the knowledge of the homeowners (Seals, unpublished data). Roughly 500,000 people lived within the sampling area at the time of study (United States Census Bureau n.d.).

The NAL bear population had a core distribution in Little River National Preserve (LIRI) in DeKalb County. The habitat in this region was mountainous and dominated by deciduous forests and pine plantations (Homer et al. 2015). In addition to federally managed lands including LIRI and Talladega National Forest, many large tracts of land were managed privately for hunting and/or timber production, providing much more continuous habitat than seen in the MRB. Similarly, human population density was lower, with only roughly 130,000 people

residing within parts of the study area potentially occupied by bears (United States Census Bureau n.d.).

Five comparison populations outside of Alabama were identified, including two different populations in Georgia previously shown to be genetically isolated from each other North Georga (NGA) and central Georgia (CGA; Hooker et al. 2015), Mississippi (MS), Florida (FL), and Tennessee (TN, Figure 2). The Mississippi samples came from throughout the state whereas Florida samples were collected solely from the panhandle region. The samples from Tennessee came from the northwestern Great Smokey Mountains National Park.

DNA Collection

Systematic sampling for black bears took place across the known range of both Alabama populations. The study areas were overlain with a sampling grid of 64 km^2 cells (Figure 1), which was approximately the home range size of male black bears in Alabama (Edwards 2002). One hair snare was placed in each cell, with micro-site selection determined by land access, and biotic and abiotic factors affecting bear movement on the landscape (topography, water sources, food sources, etc.). In cells where bear sign (e.g. hair snare hits, camera images, tracks, scat, anecdotal reports) was found, additional hair snares were set to increase the probability of detection for females, whose home ranges are closer to 7 km^2 (Edwards 2002).

Sampling was carried out from 2012-2015 by deploying minimally invasive hair snares. Hair snares consisted of a single strand of barbed wire placed around multiple trees at 45 centimeters above the ground to create a corral with a perimeter between 20-30 meters (Kendall and Mckelvey 2008, Figure 3). Bait was suspended in the center of the corral, such that it was greater than 2 meters from the barbed wire in any direction. Baits included canned fish and flavoring extracts applied to a tampon and suspended sufficiently high to prevent retrieval by a

bear and a subsequent food reward, which could cause the animal to become trap happy or otherwise affect behavior outside of the study parameters (Kendall and Mckelvey 2008).

Hair snare sampling took place from August through November when bears were in hyperphagia and most active before denning for the winter (Garshelis et al. 1983, Noyce and Garshelis 2011). Sites were checked regularly with 6-8 days between checks. Hair samples were collected from the barbed wire snares using hemostats/tweezers. Collection tools were flamed both before and after collection to prevent any possible contamination or mixing of samples. Following collection of a sample, the barb it was collected from, as well as the adjacent barbs, were flamed to prevent mixing samples (Kendall and Mckelvey 2008). Collected samples were placed in paper coin envelopes and stored in a secondary container with desiccant to prevent degradation of the sample due to moisture (Settlage et al. 2008).

The MRB study area was also sampled using scat-detection dogs during 2011 and 2012. Scat detection dogs allowed for more efficient and complete collection of scat along transects (Mackay et al. 2008). Dogs were trained to seek out bear scat by the EcoDogs program at Auburn University, and were each accompanied by a trained handler on all transects in addition to a biologist. Each transect was a triangle consisting of 0.5 kilometer segments, totaling a 1.5 kilometer transect. Transects were sampled across one month both fall seasons. Scats were also collected when located incidentally in both study areas throughout the study period. Scrapings were taken from the most desiccated section of each scat sample to minimize potential hydrolytic degradation and were stored in 1.4ml of DETs buffer to displace any remaining water (Murphy et al. 2002).

DNA samples from nearby populations were obtained using a variety of methods (Figure 2). Samples from central Georgia (CGA) were provided by Dr. Michael Chamberlain of the University of Georgia's Warnell School of Forestry and Natural Resources, as tissue samples from captured individuals. Northern Georgia (NGA) samples were obtained from the Georgia Department of Natural Resources as tissue samples from hunter-harvested bears. Dr. Jerrold L. Belant of Mississippi State University provided hair samples from a hair snaring study he conducted across Mississippi (MS). The Florida (FL) samples were hair samples collected from management activities conducted by the Florida Fish and Wildlife Commission. The samples from Tennessee (TN) were obtained from Katie Settlage, in the form of DNA extract from hair samples collected for her master's thesis research (Settlage 2005, Settlage et al. 2008).

DNA Analysis

DNA analysis of all collected samples was performed at the Laboratory for Ecological, Evolutionary and Conservation Genetics in the College of Natural Resources at the University of Idaho. DNA was extracted from samples using DNeasy Blood and Tissue Kits for hair and tissue and QIAamp Fast DNA Stool Mini Kit for scat (Qiagen Inc., Valencia, CA). All samples were extracted in a lab space dedicated to the extraction of low quality DNA, and an extraction negative was included in each extraction to monitor for contamination. Collected scat samples were verified as bear using a mitochondrial DNA fragment species identification test as described in De Barba et al. (2014). Scat samples, which were confirmed to be black bear and all hair samples were then identified to individual, using a microsatellite multiplex of 8 loci and a sex identification marker (G10C, G10H, G10M, G10P, G10X, G1D, Mu15, Mu23, and SE47+48; (Ennis and Gallagher 1994, Paetkau and Strobeck 1995, Paetkau et al. 1995, 1998, Taberlet et al. 1997, Waits et al. 1998, McCarthy et al. 2009, De Barba and Waits 2010; Table

1). PCR products were visualized on an Applied Biosystems 3130xL genetic analyzer and allele sizes were scored and evaluated in GeneMapper 5.0 (Applied Biosystems, Foster City, California). Due to the low genetic diversity of the MRB population, an additional 6 microsatellite loci were needed to identify individuals (G10B, D1A, G10L, Mu50, G10U and G1A; Paetkau and Strobeck 1994, 1995, Taberlet et al. 1997, Paetkau et al. 1998, Bellemain and Taberlet 2004, Graham 2016; Table 1.). Once NAL samples were assigned to an individual, a representative sample from each individual was run at the additional 6 markers to increase the accuracy of population assignment and structure tests.

The PID_{sibs} (probability that siblings share the same genotype) was calculated across all 14 microsatellite loci (Waits et al. 2001) using GenAlEx v6.5 (Peakall and Smouse 2012). Loci were then organized from least to most powerful PID_{sibs} value, and the product of those values calculated starting with the two least powerful and adding another locus until the product reached an acceptable threshold (Table 2). We utilized a threshold value of 0.03 that indicates 3 in 100 sibling pairs could share a genotype.

Genotype matching was carried out in GenAlEx v6.5 (Peakall and Smouse 2012). Samples were considered to be from the same individual if they were an exact match or matched at 7 of 8 loci for the NAL population and 12 of 14 for the MRB population (Table 2) and the mismatch of the 8^{th} or $13^{th}/14^{th}$ locus could be assumed to have been from either a potential allelic dropout or failure to amplify. All samples were amplified a minimum of two times to confirm a genotype. Two amplifications were required to confirm a heterozygote genotype for all three sample types (tissue, hair, scat). Two amplifications were sufficient for confirmation of homozygotic genotypes for tissue and hair samples, but a third consensus genotype was require for confirmation of homozygotic genotypes derived from scat samples. To prevent

overestimation of the population, genotypes that mismatched at one to three loci and where dropout or false alleles was suspected underwent additional amplifications (up to 4 total) to confirm or refute differences. For NAL samples with a questionable match, the second multiplex was run, and a PID_{sibs} value of 0.03 or lower was maintained (any mismatch still had to be due to potential drop out or failure to amplify).

Samples collected from comparison populations (NGA, CGA, MS, FL, TN) were run at all 14 loci. Additionally, once individuals were identified all loci and populations were evaluated as to whether they were in Hardy Weinberg Equilibrium (HWE, Hardy 1908). Linkage Equilibrium (LE) was also estimated to ensure that all measured loci were independently inherited. LE and HWE were estimated using Arlequin version 3.5.2.2 (Excoffier and Lischer 2010) and Genalex 6.5 (Peakall and Smouse 2012) respectively.

Population structure

The number of populations and grouping of individuals into populations was first evaluated to remove bias associated with any *a priori* assumption of population membership of individuals. Model-based Bayesian clustering analysis was undertaken in the program STRUCTURE (Pritchard et al. 2000, Falush et al. 2003, 2007). Admixture and correlated allele frequencies were assumed, and all simulations were run with 100,000 iterations of burn in and a 400,000 Markov Chain Monte Carlo run. We evaluated values of K (the number of populations modeled) from 1-12, with 10 replicates at each K to provide an averaged result. The smallest K value where the log likelihood of K begins to plateau was selected as the estimate of the actual number of populations (Pritchard et al. 2009) and confirmed with the Evanno method (Evanno et al. 2005). The statistics and graphs for these procedures were run in the program Structure Harvester (Earl and vonHoldt 2012). Due to the large number of individuals identified in the

MRB and its isolation, we were concerned about a potentially confounding signal from highly related family groups on the structure analysis. To reduce potential bias we ran a maximum likelihood estimate of relatedness in ML-relate (Kalinowski et al. 2006) and selected the 30 least related individuals with which to run a second structure analysis following the same parameters as above.

Population assignment from STRUCTURE was confirmed utilizing a Discriminant Analysis of Principle Components (DAPC, Jombart et al. 2010). DAPC uses a principle components analysis to describe as much of the within and between group variation, and the resulting principle component scores are fed into a discriminant analysis which identifies the between group genetic variation. Results were then represented graphically showing a center of commonality of principal components for each population circumscribed with an inertia ellipse which describes 95% of the variation of each population. All DAPC calculations were performed with the adegenet package (Jombart 2008) in RStudio (RStudio Team 2015, R Core Team 2017).

Population structure among identified groups was evaluated utilizing Fst (Weir and Cockerham 1984) and G"st (Hedrick 2005, Meirmans and Hedrick 2011) statistics; Fst and G"st estimates were generated using Genalex 6.5 (Peakall and Smouse 2012).

Results

DNA Collection and Analysis

The combined effort of sampling across all five years of study in Alabama resulted in the collection of 404 scat samples, which yielded 151 samples identified to individual and 1531 hair samples, which yielded 819 samples identified to individual. These samples identified 135

unique individuals in the MRB and 32 in NAL (Tables 3 and 4). Samples from outside of Alabama included 24 from CGA, 20 from NGA (of which 17 successfully amplified), 15 from MS, 18 from FL, and 30 from TN.

For Hardy Weinberg Equilibrium, 23 of 98 tests deviated from HWE expectations at $p < 0.05$ and 15 of 98 tests after Bonferroni correction ($p < 0.0005$, Table 5). The majority of the deviations occurred in the MRB population (11 of 14 both before and after Bonferroni correction). When excluding the MRB population, locus G10H still showed a high frequency of deviations across populations and was thus removed from the remaining analyses (Table 5). There were no deviations from LE ($p < 0.05$) out of 91 pairwise comparisons in each of the 7 populations.

Population Structure

Structure analysis was initially run on all identified individuals. A clear plateau of the L(K) value was observed after K=6 (Figure 4), while the Evanno method showed substantial support for selection of a K=2 (Figure 5). The two populations assigned for K=2, were the individuals assigned a priori to the MRB population, with the remaining populations grouped together (Figure 6). Additionally 6 bears from the MS population that were sampled close to the Alabama border were assigned to the MRB population cluster (Figure 6).

The second structure analysis with the MRB population reduced to 30 of the least related individuals showed a clear signal of K=6 for both the L(K) and Evanno methods of K selection (Figures 7 and 8). K=2 still had a higher delta K value in the Evanno graph, but the delta K at K=6 was still substantial and in agreement with the L(K) selection. The populations were identified along their a priori population assignments except for the NGA, which was assigned as a mixture of NAL and TN populations (Figure 9).

The DAPC analysis showed clear groupings of the principle components in agreement with the population assignment from STRUCTURE. To describe the data, 20 principle components were generated and 5 discriminant functions were used (Figure 10). The two populations hypothesized a priori to be isolated from surrounding populations (MRB and CGA) were shown to be distant from the core distribution of the remaining 5 populations (Figure 10). Similar to the STRUCTURE results, the NGA population sits directly between the TN and NAL populations with NGA's 95% inertia ellipses overlapping both NAL and TN.

Both the Fst and G"st statistics show a high level of genetic structure for bears in Alabama (Tables 6 and 7). The MRB population shows little to no genetic interaction with any other regional population of bears (all Fst > 0.217; all G"st > 0.754). Similarly the NAL population shows a moderate level of structure to all surrounding populations (Fst > 0.095, G"st >0.451) except for NGA, where it shows a low level structure (Fst = 0.046, G"st = 0.183). All Fst and G"st values differed from zero with a P-value of less than or equal to 0.001, based on 999 permutations.

Discussion

Our analysis found the MRB population to have the most restricted gene flow with surrounding populations. The initial STRUCTURE analysis with all MRB samples included showed a clear division of the MRB population as a unique population, so much so that the Evanno method for K selection favored 2 populations the MRB and all others (Figures 5 and 6). However the log likelihood of K showed a clear though less dramatic increase in descriptive power of higher values of K (Figure 4). When we removed the highly related individuals to eliminate any potential bias created by family groups within the MRB, the largest amount of population variation was still described at K=2, seperating MRB from all other populations (Figure 8). Ultimately however the most appropriate value of K=6 was selected where delta K

and L(K) agreed at K=6 (Figure 7 and 8), and MRB was still maintained as a single population. Our results were further supported by the DAPC which showed the MRB population well separated from all other populations (Figure 10). Pairwise comparisons of Fst and G"st also show a high degree of separation between the MRB and all other populations (Tables 6 and 7). The estimated level of structure indicates the population currently is either not interbreeding with any of its neighbors or is doing so infrequently, which could potentially lead to an inbreeding depression in the future.

Our results suggest that bears in eastern Mississippi are part of the MRB population. The initial structure analysis reassigned 6 individuals from the eastern portion of the MS population into the MRB population (Figure 5). Reassignment of these individuals also was supported by changes in the DAPC, Fst, and G"st values after populations were adjusted.

Both structure assignment and the DPAC suggest that there have been two recent migrants into the MRB population. Two individuals were shown in both the STRUCTURE assignment (Figure 6) and the DAPC (Figure 10) to be genetically from the FL population and the NGA population. However, since signal from these two populations is not seen in other individuals it is likely that these are two recent migrants who have not yet reproduced or resulting offspring have not yet been detected. Migrants such as these are promising and will be crucial to the future genetic health of this population.

The NAL population of bears shows clear signs of being founded from the NGA population with a high level of separation from other populations. The second STRUCTURE analysis and DAPC clearly showed the NAL to be a distinct population, with NGA being a mixture of NAL and TN (Figure 9). These results suggest that the NGA population was founded from the TN population, but then a limited number of individuals founded the NAL population

from NGA. The limited source stock for the NAL created a more unique genetic signature for the population and thus our analysis assigned NAL as unique, while NGA was assigned as between NAL and TN across all individuals. The DAPC also shows NGA as having clear overlap with both NAL and TN, but with no overlap between NAL an TN. In that analysis, all three populations also have their centers of principle components outside of the other populations 95% inertia ellipses, indicating that each population is distinct from the others. Ultimately, Fst and G"st pairwise comparisons indicate little to no separation between the NAL and NGA populations and only a stepwise greater structure between the NAL and TN (Tables 6 and 7); these results further support a stepping-stone model of migration from TN to NGA and most recently to NAL . Outside of the putative source populations, NAL shows a high level of genetic differentiation from all other populations (Tables 6 and 7).

The degree of genetic isolation between NAL and most other populations could be concerning depending on how the intereaction between NAL and NGA continues in the future. The current low level of structure between NAL and NGA could potentially be due to the recent founding of the NAL population. Bears have been present in NAL for roughly two generations (Onorato et al. 2004), which would not allow for current isolation to be detected through genetic tests. Thus, continued genetic monitoring will be necessary to assess whether or not there is continued interaction between NAL and NGA. Continued connectivity between the NAL and NGA (or other populations) will be necessary to prevent NAL from becoming as isolated as the MRB population.

Black bears in Alabama are at risk of complete genetic isolation from surrounding populations of bears and therefore the potential consequences from inbreeding depression. The MRB population already shows a high level of genetic structure from all surrounding

populations with a very limited number of recent migrants. These recent migrants are promising as studies have shown that a low number of effective migrants can have a profound effect in the form of genetic rescue. The status of the NAL population is more difficult to determine given its relative infancy. Continued monitoring of both populations will be necessary to quantify the effect of recent migrants to the MRB and to track whether or not the NAL will trend towards isolation over the coming years. However the potential risk of a reduction in genetic diversity is high and efforts to mitigate isolation should be undertaken.

Table 1. Volumes of primers, and reagents (for listed concentrations) as well as µM concentration of primers used per individual sample for PCR, and thermocycler profile.

Multiplex 1						
		µl	µM	Initial Denature	94°C	15 min
dH20		0.07		# of cycles :	14	
Master Mix (2x)		3.5		Denature:	94°c	30 sec
Q solution (5x)		0.7		Annealing:	57°C - 0.5°	90 sec
G10C (10x)	Paetkau *et al.* 1998	0.04	0.057143	Extension:	72°C	1 min
G10M (10x)	De Barba & Waits 2010	0.1	0.142857	# of cycles :	30	
G10P (10x)	Paetkau & Strobeck 1995; Paetkau et al. 1998	0.1	0.142857	Denature:	94°c	30 sec
G10X (10x)	Taberlet *et al.* 1997;	0.07	0.1	Annealing:	50°c	90 sec
G1D (10x)	Taberlet *et al.* 1997; De Barba & Waits 2010	0.1	0.142857	Extension:	72°C	1 min
SE 47+48 (10x)	Ennis & Gallagher 1994	0.03	0.042857	Final Extension	60°C	30 min
Mu23 (10x)	Taberlet *et al.* 1997	0.08	0.114286	Cooldown	4°C	10 min
Mu15 (10x)	Taberlet *et al.* 1997; De Barba & Waits 2010	0.06	0.085714			
G10H (10x)	Paetkau *et al.* 1998	0.15	0.214286			
DNA extract		2				
Multiplex 2						
		µl	µM	Initial Denature	94°C	15 min
dH20		0.45		# of cycles :	14	
Master Mix (2x)		3.5		Denature:	94°c	30 sec
Q solution (5x)		0.7		Annealing:	57°C - 0.5°	90 sec
G10B (10x)	Paetkau et al. 1998	0.04	0.057143	Extension:	72°C	1 min
D1A (10x)	Paetkau & Strobeck 1994	0.05	0.071429	# of cycles :	30	
G10L (10x)	Paetkau et al. 1995	0.05	0.071429	Denature:	94°c	30 sec
Mu50 (10x)	Bellemain & Taberlet 2004	0.12	0.171429	Annealing:	50°c	90 sec
G10U (10x)	Paetkau et al. 1998	0.05	0.071429	Extension:	72°C	1 min
G1A (10x)	Taberlet et al. 1997; Paetkau et al. 1998	0.04	0.057143	Final Extension	60°C	30 min
DNA extract		2		Cooldown	4°C	10 min

Table 2. The PIDsib values per locus is the probability that full siblings will share a genotype at that given locus. The product of the per locus PIDsib values gives the probability that full siblings share a genotype comprised of the included loci. We set a 0.03 threshold for a genotype PIDsib value. For the NAL population the threshold was met with 7 loci with multiplex 1, but rose to 8 when including both multiplexes due to lower diversity of some of the added markers. For the MRB population the threshold was met with 12 loci.

NAL PIDsib MP1		
Locus	PIDsib	product
Mu23_1	9.7E-01	
G10X_1	6.7E-01	6.5E-01
G10C_1	5.9E-01	3.8E-01
G1D_FIm+Rm_1	5.7E-01	2.2E-01
G10H_1	5.3E-01	1.2E-01
Mu15_1	4.9E-01	5.8E-02
G10P_1	4.8E-01	2.8E-02
G10M_1	4.3E-01	1.2E-02

NAL PIDsib MP1 & MP2		
Locus	PIDsib	product
Mu23_1	9.7E-01	
G10X_1	6.7E-01	6.5E-01
G10C_1	5.9E-01	3.8E-01
G1D_FIm+Rm_1	5.7E-01	2.2E-01
G1A_1	5.5E-01	1.2E-01
G10H_1	5.3E-01	6.4E-02
D1A_1	5.0E-01	3.2E-02
Mu15_1	4.9E-01	1.6E-02
G10L_1	4.9E-01	7.8E-03
G10P_1	4.8E-01	3.8E-03
G10U_1	4.8E-01	1.8E-03
G10B_1	4.6E-01	8.3E-04
Mu50_1	4.3E-01	3.6E-04
G10M_1	4.3E-01	1.6E-04

MRB PIDsib MP1 & MP2		
Locus	PIDsib	product
G10H_1	9.9E-01	
D1A_1	9.9E-01	9.8E-01
G10P_1	9.4E-01	9.2E-01
G1A_1	9.1E-01	8.4E-01
G10X_1	8.6E-01	7.2E-01
G10B_1	7.1E-01	5.1E-01
Mu23_1	6.8E-01	3.5E-01
G10L_1	6.7E-01	2.3E-01
G10U_1	6.5E-01	1.5E-01
G1D_FIm+	6.5E-01	9.8E-02
G10C_1	5.7E-01	5.6E-02
G10M_1	5.0E-01	2.8E-02
Mu50_1	5.0E-01	1.4E-02
Mu15_1	4.9E-01	6.8E-03

Table 3. Summary of all samples collected and successfully genotyped for individual ID in the MRB study region. Individual totals account for total unique individuals.

	MRB									
	Scat			Hair			Combined			
	Collected	Genotyped	% success	Collected	Genotyped	% success	Collected	Genotyped	% success	Individuals
2011	159	24	15%	-	-	-	159	24	15%	15
2012	157	90	57%	-	-	-	157	90	57%	30
2013	-	-	-	28	15	54%	28	15	54%	10
2014	46	15	33%	218	147	67%	264	162	61%	48
2015	32	16	50%	411	230	56%	443	246	56%	62
Totals	394	145	37%	657	392	60%	1051	537	51%	135

Table 4. Summary of all sample collected and successfully genotyped in the NAL study region. Individual totals account for total unique individuals.

NAL									
	Scat			Hair			Combined		
	Collected	Genotyped	% success	Collected	Genotyped	% success	Collected	Genotyped	% success	Individuals
2011	-	-	-	-	-		-	-	-	-
2012	-	-	-	235	115	49%	235	115	49%	11
2013	-	-	-	67	37	55%	67	37	55%	8
2014	-	-	-	407	181	44%	407	181	44%	19
2015	10	6	60%	165	94	57%	175	100	57%	22
Totals	10	6	60%	874	427	49%	884	433	49%	32

Table 5. Significant deviation from Hardy Weinberg Equilibrium by locus and population after Bonferroni correction. ns = not significant, * P<0.05(0.0005), ** P<0.01(0.0001), *** P<0.001 (1.0^-5)

Hardy Weinberg Equilibrium by locus and population							
	MRB	**NAL**	**CGA**	**NGA**	**MS**	**FL**	**TN**
D1A_1	***	ns	ns	ns	ns	ns	ns
G10B_1	***	ns	ns	ns	ns	ns	ns
G10C_1	ns	ns	ns	ns	ns	ns	ns
G10H_1	ns	**	*	ns	ns	ns	**
G10L_1	***	ns	ns	ns	ns	ns	ns
G10M_1	***	ns	ns	ns	ns	ns	ns
G10P_1	***	ns	ns	ns	ns	ns	ns
G10U_1	***	ns	ns	ns	ns	ns	ns
G10X_1	***	ns	ns	ns	ns	ns	ns
G1A_1	***	ns	ns	ns	ns	ns	ns
G1D_FIm+Rm_1	***	***		ns	ns	ns	ns
Mu15_1	ns	ns	ns	ns	ns	ns	ns
Mu23_1	***	ns	ns	ns	ns	ns	ns
Mu50_1	***	ns	ns	ns	ns	ns	ns

Table 6. Pairwise Fst values for all populations are above the diagonal And G"st values are below the diagonal. All values have a P value of ≤.001 based on 999 permutations.

	MRB	NAL	CGA	NGA	MS	FL	TN	
MRB		0.337	0.320	0.246	0.295	0.243	0.217	**MRB**
NAL	0.913		0.246	0.046	0.228	0.186	0.095	**NAL**
CGA	0.793	0.766		0.185	0.292	0.231	0.178	**CGA**
NGA	0.827	0.183	0.706		0.152	0.125	0.050	**NGA**
MS	0.761	0.738	0.812	0.595		0.208	0.135	**MS**
FL	0.754	0.742	0.784	0.627	0.736		0.098	**FL**
TN	0.760	0.451	0.700	0.270	0.542	0.506		**TN**
	MRB	**NAL**	**CGA**	**NGA**	**MS**	**FL**	**TN**	

Figure 1

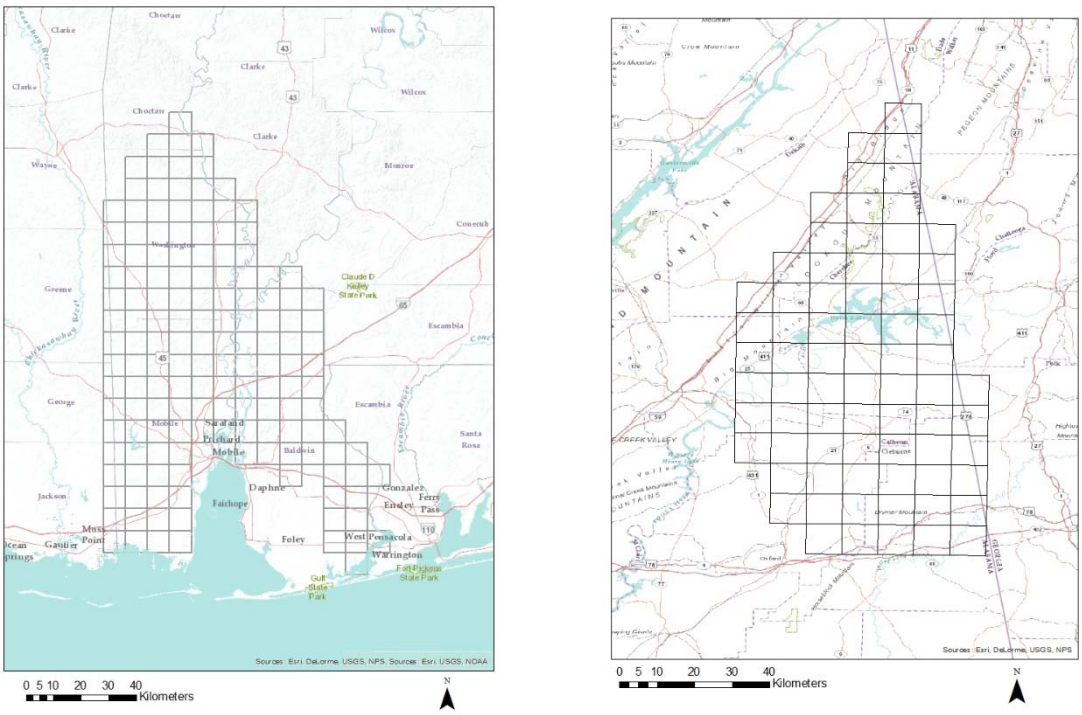


Figure 1. Grid cells (8x8 Km) overlain on the Mobile River Basin (MRB) population (left) and the North Alabama (NAL) population (left). Both grid systems extend beyond the observed presence of bears to capture the edges and reduce closed population violations.

Figure 2.

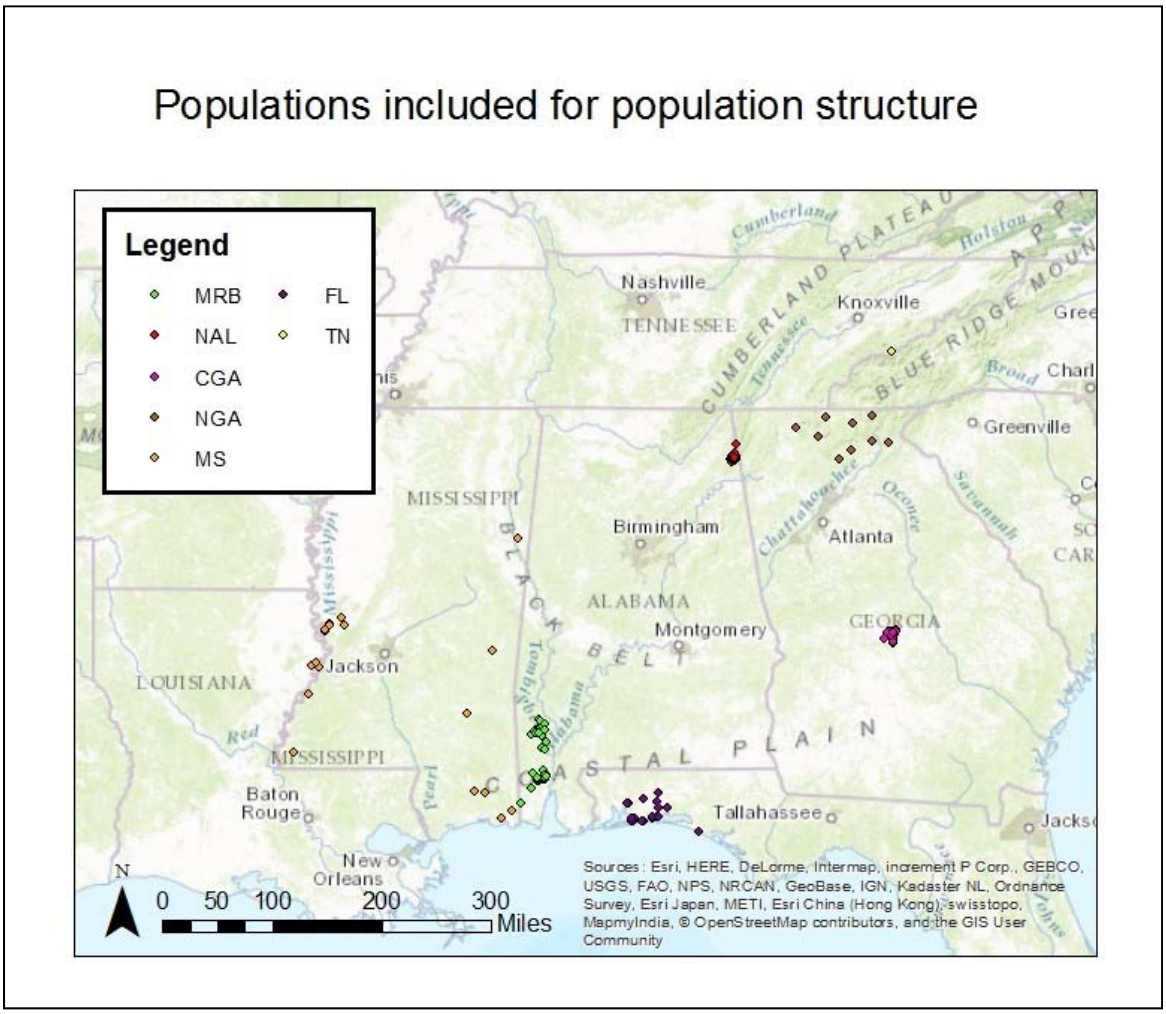


Figure 2. Locations of samples collected from MRB, NAL, MS, CGA, FL, centers of county collected for NGA and approximate center of study area for TN where location information was not available for individual samples.

Figure 3.

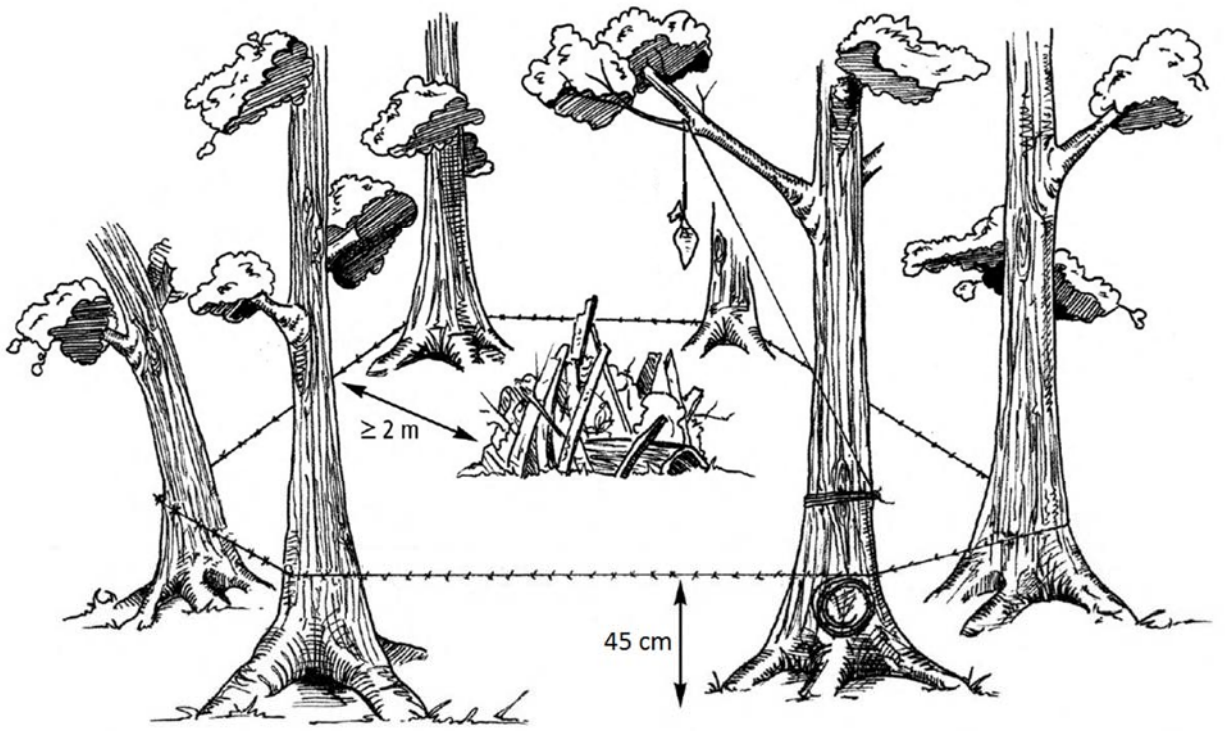


Figure 3. Hair snare station used for sampling for bears across Alabama: centrally located scent pile, suspended lure, double-stranded, four-barbed wire at a height of 45 – 50 cm. Credit: S. Harrison from Kendall and McKelvey 2008, modified by John Draper

Figure 4.

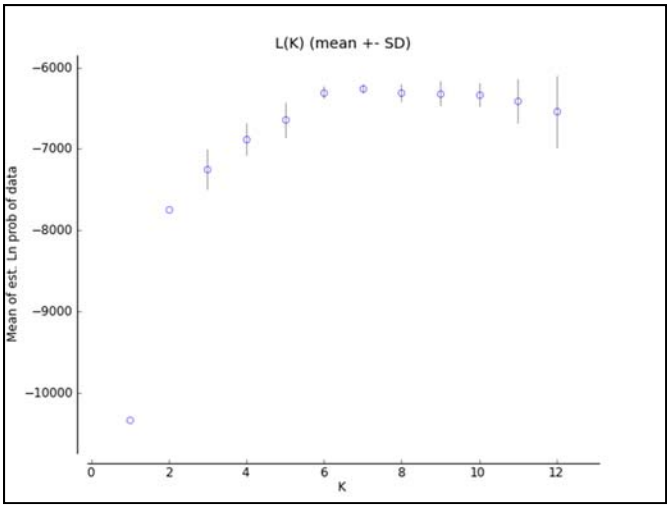


Figure 4. Log likelihood of K for STRUCTURE analysis with all samples included.

Figure 5.

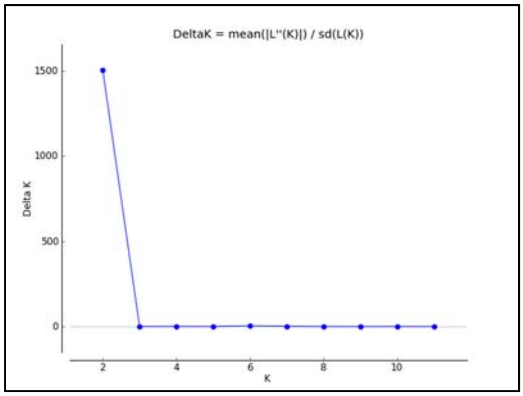


Figure 5. Delta K (Evanno Method) for STUCTURE analysis with all samples included

Figure 6.

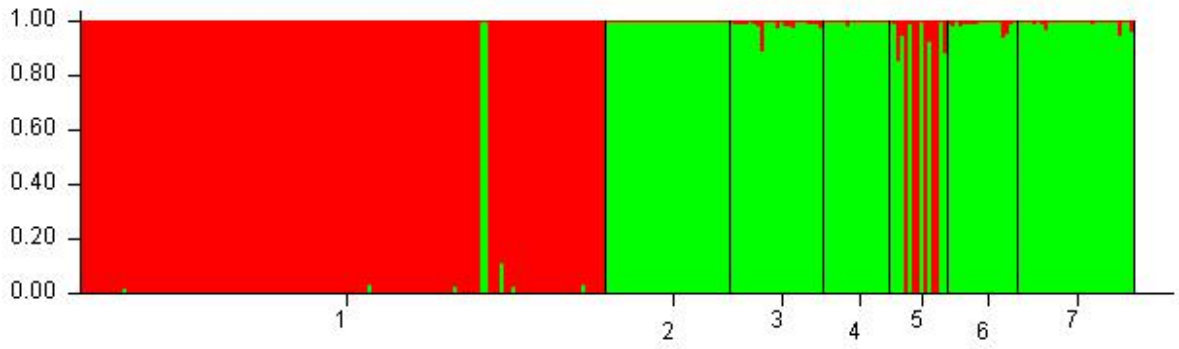


Figure 6. Bar chart for K=2 showing population assignment from STRUCTURE divided by a priori population assignment (1=MRB, 2=NAL, 3=GA, 4=NGA, 5=MS, 6=FL, 7=TN). Note the 6 individuals originally in the MS population (5), that clearly show population assignment to the MRB population, and the two potential new immigrants in the MRB (1), that show assignment to the non-MRB population cluster.

Figure 7.

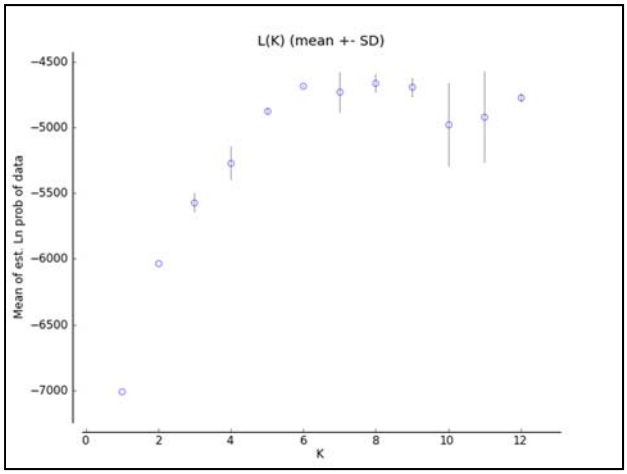


Figure 7. Log likelihood of K for STRUCTURE analysis with highly related individuals in the MRB removed.

Figure 8.

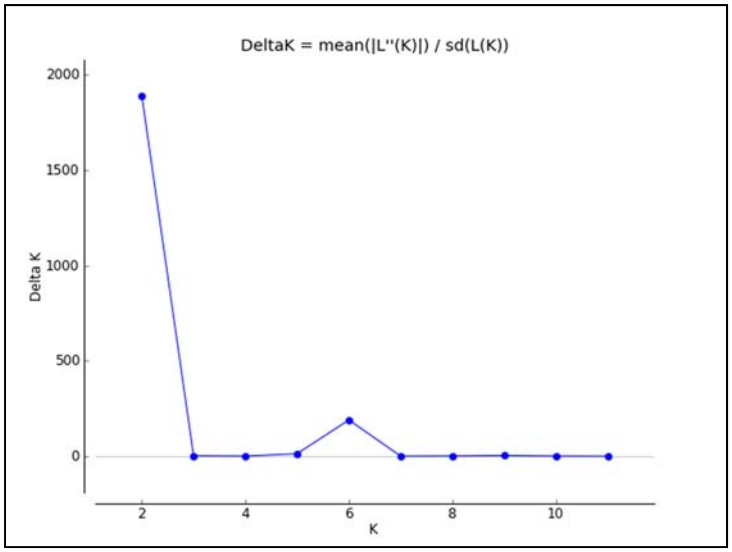


Figure 8. Delta K (Evanno Method) for STRUCTURE analysis with highly related individuals in the MRB removed.

Figure 9.

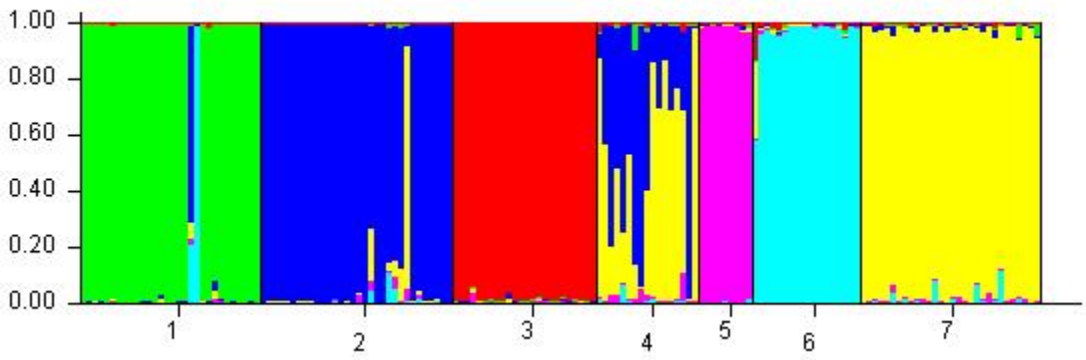


Figure 9. Bar chart showing population assignment from STUCTURE divided by a priori population assignment (1=MRB, 2=NAL, 3=GA, 4=NGA, 5=MS, 6=FL, 7=TN). Note the two potential new immigrants in the MRB (1), from FL (6) and NGA(4) (the 6 individuals originally in the MS population were reassigned to the MRB for this analysis).

Figure 10.

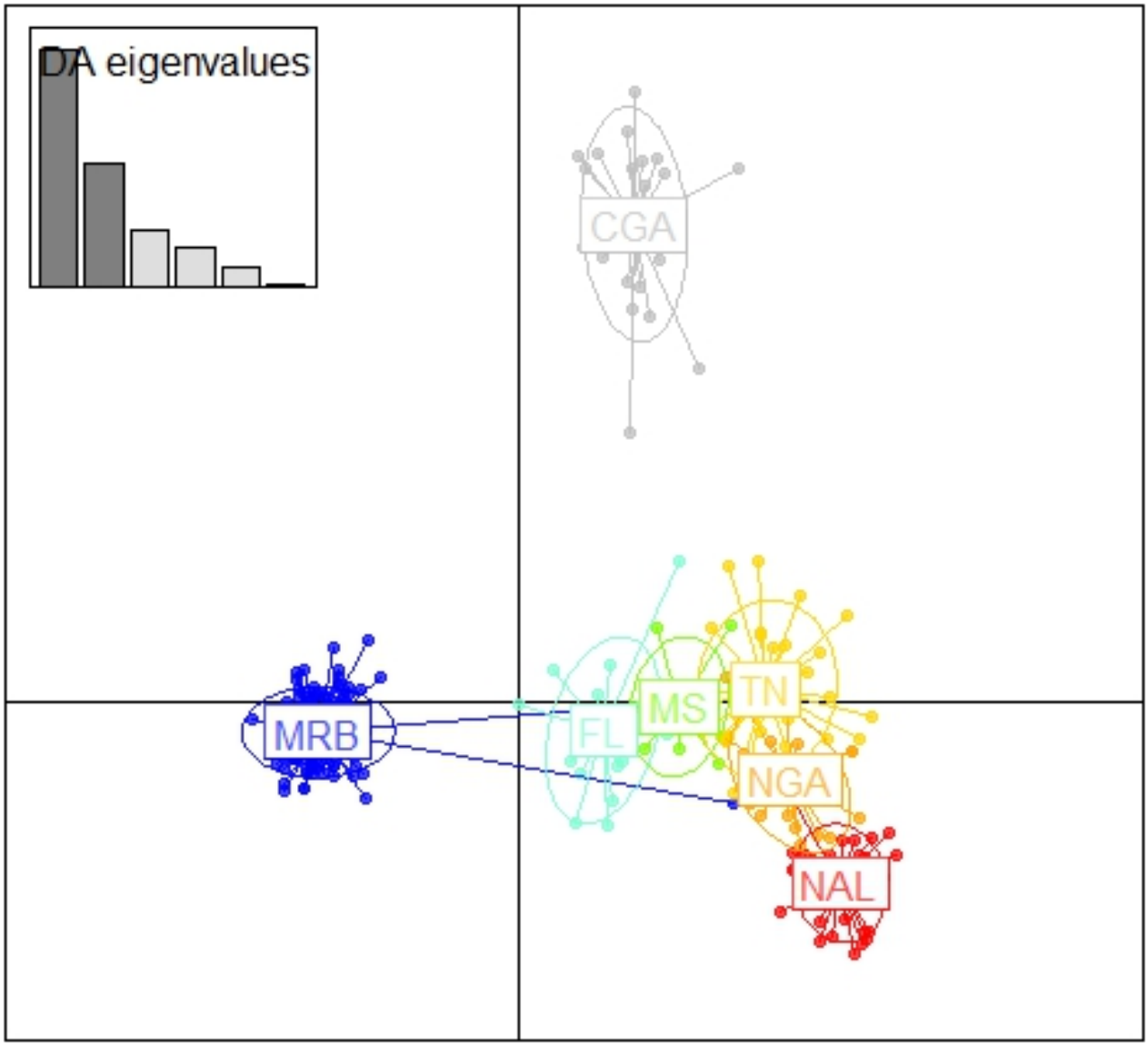


Figure 10. DAPC population grouping, 20 principle components and 5 discriminant functions were used. Power of the eigenvalues is shown in the upper left.

Chapter 2: Population modeling and genetic diversity of black bears (*Ursus americanus*) in Alabama

Intro

One of the major concerns in conservation today is the loss of genetic diversity (Reed and Frankham 2003), which is a frequent consequence of population isolation and small population sizes (Ohnishi et al. 2007). Small, isolated populations are at greater risk of loss in genetic diversity due to increased potential for genetic drift and inbreeding (Balkenhol et al. 2015). As genetic diversity is lost, fitness and fecundity of individuals in a population can be reduced, resulting in inbreeding depression (Charlesworth and Charlesworth 1987). Inbreeding depression is the first step in genetic meltdown, the fixation of deleterious mutations (Lynch et al. 1995), which in turn leads to a negative feedback loop of continued reduction of both population size and genetic diversity. Such feedback loops are known as an extinction vortex, and can ultimately lead to the loss of a population (Reed 2005). To avoid such calamities, monitoring of the genetic diversity of species or populations that are susceptible to low numbers and isolation is needed.

Various factors such as trophic level, home range size, and reproduction rate make certain taxonomic groups of species at higher risk for these adverse population and genetic outcomes (Purvis et al. 2000). For example, populations of animals from within the order *Carnivora* are especially prone to population isolation, reduced population size, and ultimately loss of genetic diversity due to greater persecution, large home ranges, and high trophic level (Mladenoff et al. 1997). Persecution of carnivores arises from actual and perceived threats to human interests and safety, and can contribute to populations being reduced (Treves and Karanth 2003). Furthermore, carnivores are typically characterized as having large home ranges, which

increases exposure to anthropogenic persecution and utilization (Woodroffe and Ginsberg 1998). Large home ranges also make carnivores more vulnerable to habitat fragmentation and consequently population isolation within protected areas or other refugia (Woodroffe and Ginsberg 1998, Purvis et al. 2000, Cardillo et al. 2005). Finally, carnivores' high trophic level, combined with their typically low recruitment rate and naturally low densities, further contribute to individuals existing in isolated populations with few individuals (Purvis et al. 2000). Once reduced to small, isolated populations, carnivores are especially vulnerable to genetic meltdown, a positive feedback loop of negative consequences from decreasing genetic diversity that leads to the local extinction of a population. Thus, wildlife managers need to estimate and monitor population sizes and genetic diversity of carnivore populations to ensure their long-term survival.

Black bears (*Ursus americanus*) have suffered particularly sharp declines in population size and range in North America, creating conditions where isolation and loss of genetic diversity can occur. Black bear ranges have been reduced 62% from the historical extent in North America, with an even more significant 80% reduction in the southeastern United States (Pelton and Van Manen 1997). As a result, black bears in Louisiana, for example, have shown extremely low genetic diversity and significant genetic population structure indicating a restriction in gene flow between populations (Triant et al. 2004). Only the populations of bears in northern Louisiana, which were artificially supplemented with outside genetic stock (translocations in the 1960's), showed levels of genetic diversity similar to other black bear populations in North America (Triant et al. 2004). Studies of other isolated bear populations in the Southeast are necessary to identify and adress similar situations.

Alabama has two small populations of black bears, one of which is geographically and genetically isolated from all other populations of bears, which creates a high potential for loss of

genetic diversity (Chapter 1). This population, which was restricted to the lowlands surrounding the Mobile River Basin (MRB; Howell 1921), has persisted through the extensive habitat conversion and persecution that accompanied European settlement of the area. However, the U.S. Fish and Wildlife Service suggested that the MRB population had a low probability for long-term persistence due to its small size and isolation from other bear populations (U.S. Fish and Wildlife Service 1998). Furthermore, Kasbohm et al. (1994, as reported in Edwards 2002) found potential physical expression of low genetic diversity, including cryptorchidism, prolapsed rectum, and kinked or absent coccygeal vertebrae . The second population of black bears in Alabama is a newly recolonizing population in northeastern Alabama (NAL), whose continuing connectivity with its source population is unknown due to its recent founding (Chapter 1). Little is known about either populations' current status; prior to recent preliminary research (Graham 2016) the MRB population was last studied over 15 years ago (Edwards 2002) and the NAL population has never been studied. Therefore, wildlife managers need to better understand the current genetic status and potential for continued genetic health for both populations.

In this study our goals were to establish baseline measurements of population size and genetic diversity for the two populations of black bears in Alabama. Our objectives were to: 1. estimate the abundance and distribution of black bears in the core of both the MRB and NAL populations; and 2. estimate the genetic diversity within each population and compare it to the genetic diversity of surrounding populations of black bears in the Southeastern United states. Such information will be useful for guiding future management actions aimed at promoting genetic diversity and in turn the survival of black bears in Alabama.

METHODS

Study area

We estimated bear population size and genetic diversity at two study areas, which cover both known breeding populations of black bears in Alabama. The southern study area surrounding the MRB population of bears extended from 3,521,473m north to 335,430m south and 360,775m west to 464,824m east (NAD 1987 UTM zone 16N) and encompassed portions of Baldwin, Choctaw, Clarke, Mobile and Washington counties (Figure 1). The northern study area extended from 3,841,979m north to 3,721,931m south and 597,022m west to 653,073m east and encompassed the majority of Cherokee and Cleburne counties with significant portions of Calhoun, DeKalb, and Etowah counties also included (Figure 1).

Natural and near natural habitat available to bears in the MRB varied from woody wetlands to pine plantations (Homer et al. 2015). Substantial portions of the study area were also covered with suburban and exurban development. Roughly 500,000 people lived within the sampling area at the time of study (United States Census Bureau n.d.).

The habitat available in the NAL region was mountainous and was dominated by deciduous forests and pine plantations (Homer et al. 2015). In addition to federally managed lands including LIRI and Talladega National Forest, many large tracts of land were managed privately for hunting and/or timber production, providing much more continuous habitat than in the MRB. Similarly, human population density was lower, with only roughly 130,000 people residing within parts of the study area potentially occupied by bears (United States Census Bureau n.d.).

DNA Collection

Systematic sampling for black bears took place across the known range of both populations. The study areas were overlain with a sampling grid of 64 km^2 cells (Figure 1), which was approximately the home range size of male black bears in Alabama (Edwards 2002). At least one hair snare was placed in each cell, with micro-site selection determined by land access, and biotic and abiotic factors affecting bear movement on the landscape (topography, water sources, food sources, etc.). In cells where bear sign (e.g. hair snare hits, camera images, tracks, scat, anecdotal reports) was found, additional hair snares were set to increase the probability of detection for females, whose home ranges are closer to 7 km^2 in size (Edwards 2002).

Sampling was carried out from 2012-2015 by deploying minimally invasive hair snares. Hair snares consisted of a single strand of barbed wire placed around multiple trees at 45 centimeters above the ground to create a corral with a perimeter between 20-30 meters (Kendall and Mckelvey 2008, Figure 3). Bait was suspended in the center of the corral, such that it was greater than 2 meters from the wire in any direction. Baits included canned fish and flavoring extracts applied to a tampon and suspended sufficiently high to prevent retrieval by a bear and a subsequent food reward, which could cause the animal to become trap happy or otherwise affect behavior outside of the study parameters (Kendall and Mckelvey 2008).

Hair snare sampling took place from August through November when bears were in hyperphagia and most active before denning for the winter (Garshelis et al. 1983, Noyce and Garshelis 2011). Sites were checked regularly with 6-8 days between checks. Hair samples were collected from the barbed wire snares using hemostats/tweezers, to prevent contamination.

Collection tools were flamed both before and after collection to prevent any possible mixing of samples. Following collection of a sample, the barb it was collected from, as well as the adjacent barbs, were flamed to prevent mixing samples (Kendall and Mckelvey 2008). Collected samples were placed in paper coin envelopes and stored in a secondary container with silica desiccant to prevent degradation of the sample from moisture (Settlage et al. 2008).

The MRB study area was also sampled using scat-detection dogs during 2011 and 2012. Triangular transects were established in the MRB in those years for the deployment of scat detection dogs, which allowed for more efficient and complete collection of scat along transects (Mackay et al. 2008). Dogs were trained to seek out bear scat by the EcoDogs program at Auburn University, and were accompanied by a trained handler in addition to a biologist. Each side of the triangle was 0.5 kilometers in length totaling 1.5 kilometers of sampled transect per occasion. Transects were sampled across one month both fall seasons. Scats were also collected when located incidentally in both study areas throughout the study period. Scrapings were taken from the most desiccated section of each located scat sample to minimize potential hydrolytic degradation and were stored in 1.4ml of DETs buffer (Murphy et al. 2002).

DNA samples were obtained for diversity comparisons from Florida (FL), Mississippi (MS), Tennessee (TN) and from two separate populations in Georgia (North Georgia - NGA and Central Georgia - CGA) that are isolated from each other (Hooker et al. 2015, Figure 11). The Florida samples were hair samples collected from management activities conducted by the Florida Fish and Wildlife Commission. Dr. Jerrold L. Belant of Mississippi State University provided hair samples from a hair snaring study he conducted across Mississippi. The samples from Tennessee were obtained from Katie Settlage, in the form of DNA extract from her master's thesis research (Settlage 2005, Settlage et al. 2008). Samples from central Georgia

were provided by Dr. Michael Chamberlain of the University of Georgia's Warnell School of Forestry and Natural Resources, as tissue samples from captured individuals. Finally northern Georgia samples were obtained from the Georgia Department of Natural Resources as hair samples from hunter harvested bears. Additional genotypes are included from previously published work by Puckett et al. (2015) to include coastal and montane North Carolina (CNC and MNC respectively) and West Virginia.

DNA Analysis

DNA analysis of all collected samples was performed at the Laboratory for Ecological, Evolutionary and Conservation Genetics in the College of Natural Resources at the University of Idaho. DNA was extracted from samples using DNeasy Blood and Tissue Kits for hair and tissue and QIAamp Fast DNA Stool Mini Kit for scat (Qiagen Inc., Valencia, CA). All samples were extracted in a lab space dedicated to the extraction of low quality DNA, and an extraction negative was included in each extraction to monitor for contamination. Collected scat samples were verified as bear using a mitochondrial DNA fragment species identification test as described in De Barba et al. (2014). Scat samples, which were confirmed to be black bear and all hair samples were then identified to individual, using a microsatellite multiplex of 8 loci and a sex identification marker (G10C, G10H, G10M, G10P, G10X, G1D, Mu15, Mu23, and SE47+48; (Ennis and Gallagher 1994, Paetkau and Strobeck 1995, Paetkau et al. 1995, 1998, Taberlet et al. 1997, Waits et al. 1998, McCarthy et al. 2009, De Barba and Waits 2010; Table 1) to avoid a negative bias in population estimates that results from subsampling by reducing capture and recapture rates (Tredick et al. 2007). PCR products were visualized on an Applied Biosystems 3130xL genetic analyzer and allele sizes were scored and evaluated in GeneMapper 5.0 (Applied Biosystems, Foster City, California). Due to the low genetic diversity of the MRB

population, an additional 6 microsatellite loci were needed to identify individuals (G10B, D1A, G10L, Mu50, G10U and G1A; Paetkau and Strobeck 1994, 1995, Taberlet et al. 1997, Paetkau et al. 1998, Bellemain and Taberlet 2004, Graham 2016; Table 1.). Once NAL samples were assigned to an individual, a representative sample from each individual was run at the additional 6 loci to increase the accuracy of genetic diversity tests.

Genotype matching was carried out in GenAlEx v6.5 (Peakall and Smouse 2012). Samples were considered to be from the same individual if they were an exact match or matched at 7 of 8 loci for the NAL population and 12 of 14 for the MRB population (Table 2) and the mismatch of the 8^{th} or $13^{th}/14^{th}$ locus could be assumed to have been from either a potential allelic dropout or failure to amplify. All samples were amplified a minimum of two times to confirm a genotype. Two amplifications were required to confirm a heterozygotic genotype for all three sample types (tissue, hair, scat). Two amplifications were sufficient for confirmation of homozygotic genotypes for tissue and hair samples, but a third consensus genotype was require for confirmation of homozygotic genotypes derived from scat samples. To prevent overestimation of the population, genotypes that mismatched at one to three loci and where dropout or false alleles was suspected underwent additional amplifications (up to 4 total) to confirm or refute differences. For NAL samples with a questionable match, the second multiplex was run, and a PID_{sibs} value of 0.03 or lower was maintained (any mismatch still had to be due to potential drop out or failure to amplify).

Samples collected from comparison populations (NGA, CGA, MS, FL, TN) were run at all 14 loci. Additionally, once individuals were identified all loci and populations were evaluated as to whether they were in Hardy Weinberg Equilibrium (HWE, Hardy 1908). Linkage Equilibrium (LE) was also estimated to ensure that all measured loci were

independently inherited. LE and HWE were estimated using Arlequin version 3.5.2.2 (Excoffier and Lischer 2010) and Genalex 6.5 (Peakall and Smouse 2012) respectively. Samples (n=5) representing the observed diversity within the Puckett library were run at the 9 shared loci (G10L, G10P, G1D, G10C, G10M, G1A, G10B, G10U and Mu23), and a correction factor was calculated for each locus, then the genotypes for each sample from the CNC, MNC and WV populations were transformed to be used for comparison.

Population Models

Once samples were identified to individual, capture histories of each individual were created for analysis in capture-mark-recapture (CMR) models. The study areas extended well beyond the observed range of bears in Alabama, which was further supported by the distribution of snare success (Figures 4 and 5). The extent of the sampling study areas allowed us to assume closure within each year's sampling period. Hair snares were not distributed in a uniform fashion on the landscape due to the hierarchical snare layout, intent on maximizing individuals sampled and the heterogeneous access to private land. To address the potential for unequal capture probability due to an uneven density of traps across the study area, a spatial covariate was calculated. The covariate was calculated as the distance between the snare where an individual was detected and the next nearest snare, which was then averaged across all detections in an individual's capture history.

Capture histories with the calculated spatial covariate and a sex covariate were then input into the program MARK (White and Burnham 1999). A Huggins closed capture model was used to estimate within-year population size ($\hat{N}$) for both the MRB and NAL populations (Huggins 1989, 1991). To account for possible between year immigration/emigration, a robust design framework was applied to the NAL population's estimates (White 2008) and $\hat{N}$ was derived for

2012-2015. Only the 2015 MRB capture histories had a sufficient recapture rate to provide a reliable estimate of $\hat{N}$. Multiple a-priori models were tested to account for the effects of the sex and spatial covariates on capture and recapture probabilities, as well as between year variation on survival and capture and recapture for the NAL population. A likelihood ratio test (LRT) was applied to all nested pairs of models to remove more complicated models that did not account for a significant increase in explanatory power. All remaining models were then evaluated and ranked using Akaike's Information Criterion corrected for small sample sizes (AIC_c, ,Anderson and Burnham 2002).

Additionally, we analyzed the data with continuous occasion CMR models in the package Capwire (Pennell et al. 2013) in R studio (RStudio Team 2015, R Core Team 2017). The likelihood function in Capwire was developed for use with non-invasive genetic sampling studies, where individuals can be sampled multiple times during a single occasion as defined by traditional CMR models (Miller et al. 2005). The pooling of all capture events into a continuous occasion helps when estimating small population sizes, and using datasets with sparse capture histories. Within Capwire, two different assumptions about the innate capture rate of individuals were modeled. The first assumption is an even capture model (ECM) where all individuals have equal capture probabilities; and the second is a two innate rates model (TIRM), which assumes that there were two undefined groups within the sampled population that had different capture probabilities. LRTs were used to select between the ECM and TIRM models.

Genetic Diversity Analysis

Genetic diversity of all populations was estimated using observed and unbiased expected levels of heterozygosity (H_o, H_e) (Hartl and Clark 1997) and allelic richness, in GenAlEx v6.5 (Peakall and Smouse 2012) and FSTAT (Goudet 2002), respectively. Differences among

populations for all measures of diversity were assessed with a Kruskal Wallis Rank Sum test with a Nemenyi correction for multiple comparisons (Hollander and A. Wolfe 1973, Sachs 1997).

RESULTS

DNA Collection and Analysis

A total of 1935 samples of scat and hair were collected, of which 970 yielded consensus genotypes. In the MRB study area, 135 unique individuals were identified and in the NAL 32 unique individuals were identified (tables 3 and 4). All of these individuals were used for genetic diversity analysis.

Samples used for population analysis came strictly from hair snares; scat collection did not yield sufficient additional samples to justify the increased complexity of models necessary for its inclusion. Due to low recapture success in the MRB, only results from 2015 were used, which included 228 samples successfully identified to individual (Table 3) collected from 29 of 130 deployed snares (Figure 12) and comprised of 62 unique bears. All four years of hair snare data from the NAL provided adequate capture and recapture rates for models to optimize. All four years of hair snare data yielded 874 samples, of which 427 yielded consensus genotypes which were matched to 32 unique individuals (Table 4).

Both study areas showed a restricted distribution of detections. Though trap density, extent, and layout varied year to year in NAL, the concentration of successful snares was consistently in and around Little River Canyon National Preserve (Figure 13). The MRB population also saw a relatively limited distribution of successful snares. Despite a broad deployment of snares, bear detections were concentrated in two disjoint areas: one near Saraland in Mobile county and the other near Wagarville and Chatom in Washington county, with no

detections between them (Figure 12). These two sub-populations however do not show any genetic clustering separating them (unpublished data –chapter 1).

Population Models

MRB

The Huggins and Capwire closed capture models for the MRB provided qualitatively similar results. The two a priori Huggins models that remained after removing insignificant nested models as tested with LRT were a trap response only model and a trap response model with a constant effect from the spatial covariate (Table 7). The latter of the two models was selected by AICc, and the model estimated the population had 86.41 individuals (95% CI 63.95-165.22, Table 8). Similarly, the TIRM Capwire model was selected by LRT, and estimated 86 individuals (95% CI 76-124, Table 9).

NAL

The Huggins and Capwire models for the NAL showed similar qualitative agreement, and additionally showed a general agreement in the population trend from 2012-2015. After LRT, 5 Huggins models remained for consideration, of which the clear top model accounted for variation in survival between sexes, a trap response, and an equal effect of sex on both capture and recapture probabilities (Table 10). The Huggins Robust Design model showed a clear increasing trend in $\hat{N}$ across all four years of sampling culminating in a final estimate of 24.77 bears (95% CI 22.53-36.57, Table 11). The TIRM Capwire model was selected by LRT and the population estimates support the findings of the Huggins model with a clear increasing trend across all four years, culminating in a final estimate of $\hat{N}$ of 34 bears (95% CI 26-43, Table 12).

Genetic Diversity Analysis

Black bears in Alabama show low to moderate genetic diversity when compared to surrounding populations. The MRB population had the lowest richness at 2.33 alleles per locus, with all comparison populations ranging from 2.43 – 6.00 alleles per locus (Table 13). Observed and expected heterozygosity was also lowest in the MRB population at 0.33, with all comparison populations ranging from 0.45-0.69 observed and, from 0.48-0.72 for expected. Richness and H_e for the MRB were significantly different for 5 of 9 pairwise population comparisons (Tables 14 and 15) and 3 of 9 pairwise comparisons for H_o. NAL showed a more moderate level for all three measures with a richness of 3.32 alleles per locus (observed range 2.33-6.00), H_o of 0.53 (observed range 0.33-0.69) and a H_e of 0.65 (observed range 0.33-0.72). Indices of diversity for NAL did differ significantly from that of other populations at one pairwise comparison of richness and two of H_o. However, we would argue that the differences in H_o were due to an artifact from the Kruskal-Wallis ranking procedure, one locus in the NAL population was nearly monomorphic giving it a rank of 2 and lowering its rank score more substantially than the actual effect on the H_o value, which differed by 5 fold less than other comparisons that were not found to be significantly different (Table 14 and 16).

Discussion

Black bear populations in Alabama were extremely small. Both the NAL and MRB populations were estimated to be less than 100 individuals, with upper confidence limits on the estimates of less than 175 individuals (Tables 8, 9, 11, and 12). However, we stress that these estimates only included the core distribution of bears in the state; reports of individual male bears from outside our sampled population are often collected. Furthermore, while small, the NAL population showed a clear growth trend, more than doubling in 3 years (Tables 11 and 12),

which is promising for the continued persistence and genetic health of the population. In the MRB, however, we were unable to determine if the population was stable, growing, or declining. With only 86 individuals estimated for the core population (Tables 8 and 9), determining the trajectory of the population will be critical to assessing the viability of the population. To better estimate the population in future studies, we suggest that researchers focus on intensely sampling in and immediately surrounding where we have identified the core distribution of bears in the MRB to increase capture and recapture rates (Figure 12).

Our spatially extensive sampling scheme allowed us to establish distribution patterns for both populations of black bears in Alabama. While previous analysis have suggested extensive suitable habitat for black bears in Alabama (Silvano et al. 2007), both populations showed relatively restricted distributions. The MRB population seems to be restricted to two pockets within its available habitat (Figure 12); however no genetic structure exists between the two (unpublished data) and active travel corridors have been identified between them (Chris Seals, Auburn University, personal communication). With no observed barriers between and around these two concentrations of activity, further research is needed to explore why bears are not expanding beyond them. With the limited but increasing population seen in NAL, spatial expansion should be expected as well. Currently, the population is located in and immediately surrounding LIRI (Figure 13). However, ample habitat is available for the bear population to expand into, and incidental reports of explorations by single individuals outside of the current distribution frequently are collected. Unlike the MRB, the NAL population was recently founded, and its limited distribution is more likely due to the rate of natural expansion from its core rather than an anthropogenic or biological restriction preventing it from expanding.

The observed low to moderate genetic diversity of black bears in Alabama is probably due to multiple factors. The MRB population is significantly isolated genetically from surrounding populations (Chapter 1), so much so that one clustering procedure favored only two regional populations: the MRB population and all other populations considered as one. This isolation, combined with its small population size, easily explains the bears' currently low genetic diversity. However, recent migrants have been detected in the MRB (Chapter 1), and could potentially bolster the future genetic diversity of the population. Additionally, these migrants provide evidence that there is some permeability to the landscape separating the MRB from other populations. Bears in NAL display a more moderate level of genetic diversity, but that diversity is still in the lower range of observed diversity of bears in the southeastern U.S. The lower diversity of NAL bears is likely due to a founder effect. However, the NAL population still appears to have some level of connection with CGA (Chapter 1), and the population is growing (Tables 9 and 10), both of which will help to maintain and improve the genetic diversity of the population. Both populations will require continued monitoring to determine any trends in genetic diversity over time.

Efforts should be made to continue to monitor the population size, distribution, and genetic diversity of both populations. Population size and genetic diversity are intrinsically tied to each other and monitoring both population size and genetic diversity going forward will allow managers to detect improvement or declines in population health, and respond accordingly. If barriers restricting range expansion exist for either population, further population growth will be hampered. Thus, to ensure that population growth can continue, researchers need to determine if there are any biotic or anthropogenic limitations to further range expansion for bears in Alabama. Similarly, continued immigration of genetic material into Alabama, like the founding of the NAL

population and the two recent migrants to the MRB, will also be crucial. To allow for a natural infusion of new migrants to both populations, natural corridors should be identified between populations and efforts be made to secure and improve them. Ideally removing barriers to range expansion and immigration should ensure the improvement in genetic health and resulting persistence of black bears in Alabama. However, if genetic diversity declines in the MRB, translocations may need to be considered for temporary infusion of novel genetic material. Of course, there are numerous social, political and biological concerns with translocations, and they should only be considered as a last resort (Miller et al. 1999, Bouzat et al. 2008).

Table 7. Huggins closed capture models considered for the MRB population in 2015 after removal of more complex models that lacked significance in a likelihood ratio test to their reduced model pair. P is rate of capture, C is rate of recapture, Avmin_dis is a spatial covariate that is the average minimum distance between an individuals detection location and the next nearest available snare of all detections which was applied as a constant covariate to both P and C equally.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{P(.t)C(.)Avmin_dis}	609.53	0.00	0.96	1.00	3.00	603.50
{P(.)C(.)}	615.72	6.19	0.04	0.05	2.00	611.71

Table 8. Huggins closed Capture estimate of N̂ for the MRB population in 2015, the Standard error of N̂ and the 95% confidence interval of N̂.

			95% confidence interval	
	N̂	SE	Lower	Upper
{P(. t)C(.)Avmin_dis}	86.41	22.37	63.95	165.22

Table 9. Capwire estimate of $\hat{N}$ and 95% confidence interval of $\hat{N}$.

Capwire		95% Confidence Interval	
	$\hat{N}$	Lower	Upper
2015	86	76	124

Table 10. Huggins Robust Design model selection for the NAL population for 2012-2015. S is survival between years. G" is the probability of temporary emigration in primary period i given NOT a temporary emigration at i-1, and G' is the probability of temporary emigration in primary period i given temporary emigration at i-1, for all included models both were held constant between years, but not equal to each other for a Markovian assumption of migration. P is the probability of capture and C is the probability of recapture. Y is the anual variation in either captrue or recapture probability, and sex is the effect of sex on either survival, or as an equal effect on both P and C as indicated in the model name.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{S(.sex)G"(.)G'(.)(P(y.)C(y.)sex)}	956.13	0.00	0.99	1.00	11.00	932.70
{S(.)G"(.)G'(.)(P(..)C(..)sex)}	966.58	10.45	0.01	0.01	5.00	956.27
{S(.sex)G"(.)G'(.)P(y.)C(y.)}	967.89	11.76	0.00	0.00	10.00	946.71
{S(.)G"(.)G'(.)P(y.)C(y.)}	970.54	14.41	0.00	0.00	10.00	949.36
{S(.sex)G"(.)G'(.)P(..)C(..)}	983.03	26.90	0.00	0.00	5.00	972.72
{S(.)G"(.)G'(.)P(..)C(..)}	985.19	29.06	0.00	0.00	5.00	974.88

Table 11. Huggins Robust Design top model estimate of $\hat{N}$ for the NAL population for 2012-2015. The model assumes differential survival between sexes, but constant between years, Markovian estimates of emigration, and an equal effect of sex on both P (capture) and C (recapture).

			95% Confidence Interval	
{S(.sex)G"(.)G'(.)(P(y.)C(y.)sex)}	$\hat{N}$	SE	Lower	Upper
2012	12.23	1.90	11.14	21.67
2013	12.86	4.41	9.06	30.18
2014	19.38	0.74	19.03	23.40
2015	24.77	2.84	22.53	36.57

Table 12. Capwire estimates of $\hat{N}$ for the NAL population for 2012-2015

Capwire		95% Confidence Interval	
	$\hat{N}$	Lower	Upper
2012	11	11	12
2013	9	8	12
2014	19	19	20
2015	34	26	43

Table 13. Sample populations are listed in ascending order as determined by allelic richness (Richness). N is the number of individuals contributing to each estimate, values are non integers because some genotypes were incomplete due to allelic dropout. Subsequent estimates are allelic richness (Richness), observed heterozygosity (Ho) and expected heterozygosity (He).

Pop	N	SE	Richness	SE	Ho	SE	He	SE
MRB	133.29	0.44	2.33	0.19	0.33	0.06	0.33	0.06
CGA	23.50	0.25	2.43	0.28	0.45	0.05	0.56	0.05
MS	14.86	0.10	2.89	0.20	0.51	0.04	0.66	0.03
NAL	31.93	0.07	3.32	0.29	0.53	0.05	0.65	0.03
FL	17.14	0.29	4.33	0.32	0.63	0.05	0.59	0.05
NGA	16.57	0.14	4.92	0.41	0.60	0.04	0.71	0.02
TN	28.86	0.48	5.03	0.31	0.69	0.03	0.72	0.03
NCC	8.21	1.74	5.53	0.45	0.51	0.11	0.48	0.10
WV	11.79	2.49	5.82	0.38	0.48	0.10	0.49	0.10
NCM	10.50	2.19	6.00	0.32	0.52	0.11	0.49	0.10

Table 14. Pairwise Nemenyi post hoc p-values of a Kruskal-Wallis rank sum ANOVA of allelic richness estimates.

	MRB	NAL	CGA	NGA	MS	FL	TN	NCC	NCM	WV
MRB	-	-	-	-	-	-	-	-	-	-
NAL	0.87879	-	-	-	-	-	-	-	-	-
CGA	1	0.90716	-	-	-	-	-	-	-	-
NGA	0.01241	0.56143	0.01601	-	-	-	-	-	-	-
MS	0.99797	0.99955	0.999	0.15503	-	-	-	-	-	-
FL	0.11975	0.95	0.14356	0.99929	0.5905	-	-	-	-	-
TN	0.00493	0.38838	0.0065	1	0.08142	0.99322	-	-	-	-
NCC	0.00117	0.19344	0.0016	0.99987	0.0281	0.94561	1	-	-	-
NCM	4.30E-05	0.02688	6.20E-05	0.95209	0.00204	0.5679	0.98832	0.99947	-	-
WV	0.00012	0.05229	0.00017	0.9852	0.00476	0.71514	0.99779	0.99997	1	-

Table 15. Pairwise Nemenyi post hoc p-values of a Kruskal-Wallis rank sum ANOVA of He estimates.

	MRB	NAL	CGA	NGA	MS	FL	TN	NCC	NCM	WV
MRB	-	-	-	-	-	-	-	-	-	-
NAL	0.39433	-	-	-	-	-	-	-	-	
CGA	0.87096	0.99925	-	-	-	-	-	-	-	
NGA	0.02649	0.98722	0.72687	-	-	-	-	-	-	
MS	0.42461	1	0.99955	0.98295	-	-	-	-	-	
FL	0.20145	1	0.98644	0.99932	1	-	-	-	-	
TN	0.00385	0.84572	0.35921	0.99995	0.82273	0.96164	-	-	-	
NCC	0.00322	0.82273	0.33112	0.9999	0.79804	0.95209	1	-	-	
NCM	0.00014	0.34784	0.05977	0.9561	0.32021	0.58405	0.99909	0.99945	-	
WV	0.00012	0.32564	0.05372	0.94783	0.29897	0.55819	0.99868	0.99917	1	

Table 16. Pairwise Nemenyi post hoc p-values of a Kruskal-Wallis rank sum ANOVA of Ho estimates.

	MRB	NAL	CGA	NGA	MS	FL	TN	NCC	NCM	WV
MRB	-									
NAL	0.9852	-								
CGA	0.99998	0.99982	-							
NGA	0.39732	0.97391	0.72687	-						
MS	0.99885	1	1	0.88999	-					
FL	0.06901	0.62893	0.22683	0.999	0.40634	-				
TN	0.09103	0.69723	0.27855	0.99967	0.4746	1	-			
NCC	0.00035	0.02688	0.00273	0.47778	0.00895	0.92674	0.89179	-		
NCM	0.00032	0.02496	0.0025	0.46196	0.00824	0.91957	0.8826	1	-	
WV	0.00245	0.10154	0.0148	0.78247	0.04082	0.99449	0.98867	0.99999	0.99998	-

Figure 11.

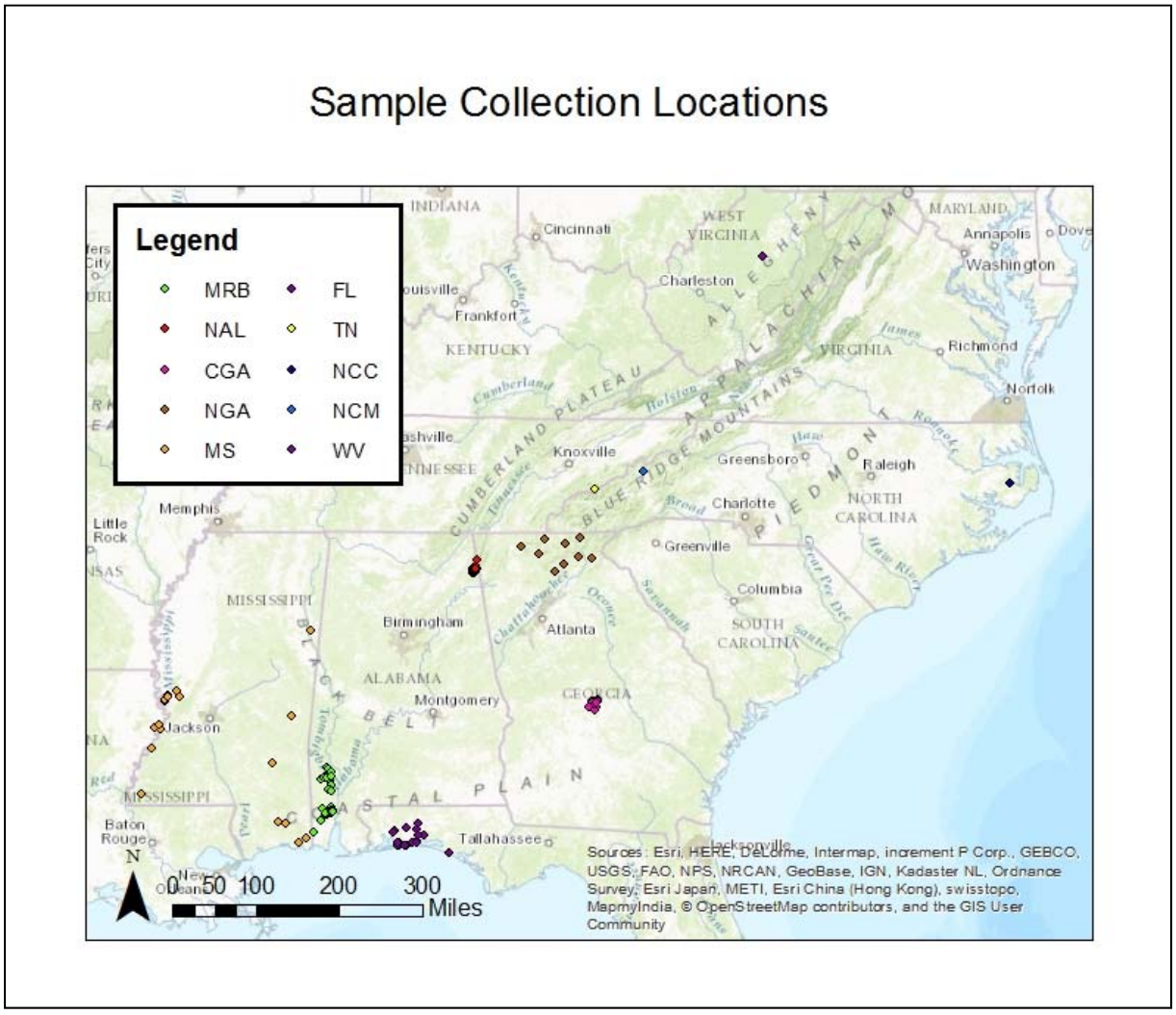


Figure 11. Locations of samples collected from MRB, NAL, MS, CGA, FL, centers of county collected for NGA and approximate center of study area for TN where location information was not available for individual samples. Approximate centers of sample collection locations for NCC, NCM and WV genotypes obtained from Puckett et al. 2015

Figure 12.

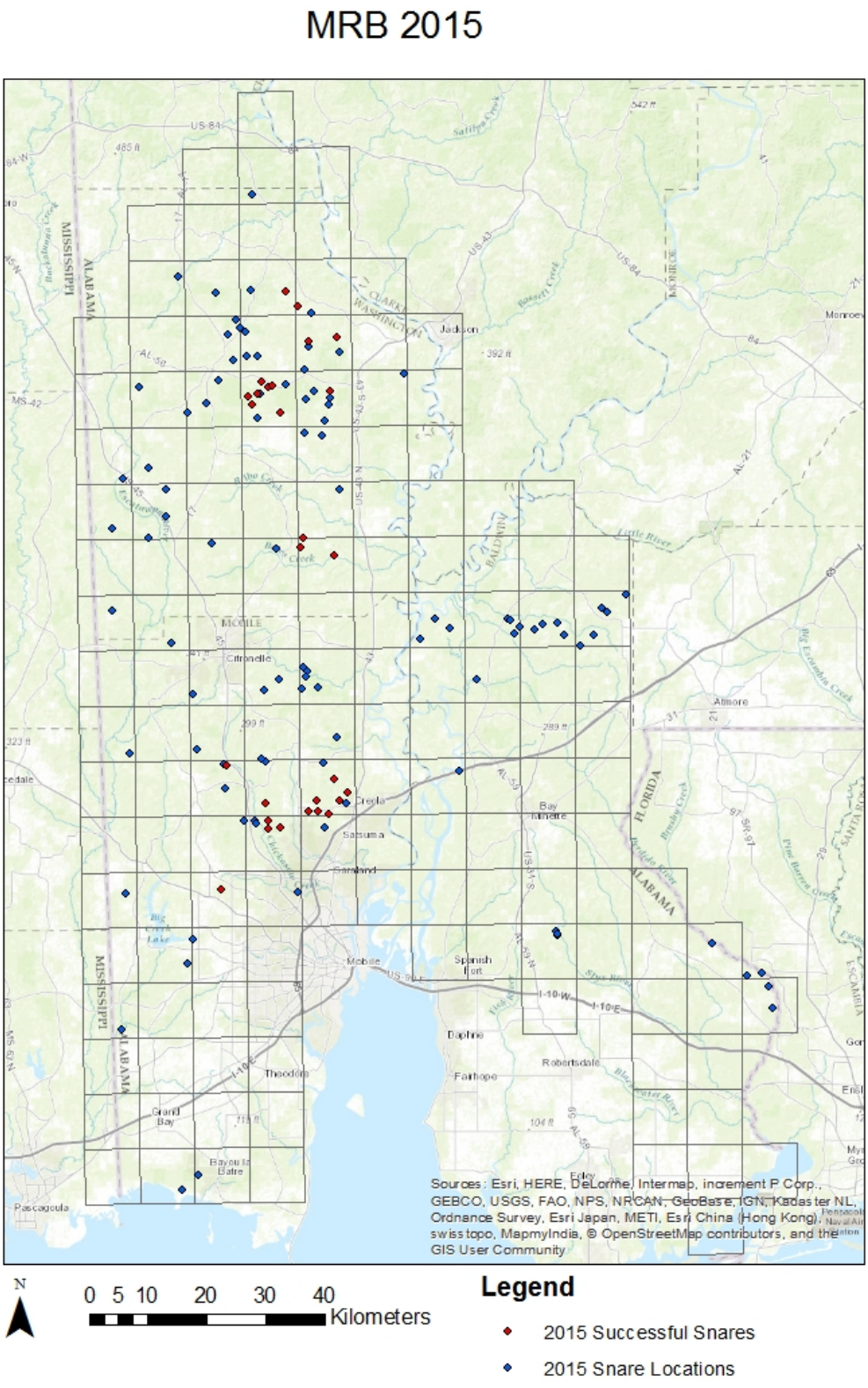
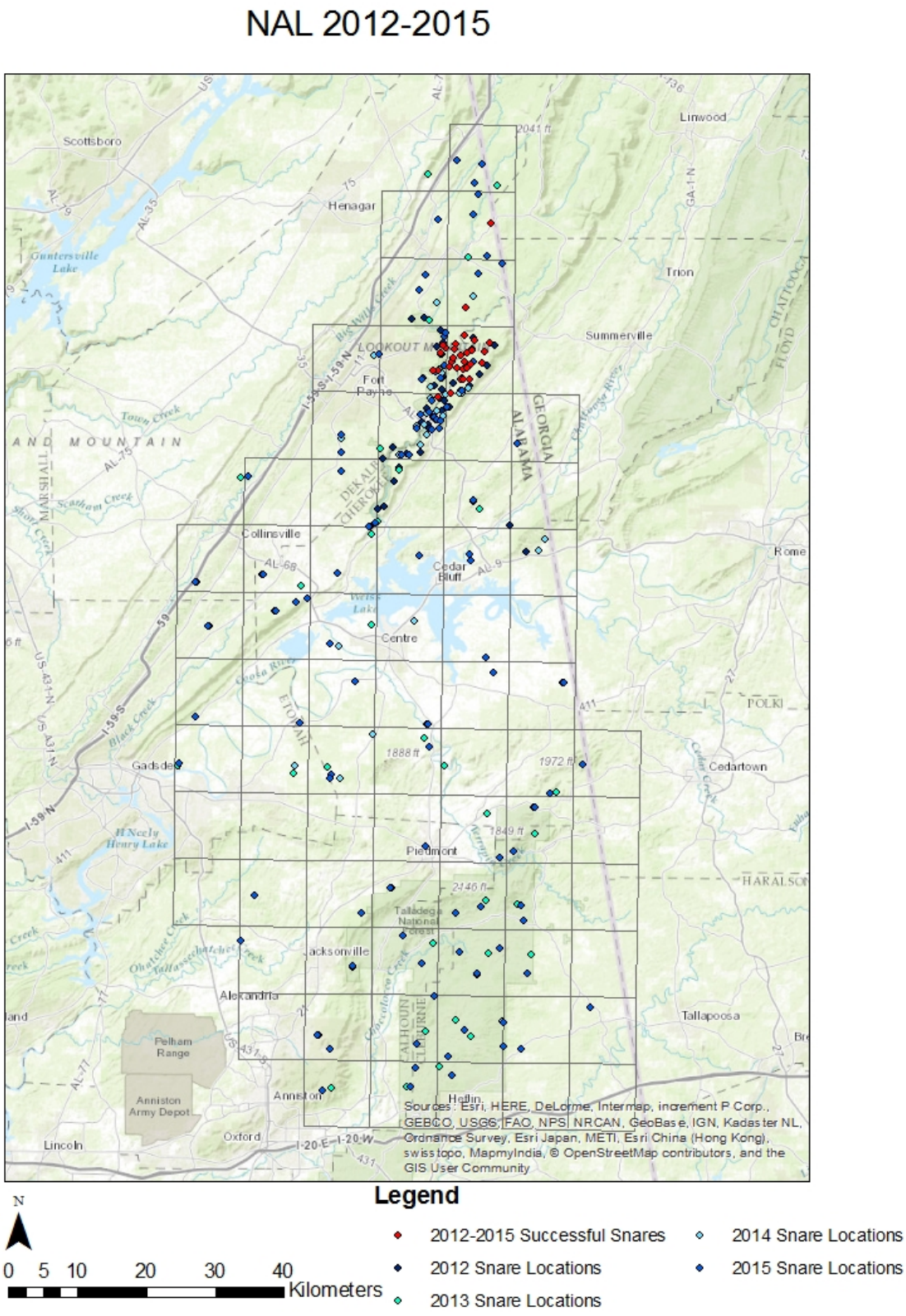

MRB 2015
Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community
N
0 5 10 20 30 40
Kilometers
Legend
2015 Successful Snares
2015 Snare Locations

Figure 13.


NAL 2012-2015
Scottsboro
Henagar
Guntersville Lake
Linwood
Trion
Summerville
LOOKOUT MT
Fort Payne
Collinsville
Cedar Bluff
Centre
Rome
Gadsden
Cedartown
Piedmont
Talladega National Forest
Jacksonville
Alexandria
Pelham Range
Anniston Army Depot
Anniston
Oxford
Lincoln
Tallapoosa
Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community
Legend
2012-2015 Successful Snares
2012 Snare Locations
2013 Snare Locations
2014 Snare Locations
2015 Snare Locations
N
0 5 10 20 30 40
Kilometers

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Appendix 1. MRB Genotypes

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MRB_1	240	240	165	165	150	150	105	107	243	243	130	146	120	120	156	156	182	182	149	149	189	189	117	117	131	137	154	170	142	142
MRB_2	240	240	165	165	150	160	105	105	243	243	146	146	120	130	156	156	182	182	149	149	189	189	117	117	131	131	154	170	122	142
MRB_3	240	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	131	137	154	154	122	142
MRB_4	188	240	165	165	150	150	105	111	243	243	146	146	124	130	156	156	182	182	149	149	189	189	117	117	133	137	154	154	122	146
MRB_5	188	240	165	165	150	150	105	107	243	243	146	146	120	120	156	156	180	182	149	149	189	189	117	117	131	137	154	170	122	142
MRB_6	240	240	165	165	150	160	107	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	137	137	154	170	142	142
MRB_7	188	240	165	165	150	150	105	107	243	243	146	146	124	130	156	156	180	182	149	149	189	189	117	117	131	137	154	154	142	142
MRB_8	240	240	165	165	150	150	105	105	243	243	132	146	120	124	156	156	182	182	149	149	189	189	117	117	131	133	154	170	122	142
MRB_9	240	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	182	149	161	189	189	117	117	137	137	154	170	122	142
MRB_10	240	240	165	165	150	160	105	107	243	243	130	146	120	120	156	156	182	182	149	149	189	189	107	117	133	133	154	154	142	142
MRB_11	240	240	165	165	150	160	105	105	243	243	130	146	120	120	156	156	182	182	149	149	189	189	107	117	133	137	154	154	142	142
MRB_12	240	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	149	185	189	117	117	131	133	154	170	122	122
MRB_13	240	240	165	165	150	150	105	111	243	243	130	146	120	124	156	156	182	182	149	149	189	189	117	117	131	137	154	154	122	146
MRB_14	240	240	165	165	150	160	105	107	243	243	130	146	120	120	156	156	182	182	149	161	189	189	117	117	131	133	154	154	142	142
MRB_15	188	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	149	189	189	107	107	131	131	154	154	142	142
MRB_16	188	240	165	165	150	150	105	111	243	243	132	146	120	120	156	156	180	182	149	149	189	189	117	117	131	131	154	154	122	142
MRB_17	240	240	165	165	150	160	105	107	243	243	130	146	120	124	156	156	180	180	149	149	189	189	107	117	131	133	154	154	142	146
MRB_18	240	240	165	165	150	150	105	111	243	243	132	146	120	120	156	156	180	182	149	149	189	189	107	117	131	137	154	170	122	142
MRB_19	188	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	131	133	154	154	142	142
MRB_20	188	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	149	149	189	189	107	117	131	133	154	154	142	142
MRB_21	188	240	165	165	150	160	105	107	243	243	146	146	120	120	156	156	180	182	149	149	189	189	107	107	131	137	154	170	146	146
MRB_22	240	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	133	137	154	154	122	122
MRB_23	240	240	165	165	150	150	105	111	243	243	132	146	120	120	156	156	182	182	149	149	189	189	107	107	131	137	154	154	122	146
MRB_24	240	240	165	165	150	160	105	107	243	243	132	146	120	124	156	156	180	182	149	149	189	193	107	117	131	137	154	154	142	146
MRB_25	240	240	165	165	150	160	105	107	243	243	132	146	120	124	156	156	180	182	149	149	189	193	107	107	131	131	154	154	142	146
MRB_26	240	240	165	165	160	160	105	0	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	137	137	154	154	122	142
MRB_27	188	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	137	137	154	154	122	142

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MRB_28	240	240	165	165	150	160	105	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	131	131	154	154	142	146
MRB_29	188	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	131	137	154	154	122	122
MRB_30	188	240	165	165	150	150	105	111	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	133	137	154	154	142	142
MRB_31	188	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	170	122	142
MRB_32	240	240	165	165	150	150	105	111	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	154	142	146
MRB_33	240	240	165	165	150	150	105	111	243	243	132	146	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	154	146	146
MRB_34	240	240	165	165	150	150	105	111	243	243	146	146	124	124	156	156	182	182	149	149	189	189	117	117	137	137	154	154	142	146
MRB_35	240	240	165	165	150	160	105	107	243	243	146	146	124	130	156	156	180	180	149	149	189	189	107	117	131	133	154	154	142	142
MRB_36	188	240	165	165	150	150	105	111	243	243	146	146	124	130	156	156	180	182	149	149	189	189	117	117	131	137	154	154	122	142
MRB_37	240	240	165	165	150	160	105	111	243	243	130	132	120	120	156	156	180	182	149	149	189	189	107	117	131	133	154	154	122	146
MRB_38	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	117	117	137	137	154	154	142	142
MRB_39	240	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	182	182	149	149	189	189	107	117	131	137	154	154	122	142
MRB_40	240	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	117	117	131	137	154	154	122	142
MRB_41	240	240	165	165	150	150	105	105	243	243	132	146	124	130	156	156	182	182	149	149	189	189	107	117	131	137	154	170	122	142
MRB_42	240	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	149	149	189	189	117	117	131	131	154	154	122	142
MRB_43	240	240	165	165	150	150	107	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	154	122	142
MRB_44	240	240	165	165	150	160	105	111	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	133	137	154	170	122	122
MRB_45	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	182	182	149	161	189	189	117	117	131	137	154	154	142	142
MRB_46	188	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	149	189	189	117	117	131	137	154	154	142	142
MRB_47	240	240	165	165	150	150	107	107	243	243	146	146	124	124	156	156	180	182	149	149	189	189	117	117	133	137	154	170	142	146
MRB_48	188	240	165	165	150	150	105	105	243	243	146	146	124	124	156	156	180	180	149	161	189	189	117	117	131	131	154	154	142	142
MRB_49	188	240	165	165	150	150	107	107	243	243	130	146	124	124	156	156	182	182	149	149	189	189	117	117	131	137	154	154	142	142
MRB_50	240	240	165	165	150	160	107	107	243	243	146	146	124	130	156	160	180	182	149	149	189	189	117	117	137	137	154	170	122	142
MRB_51	188	240	165	165	150	160	105	107	243	243	146	146	124	130	156	160	180	182	149	149	189	189	117	117	137	137	154	170	122	142
MRB_52	188	240	165	165	150	150	107	111	243	243	146	146	120	130	156	156	182	182	149	149	189	189	117	117	137	137	154	154	122	142
MRB_53	188	240	165	165	160	160	105	105	243	243	130	132	120	120	156	156	182	182	149	149	189	189	117	117	133	137	154	154	142	142
MRB_54	240	240	165	165	150	150	105	105	243	243	146	146	124	124	156	156	182	182	149	149	189	189	107	117	131	137	154	170	122	122
MRB_55	240	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	149	149	189	189	107	117	137	137	154	170	122	122
MRB_56	240	240	165	165	150	160	105	107	243	243	146	146	120	120	156	156	182	182	149	161	189	189	107	107	131	137	154	170	142	146

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MRB_57	188	240	165	165	160	160	105	107	243	243	130	146	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	170	122	146
MRB_58	188	240	165	165	150	150	105	105	243	243	130	146	120	124	156	156	182	182	149	149	189	189	107	107	137	137	154	170	142	142
MRB_59	188	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	149	149	189	189	117	117	131	137	154	170	122	122
MRB_60	188	240	165	165	150	150	105	105	243	243	146	146	130	130	156	156	182	182	149	149	189	189	107	117	131	131	154	170	122	142
MRB_61	188	240	165	165	150	160	105	105	243	243	130	146	120	124	156	156	182	182	149	149	189	189	107	107	131	137	154	170	146	146
MRB_62	240	240	165	165	150	160	105	107	243	243	146	146	120	130	156	156	180	182	149	149	189	189	107	117	137	137	154	154	142	142
MRB_63	240	240	165	165	150	160	105	105	243	243	146	146	120	130	156	156	180	182	149	161	189	189	107	117	131	137	154	154	142	142
MRB_64	240	240	165	165	150	150	105	107	243	243	146	146	120	120	156	156	182	182	149	149	189	189	117	117	137	137	154	154	122	122
MRB_65	240	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	180	149	161	189	189	107	117	131	137	154	170	142	142
MRB_66	188	240	165	165	150	150	107	107	243	243	130	146	124	124	156	156	180	182	149	149	189	189	117	117	133	137	154	154	142	146
MRB_67	188	240	165	165	150	150	107	111	243	243	146	146	124	124	156	156	180	180	149	149	189	189	117	117	137	137	154	154	122	142
MRB_68	188	240	165	165	150	160	105	105	243	243	130	146	120	120	156	156	180	182	149	149	189	189	117	117	131	131	154	154	142	146
MRB_69	240	240	165	165	150	160	105	105	243	243	146	146	120	120	156	156	182	182	149	149	189	189	117	117	131	133	154	170	142	142
MRB_70	188	240	165	165	150	160	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	193	107	117	131	137	154	170	142	142
MRB_71	188	240	165	165	150	160	105	111	243	243	130	132	120	120	156	156	180	182	149	149	189	189	107	117	131	131	154	170	142	146
MRB_72	188	240	165	165	150	160	107	111	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	131	133	154	170	142	146
MRB_73	188	240	165	165	150	160	107	111	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	131	133	154	170	122	142
MRB_74	240	240	165	165	150	160	105	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	107	117	137	137	154	154	122	146
MRB_75	240	240	165	165	150	150	105	105	243	243	146	146	120	124	156	170	182	182	149	149	189	189	117	0	137	137	170	170	122	142
MRB_76	188	240	165	165	160	160	105	111	243	243	130	146	120	120	156	156	180	182	149	149	189	189	117	117	131	133	170	170	122	142
MRB_77			165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	180	149	161	189	189	107	117	137	137	154	154	142	146
MRB_78	188	240	165	165	150	150	105	107	243	249	132	146	124	130	156	156	180	182	149	149	189	189	117	117	133	137	154	170	142	146
MRB_79	188	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	131	137	154	170	142	142
MRB_80	188	240	165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	161	189	189	107	117	137	137	154	154	142	146
MRB_81	240	240	165	165	150	150	105	107	243	243	130	146	120	124	156	156	180	182	149	161	189	189	117	117	133	137	154	170	142	142
MRB_82	188	240	165	165	150	160	105	111	0	0	132	146	120	124	0	0	182	182	149	149	189	189	107	117	133	137	154	170	122	122
MRB_83	240	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	137	137	0	170	142	142
MRB_84	188	240	165	165	150	160	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	117	117	131	131	154	170	142	142
MRB_85	240	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	180	149	149	189	189	117	117	137	137	154	170	142	146

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MRB_86	188	240	165	165	150	160	105	107	243	243	130	146	120	120	156	156	182	182	149	149	189	189	117	117	133	137	154	154	142	142
MRB_87	240	240	165	165	150	150	105	111	243	243	130	146	120	124	156	156	182	182	149	149	189	189	117	117	131	137	154	154	122	142
MRB_88	188	240	165	165	150	150	105	111	243	243	132	146	120	120	156	156	180	182	149	149	189	189	117	117	131	137	154	154	122	142
MRB_89	240	240	165	165	150	160	105	107	243	243	132	146	120	124	156	156	180	182	149	149	189	193	107	117	131	137	154	154	122	142
MRB_90	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	0	0	133	137	154	154	122	142
MRB_91	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	180	149	149	189	189	117	117	131	137	154	154	142	142
MRB_92	240	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	182	182	149	149	189	189	107	117	131	137	154	170	122	142
MRB_93	188	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	149	149	189	189	107	117	131	137	154	170	122	142
MRB_94	240	240	165	165	150	150	105	107	243	243	146	146	120	120	156	156	182	182	149	149	189	189	117	117	137	137	154	154	122	142
MRB_95	188	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	149	189	189	117	117	133	137	154	154	122	122
MRB_96	240	240	165	165	0	0	105	105	243	243	132	146	120	120	156	156	182	0	149	149	189	189	117	117	133	137	154	154	122	122
MRB_97	188	240	165	165	150	160	105	107	243	243	146	146	120	124	156	156	180	182	149	149	189	189	117	117	137	141	154	170	142	142
MRB_98	240	240	165	165	150	150	105	105	243	243	146	146	124	130	156	156	182	182	0	0	189	189	117	117	133	137	154	154	142	142
MRB_99	188	240	165	165	150	160	105	111	243	243	132	146	120	130	156	156	180	182	149	161	189	189	107	117	131	137	154	154	122	142
MRB_100	240	240	165	165	150	160	105	111	243	243	132	146	120	130	156	156	180	182	149	161	189	189	107	117	131	137	154	170	122	142
MRB_101	0	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	161	189	189	107	117	131	137	154	154	142	146
MRB_102	188	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	149	189	189	107	117	131	137	170	170	142	146
MRB_103			165	165	150	150	105	105	243	243	146	146	120	124	156	156	182	182	149	161	189	189	107	107	137	137	154	154	122	146
MRB_104	188	240	175	177	152	152	107	111	0	0	130	148	124	133	164	170	180	180	145	147	191	195	107	121	137	137	154	164	120	120
MRB_105	188	240	165	165	150	158	111	111	243	243	148	148	126	128	168	172	178	184	145	155	191	195	109	121	137	141	162	172	126	126
MRB_106	188	240	165	165	150	150	105	105	243	243	0	146	120	124	156	156	180	180	149	149	189	189	117	117	131	131	154	170	142	0
MRB_107	188	240	165	165	150	150	105	105	243	243	132	146	120	124	156	156	182	182	149	149	189	193	107	117	131	137	154	154	146	146
MRB_108	188	240	165	165	150	160	105	107	243	243	146	146	124	130	156	156	182	182	149	149	189	189	107	107	137	137	154	0	122	142
MRB_109	188	188	165	165	150	150	105	111	0	0	132	146	120	120	156	156	180	182	147	149	189	189	115	117	131	131	154	154	122	142
MRB_110	240	240	165	165	150	150	0	0	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	133	133	154	170	142	142
MRB_111	188	240	165	165	150	160	105	107	243	243	130	146	124	124	156	160	180	180	149	149	189	189	117	117	133	137	154	154	142	146
MRB_112	188	240	165	165	150	150	105	115	243	243	146	146	120	120	156	156	182	0	149	149	189	189	107	117	137	137	154	154	122	142
MRB_113	188	240	165	165	150	150	105	105	243	243	130	146	120	124	156	156	182	182	149	149	189	189	107	107	131	137	154	154	142	142
MRB_114	188	240	165	165	150	150	107	107	243	243	130	132	120	124	156	156	182	182	149	149	189	189	117	117	133	137	154	170	122	142

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MRB_115	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	180	149	149	189	189	107	117	131	131	154	154	122	142
MRB_116	240	240	165	165	150	150	107	107	243	243	146	146	120	124	156	156	182	182	149	149	189	189	117	117	131	137	154	154	142	146
MRB_117	188	240	165	165	150	150	105	105	243	243	132	146	120	124	156	156	182	182	149	149	0	189	107	117	131	133	154	154	122	146
MRB_118	240	240	165	165	150	150	105	107	243	243	146	146	120	130	156	156	182	182	149	149	189	189	107	117	131	137	154	170	142	146
MRB_119	188	240	165	165	150	150	105	107	243	243	132	146	120	130	156	156	180	182	149	149	189	189	117	117	131	133	154	170	122	142
MRB_120	188	240	165	165	150	160	105	111	243	243	146	146	120	124	156	156	180	182	149	161	189	189	107	117	131	137	154	170	142	0
MRB_121	240	240	165	165	150	160	105	111	0	0	132	146	124	124	156	156	182	182	149	149	189	189	107	117	137	137	154	154	122	142
MRB_122	188	240	165	165	160	160	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	107	117	137	137	154	170	142	0
MRB_123	240	240	165	165	150	150	105	107	243	243	132	146	120	130	156	156	180	182	149	149	189	193	107	117	133	137	154	154	142	146
MRB_124	188	240	165	165	150	160	105	107	243	243	130	132	120	124	156	156	182	182	149	149	189	189	107	117	131	137	154	170	122	146
MRB_125	240	240	165	165	150	150	105	107	243	243	132	146	120	124	156	156	180	182	149	149	189	189	107	117	133	133	154	154	146	146
MRB_126	240	240	165	165	150	160	105	111	243	243	132	146	120	124	156	156	180	182	149	149	189	193	107	117	131	133	154	154	142	146
MRB_127	240	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	180	180	149	149	189	189	107	117	137	137	154	170	122	142
MRB_128	188	240	165	165	150	160	107	107	243	243	130	146	120	120	156	156	182	182	149	161	189	189	117	117	131	137	154	154	122	142
MRB_129	240	240	165	165	150	160	105	107	243	243	132	146	120	124	156	156	182	182	149	149	189	189	117	117	137	137	154	170	122	142
MRB_130	240	240	165	165	150	150	105	107	243	243	146	146	120	124	156	156	180	180	149	149	189	189	117	117	137	139	154	170	142	146
MRB_131	188	240	165	165	150	160	105	105	243	0	132	146	124	124	156	156	180	182	149	149	189	189	117	117	131	137	154	170	122	146
MRB_132	188	240	165	165	150	150	105	105	243	243	146	146	124	124	156	156	180	182	149	149	189	189	117	117	131	133	154	170	142	142
MRB_133	240	240	165	165	150	150	105	105	243	243	130	146	120	124	156	156	182	182	149	149	189	189	0	0	131	131	0	0	122	142
MRB_134	240	240	165	165	150	160	105	107	243	0	130	130	120	124	156	156	182	182	149	149	189	189	117	117	133	133	154	154	122	142
MRB_135	188	240	165	165	150	160	107	111	243	243	130	146	124	124	156	156	180	182	149	149	189	189	117	117	131	133	154	154	122	146

Appendix 2. NAL Genotypes

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
NAL_1	240	240	157	177	150	150	107	107	241	249	148	148	120	133	170	170	184	184	147	147	193	195	103	107	135	143	164	164	126	148
NAL_2	188	240	157	177	150	150	107	111	249	249	130	148	120	133	170	170	184	184	147	147	193	193	103	107	135	143	164	164	126	148
NAL_3	240	240	177	179	150	158	107	111	249	249	148	148	128	128	170	172	180	184	147	147	189	195	107	107	135	137	164	164	120	126
NAL_4	240	240	177	177	150	158	107	107	249	249	148	148	120	133	170	172	182	184	147	147	193	193	103	107	137	143	164	164	120	126
NAL_5	188	240	157	177	150	152	107	111	241	249	130	148	120	133	170	172	178	180	147	147	193	193	107	107	137	143	164	164	126	148
NAL_6	240	240	177	179	152	158	107	111	249	253	130	148	128	133	154	170	184	184	147	147	189	193	107	107	135	137	164	164	120	148
NAL_7	240	240	179	179	152	158	107	107	241	249	130	150	126	133	168	170	180	180	147	165	189	193	107	115	135	137	164	164	126	148
NAL_8	240	240	177	179	152	152	107	107	241	241	130	150	126	133	170	170	180	184	147	165	189	195	107	115	135	137	164	164	126	126
NAL_9	240	240	177	179	150	152	107	107	241	249	130	130	128	133	168	170	180	184	147	165	189	193	107	115	135	135	164	164	126	126
NAL_10	240	240	157	177	152	158	111	111	241	241	130	148	128	128	170	170	178	184	147	147	193	195	103	107	135	143	164	164	120	126
NAL_11	188	240	177	177	152	158	107	107	241	249	130	148	128	128	154	170	184	184	147	147	193	193	107	107	135	137	164	164	126	148
NAL_12	188	240	177	177	150	150	107	107	241	249	148	148	120	128	170	170	178	180	147	147	193	195	107	107	135	137	164	164	126	148
NAL_13	188	240	177	179	152	158	107	107	241	249	130	150	128	128	172	172	180	184	145	165	195	195	107	107	135	137	164	164	122	148
NAL_14	188	240	177	177	152	152	111	111	241	253	130	130	128	133	170	170	180	184	147	147	193	193	107	107	135	135	164	164	120	148
NAL_15	240	240	177	179	152	152	107	107	249	249	130	150	126	128	168	172	180	180	147	165	193	195	107	115	135	135	164	164	126	148
NAL_16	188	240	177	177	150	158	107	107	249	249	148	148	128	133	154	172	184	184	147	147	193	195	107	107	135	135	164	164	148	148
NAL_17	188	240	177	179	152	158	107	109	241	253	130	148	133	133	154	172	182	184	147	147	191	193	107	107	135	137	164	164	120	126
NAL_18	240	240	157	177	150	158	107	111	241	249	148	148	120	128	170	170	178	184	147	147	193	193	103	107	135	137	164	164	120	148
NAL_19	240	240	157	165	152	158	107	111	241	241	148	150	126	128	168	170	178	182	145	147	193	193	107	117	135	137	164	164	148	150
NAL_20	240	240	157	177	152	158	107	111	241	241	148	148	128	133	170	170	184	184	147	147	193	193	107	107	143	143	164	164	126	148
NAL_21	188	240	177	177	150	152	107	111	241	249	130	148	128	133	170	172	180	184	147	147	193	195	107	107	135	143	164	164	126	148
NAL_22	188	240	165	177	150	158	107	107	241	249	150	150	126	128	168	172	182	184	145	147	193	195	107	117	135	143	164	164	126	148
NAL_23	188	240	165	177	152	158	107	111	241	249	148	150	126	133	168	170	182	184	145	147	193	193	107	117	137	143	164	164	126	150
NAL_24	240	240	157	177	150	158	105	109	241	249	148	148	118	128	170	170	178	184	147	147	193	193	101	105	135	137	164	164	120	148
NAL_25	188	240	175	177	154	158	101	109	243	243	130	130	126	133	170	172	182	182	147	165	195	195	0	0	137	137	158	164	122	146
NAL_26	188	240	177	179	152	152	107	107	249	249	130	150	126	128	168	172	180	184	147	165	193	195	107	115	135	135	164	164	126	148
NAL_27	188	240	177	179	152	158	107	109	241	249	130	150	128	133	154	172	182	184	145	147	195	195	107	107	135	137	164	164	122	126

Sample	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
NAL_28	188	240	177	179	152	158	107	107	0	0	148	150	126	133	168	172	180	180	147	147	193	195	107	115	135	135	164	164	126	148
NAL_29	240	240	157	177	150	158	107	107	241	241	148	148	126	133	170	0	178	184	147	165	193	193	107	115	0	137	164	164	120	148
NAL_30	188	240	177	179	152	158	107	111	241	249	130	150	128	128	170	172	180	184	145	147	193	195	107	107	135	137	164	164	122	148
NAL_31	188	240	177	177	150	158	107	107	249	249	148	148	120	128	0	0	184	184	147	147	193	193	103	107	137	143	164	164	120	126
NAL_32	240	240	157	177	150	158	107	111	249	249	148	148	120	133	170	172	184	184	147	147	193	193	103	107	137	143	164	164	120	126

Appendix 3: CGA, NGA, MS, FL, TN, Genotypes

Sample	Pop	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
GA-101	CGA	240	240	181	181	154	154	107	107	245	249	132	150	126	130	156	156	178	180	149	149	191	197	107	107	133	135	174	174	124	124
GA-102	CGA	240	240	163	181	154	158	107	107	249	249	132	150	126	128	156	156	178	180	149	149	189	197	107	107	135	137	172	174	124	126
GA-103	CGA	188	240	175	181	158	158	107	107	249	249	130	132	130	130	156	156	180	180	149	149	197	197	107	107	135	137	172	172	126	126
GA-104	CGA	188	240	165	181	154	154	107	109	249	249	130	132	126	128	156	176	182	182	149	149	185	197	107	107	135	137	174	174	126	126
GA-106	CGA	240	240	165	175	154	154	107	107	249	249	130	132	126	128	156	156	180	180	149	149	191	191	107	107	135	137	172	174	126	126
GA-107	CGA	188	240	181	181	154	154	107	107	249	249	132	132	128	130	156	176	180	180	149	149	191	191	107	107	137	137	172	172	124	124
GA-108	CGA	188	240	165	175	154	154	105	0	241	249	130	150	126	130	156	0	180	180	155	155	185	197	107	107	135	135	172	174	126	126
GA-109	CGA	188	240	165	181	154	154	107	107	249	249	132	150	128	130	156	156	180	180	149	149	191	191	107	107	137	137	172	172	126	126
GA-110	CGA	188	240	165	181	154	154	105	107	241	249	132	132	130	130	156	156	178	180	149	149	197	197	107	107	137	139	172	172	126	126
GA-111	CGA	188	240	165	181	154	154	107	109	241	249	150	150	128	128	156	156	178	180	149	149	191	191	107	107	135	135	172	174	120	126
GA-112	CGA	240	240	163	181	154	154	107	107	249	249	132	150	128	130	156	176	178	180	147	149	191	191	107	107	135	135	172	172	124	124
GA-113	CGA	240	240	181	181	154	154	107	107	249	249	132	132	128	130	156	176	180	180	149	149	191	197	107	107	137	137	172	174	124	124
GA-114	CGA	188	240	165	181	154	154	107	107	241	249	132	150	130	130	156	156	180	180	149	155	189	197	107	107	135	137	172	174	126	126
GA-115	CGA	240	240	165	175	154	154	107	109	241	249	132	150	126	130	156	176	178	178	149	149	191	191	107	107	137	137	172	172	124	124
GA-116	CGA	240	240	163	175	154	158	107	107	241	249	130	132	130	130	156	156	180	182	147	149	189	197	107	107	133	133	172	172	124	124
GA-117	CGA	240	240	165	175	154	154	0	0	249	249	130	132	128	128	156	156	178	182	149	149	185	191	107	107	135	137	172	174	124	124
GA-118	CGA	240	240	165	181	154	154	107	107	249	249	132	150	130	130	156	156	178	180	149	149	191	191	107	107	137	137	172	172	126	126
GA-119	CGA	188	240	163	181	154	158	107	107	249	249	132	132	128	130	156	156	178	178	149	149	191	197	107	107	133	135	172	174	124	124
GA-120	CGA	0	0	181	181	154	154	107	107	249	249	132	150	0	0	0	0	178	180	147	155	189	189	0	0	0	0	0	0	124	124
GA-121	CGA	240	240	181	181	154	154	107	107	247	247	132	150	128	128	156	156	180	180	147	149	191	191	107	107	137	137	172	172	126	126
GA-123	CGA	240	240	165	181	154	158	107	107	249	249	150	150	128	128	156	176	180	182	149	149	185	191	107	107	133	137	172	172	126	126
GA-124	CGA	240	240	163	181	154	154	107	107	249	249	132	132	128	130	156	156	178	0	147	149	189	191	107	107	137	139	172	174	124	124
GA-125	CGA	240	240	175	181	154	158	107	107	249	249	132	132	128	130	156	156	180	180	149	149	191	191	107	107	135	135	172	174	124	126
GA-126	CGA	188	240	165	181	154	158	107	107	247	247	132	132	128	130	156	156	180	180	149	155	189	191	107	107	133	135	172	174	124	124
7408	NGA	188	240	179	179	150	150	111	111	243	243	132	148	130	133	172	174	178	178	147	153	189	195	107	115	137	139	166	172	124	148

Sample	Pop	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
7424	NGA	188	240	177	179	152	158	109	109	243	249	130	146	133	133	170	170	180	180	147	165	191	195	107	117	135	139	166	166	120	126
8020	NGA	188	240	177	177	150	158	107	109	241	241	130	148	120	128	170	174	180	180	145	147	193	195	107	117	135	137	164	164	124	148
8022	NGA	188	240	177	177	150	152	109	111	241	241	146	150	128	130	170	170	180	184	153	165	187	195	107	115	135	139	154	164	126	148
8024	NGA	188	240	179	179	158	158	107	111	241	249	150	152	120	124	170	172	180	180	147	147	193	195	107	107	135	139	164	172	120	148
8246	NGA	188	240	177	179	150	150	107	109	241	241	130	150	128	133	168	170	182	182	147	165	193	195	103	115	135	137	162	164	120	126
8252	NGA	188	240	177	177	152	158	107	111	241	249	130	146	128	133	170	170	178	180	147	147	191	195	111	115	137	137	154	164	126	146
8258	NGA	188	240	177	177	150	152	107	109	243	243	130	130	133	133	170	170	178	180	147	147	191	195	103	107	135	137	154	164	120	148
8385	NGA	240	240	157	181	150	152	107	111	241	241	148	148	128	133	154	164	182	182	147	147	187	195	107	117	137	137	154	164	120	148
8659	NGA	188	240	157	177	150	150	107	111	241	253	144	148	128	133	168	168	178	180	145	145	187	189	103	107	137	137	164	166	126	126
10778	NGA	188	240	175	177	152	158	107	109	241	249	148	152	120	133	170	176	178	184	147	165	193	195	103	107	135	139	164	172	122	124
11088	NGA	188	240	177	177	150	150	103	107	241	253	0	0	126	126	170	170	178	182	147	147	195	0	103	107	139	139	164	166	126	150
11090	NGA	188	240	175	177	152	158	107	109	241	249	148	152	120	133	170	176	178	184	147	165	193	195	103	107	135	139	164	172	122	124
11801	NGA	188	240	177	179	150	150	107	109	243	243	138	146	128	128	164	172	184	184	147	147	185	187	103	103	135	137	154	166	126	126
11802	NGA	188	240	177	177	152	158	111	111	247	253	130	152	133	133	168	170	182	182	161	161	191	193	107	107	135	135	174	180	124	126
11818	NGA	188	240	179	179	150	152	107	107	243	243	130	148	120	133	170	170	178	178	147	147	191	195	107	117	137	137	164	164	126	148
12147	NGA	188	240	157	157	150	152	107	109	243	249	150	152	126	133	154	174	180	182	145	165	189	189	115	117	137	137	162	166	126	150
MS-AR1	MS	240	240	165	177	158	158	109	109	241	243	152	152	126	126	160	160	182	182	147	153	191	191	107	107	141	141	154	158	148	150
MS-F920	MS	240	240	177	177	150	158	109	109	241	243	130	130	126	128	160	160	182	182	147	153	189	191	107	115	141	141	154	154	132	150
MS-G470	MS	240	240	175	177	158	158	109	109	243	243	130	130	124	128	156	160	182	182	147	147	189	189	107	117	135	137	154	154	144	144
MS-I789	MS	188	240	175	177	150	156	109	109	241	241	130	130	128	128	156	172	182	182	153	153	189	191	107	117	137	141	154	154	124	150
MS-J320	MS	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	180	182	149	149	189	189	117	117	137	137	154	154	142	142
MS-KK31	MS	240	240	165	177	150	150	109	113	241	243	130	130	126	126	160	160	182	182	153	153	189	191	107	107	135	141	154	158	132	132
MS-O800	MS	188	240	165	165	150	150	105	105	243	243	146	146	120	120	156	156	182	182	149	161	189	189	107	117	131	137	154	154	142	146
MS-U755	MS	188	240	165	165	150	150	105	107	243	243	146	146	124	130	156	156	180	182	149	149	189	189	117	117	131	137	154	154	142	146
MS-W23	MS	188	240	165	177	158	158	109	109	241	241	130	130	126	128	160	160	182	182	153	153	189	191	107	107	137	141	154	158	132	150
MS-II28	MS	188	240	165	165	150	150	105	105	243	243	146	146	120	130	156	156	182	182	149	161	189	189	117	117	131	137	154	154	142	142
MS-JJ30	MS	188	240	177	177	150	158	109	109	241	243	130	130	124	128	166	172	182	182	153	153	189	189	107	107	137	137	154	154	124	144
MS-K515	MS	188	240	165	165	150	160	107	107	243	243	146	146	124	124	156	156	180	182	149	149	189	189	117	117	137	137	154	154	122	142

Sample	Pop	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
MS-N528	MS	188	240	165	165	160	160	105	105	243	243	146	146	124	124	156	156	180	182	149	149	189	189	107	117	133	137	154	170	146	146
MS-P16	MS	188	240	175	177	150	158	109	109	241	243	130	148	126	128	156	160	178	182	153	153	189	189	107	107	135	135	158	158	124	144
MS-RCB9	MS	240	240	175	177	150	156	111	111	241	243	130	130	126	126	156	160	182	182	147	153	189	189	117	117	135	137	154	172	144	144
PZV6	FL	240	240	165	175	150	154	103	105	243	243	134	148	120	128	172	172	182	188	155	155	185	197	107	113	135	137	162	172	126	126
PZW6	FL	240	240	165	165	150	150	111	111	243	0	150	152	124	124	164	168	180	182	145	147	191	195	107	117	133	141	162	162	124	144
PZX6	FL	240	240	165	165	158	158	111	111	243	243	130	150	124	133	172	172	182	184	145	155	191	195	109	0	137	141	162	168	122	126
PZY6	FL	240	240	165	165	154	0	107	111	0	0	144	144	124	130	172	172	182	182	142	147	0	0	109	0	133	141	162	0	122	122
PZZ6	FL	188	240	165	165	146	150	111	113	243	243	0	0	126	133	156	172	180	182	142	142	191	197	107	121	133	137	162	172	122	144
QZ16	FL	240	240	157	165	150	158	111	113	243	243	144	148	124	128	156	172	182	0	142	145	191	191	107	109	133	133	162	162	122	122
QZ26	FL	188	240	165	165	150	158	111	113	243	0	150	150	124	126	168	172	182	0	142	145	195	195	109	117	137	141	162	162	122	126
QZ36	FL	240	240	165	165	150	154	111	113	243	243	148	150	124	130	172	172	182	182	145	147	191	195	109	121	133	137	166	168	122	122
QZ46	FL	240	240	165	175	150	158	111	113	243	243	148	150	126	128	172	172	180	182	145	147	191	195	109	117	133	133	162	166	122	126
QZ56	FL	240	240	165	165	150	158	111	111	243	249	144	152	124	128	168	172	178	184	142	145	191	191	107	109	133	141	162	168	122	144
QZ66	FL	188	240	165	175	154	158	111	111	243	249	144	152	120	120	168	172	182	184	142	147	195	195	107	107	133	133	162	162	122	144
QZ76	FL	188	240	165	165	150	154	111	113	243	243	144	152	124	126	172	172	182	182	142	145	191	191	109	121	133	137	162	162	122	144
QZ86	FL	188	240	165	165	150	158	111	111	243	243	148	148	128	133	168	168	178	178	142	142	191	195	107	109	133	137	166	168	122	122
QZ96	FL	188	240	165	165	158	158	111	113	243	249	150	150	120	128	168	172	178	184	145	147	195	197	109	117	133	133	162	172	122	126
QZA6	FL	240	240	165	165	154	158	111	111	243	243	146	146	120	126	172	172	180	180	142	145	191	191	109	109	133	133	162	162	122	126
QZB6	FL	188	240	165	165	150	150	105	111	243	243	148	152	126	130	168	172	182	182	142	155	185	191	107	117	137	141	166	172	126	144
QZD6	FL	240	240	165	165	150	158	111	113	243	243	146	150	120	128	172	172	180	0	142	145	191	195	109	121	137	141	162	168	126	144
QZF6	FL	240	240	165	175	150	158	111	113	243	243	148	148	128	128	168	168	180	182	142	155	195	197	107	109	133	133	168	172	122	122
BR10-004	TN	188	240	157	157	150	152	103	107	249	249	138	144	126	128	170	176	182	182	147	153	191	193	115	115	133	139	0	0	142	142
BR10-006	TN	188	240	157	157	150	158	109	109	247	249	144	144	126	132	172	174	178	182	147	147	193	193	103	107	0	0	162	164	126	126
BR10-007	TN	240	240	157	175	150	150	107	107	243	253	148	148	126	130	168	0	178	182	147	165	189	193	107	107	139	141	172	172	122	148
BR10-008	TN	188	240	175	175	152	158	107	109	243	243	148	152	128	132	170	172	182	182	147	165	193	195	111	111	135	139	164	172	120	150
BR10-009	TN	188	240	157	179	150	150	115	115	249	249	130	148	126	128	154	174	182	182	149	165	189	191	107	115	135	137	162	164	126	142
BR10-012	TN	240	240	175	177	150	150	107	107	249	253	138	148	128	128	170	170	180	182	145	153	191	191	109	0	135	141	0	0	126	134
BR10-013	TN	240	240	157	165	150	152	109	113	249	257	138	146	128	132	170	174	180	182	145	165	191	193	103	115	135	139	0	0	124	142

Sample	Pop	SE47-48		D1A		G10B		G10C		G10H		G10L		G10M		G10P		G10U		G10X		G1A		G1D_FIm+Rm		Mu15		Mu23		Mu50	
BR10-024	TN	240	240	165	175	150	150	107	109	249	253	146	152	120	128	172	176	182	182	145	165	187	191	103	117	135	137	164	172	122	126
BR10-026	TN	240	240	157	175	150	152	107	109	241	249	130	144	130	132	176	176	178	182	145	145	191	191	103	107	137	137	164	172	148	150
BR10-033	TN	188	240	165	165	150	158	107	109	243	249	130	144	120	132	170	176	178	184	147	165	187	187	107	117	135	137	164	172	122	126
BR10-044	TN	188	240	157	179	158	158	107	109	241	253	130	152	124	132	168	174	182	182	147	165	189	191	111	115	135	137	158	172	126	142
BR10-048	TN	188	240	165	175	150	150	107	115	249	253	130	144	120	126	154	174	182	0	147	165	187	193	107	107	133	135	158	172	126	142
BR10-049	TN	188	240	157	165	152	152	109	111	243	243	144	148	124	132	168	168	180	0	145	147	187	193	107	121	135	137	162	164	124	146
BR10-055	TN	188	240	157	175	150	150	107	109	243	243	148	150	126	128	168	170	182	182	165	165	187	193	107	115	133	135	162	162	142	148
BR10-056	TN	188	240	157	175	150	150	101	107	243	243	130	148	120	126	168	168	180	182	147	165	193	195	107	117	135	135	172	172	120	126
BR10-059	TN	188	240	157	179	150	158	109	109	249	253	148	150	126	132	172	0	182	182	147	149	191	195	107	117	137	139	162	168	126	142
BR10-060	TN	188	240	165	175	150	154	101	109	249	253	130	148	126	132	0	0	182	182	147	147	191	193	107	117	135	135	158	172	120	126
BR10-063	TN	188	240	157	175	150	150	107	107	257	257	144	144	120	126	172	174	182	182	147	149	193	193	107	115	133	137	0	0	126	134
BR10-069	TN	240	240	157	165	152	158	103	107	243	249	130	138	126	128	174	176	178	182	145	165	189	193	107	111	139	139	162	162	142	142
BR10-070	TN	188	240	175	175	150	152	111	111	253	253	130	132	124	132	168	168	182	184	145	153	195	0	115	115	133	135	162	166	142	142
BR10-077	TN	240	240	157	177	152	158	107	111	263	263	138	144	120	128	172	172	178	182	147	147	189	191	115	117	135	137	172	172	126	134
BR10-100	TN	188	240	157	177	150	150	109	113	241	249	130	130	126	126	0	0	182	182	165	170	193	193	107	107	137	139	162	162	126	142
BR10-112	TN	188	240	175	175	150	154	103	111	243	243	138	144	126	126	168	168	180	180	147	161	189	189	107	107	135	137	164	164	120	122
BR10-126	TN	188	240	175	179	150	154	107	107	243	243	130	144	124	128	168	172	182	182	165	0	187	191	103	109	133	139	162	168	126	126
BR10-130	TN	240	240	157	157	158	158	109	115	249	253	130	150	124	128	170	174	182	182	147	165	193	193	111	117	135	137	158	168	126	126
BR10-132	TN	240	240	157	165	150	150	107	109	243	243	130	144	120	132	160	168	178	182	165	165	191	193	103	111	135	137	164	168	126	148
BR10-141	TN	188	240	157	175	150	152	107	109	249	249	130	130	120	124	168	170	182	182	149	165	191	193	109	117	133	137	164	164	122	142
BR10-142	TN	188	240	157	175	150	150	109	109	247	249	144	144	126	132	172	0	182	182	147	147	191	193	103	103	137	141	162	164	126	126
BR10-163	TN	188	240	157	175	150	158	107	107	243	249	144	150	120	126	170	176	182	182	147	153	193	193	103	107	135	135	172	172	126	142
BR10-246	TN	188	240	157	165	150	160	107	107	249	249	130	130	128	132	174	0	178	182	147	165	189	193	103	117	137	139	162	164	124	126

Appendix 4. Calibrated samples from Puckett et al. 2015

Sample	Population	G10B		G10C		G10L		G10M		G10P		G10U		G1A		G1D_FIm+Rm		Mu23	
NCC21	NCC	150	152	0	0	146	154	122	128	170	172	180	184	187	195	107	115	154	171
NCC22	NCC	154	158	107	111	144	154	122	126	172	172	178	178	189	191	0	0	162	170
NCC23	NCC	150	152	109	111	132	150	120	130	170	172	184	184	195	195	107	117	162	164
NCC24	NCC	146	152	107	107	132	148	122	126	170	170	182	182	189	191	107	117	164	172
NCC25	NCC	146	150	0	0	148	154	126	126	168	168	182	184	0	0	107	107	171	172
NCC26	NCC	150	154	109	111	154	154	126	126	168	170	178	184	187	187	0	0	158	170
NCC27	NCC	152	152	0	0	130	132	124	126	168	168	178	180	0	0	107	115	162	172
NCC28	NCC	150	150	109	111	144	150	126	126	170	172	182	184	191	193	103	107	154	172
NCC29	NCC	152	154	111	115	150	150	126	128	170	172	178	182	191	195	115	117	171	171
NCC30	NCC	150	152	109	111	130	134	124	126	168	172	178	184	193	193	103	107	162	171
NCC31	NCC	150	152	111	111	134	138	124	128	168	168	178	184	0	0	103	107	162	171
NCC32	NCC	152	158	111	111	134	150	126	130	166	170	182	184	0	0	107	111	154	156
NCC33	NCC	150	160	105	111	0	0	122	126	168	170	178	182	189	195	115	117	162	171
NCC34	NCC	150	158	0	0	134	138	120	126	156	176	178	182	193	195	107	117	172	172
NCMt01	NCM	150	158	107	109	134	144	126	132	166	166	178	180	189	193	107	115	164	172
NCMt02	NCM	150	158	107	107	132	144	124	126	168	170	178	180	191	193	113	115	164	168
NCMt03	NCM	150	150	111	111	134	144	126	126	168	172	182	184	0	0	109	117	162	162
NCMt04	NCM	152	160	107	111	130	134	130	132	166	168	182	182	193	195	107	109	158	172
NCMt05	NCM	152	160	107	111	144	150	124	132	154	170	0	0	193	195	111	117	162	162
NCMt06	NCM	150	150	107	117	134	138	128	132	166	166	182	182	189	193	115	115	172	172
NCMt07	NCM	150	150	101	107	132	150	124	132	154	172	178	178	189	195	107	117	154	164
NCMt09	NCM	150	158	101	107	138	148	120	132	172	172	180	184	0	0	107	111	162	172
NCMt10	NCM	146	150	107	111	148	148	126	132	168	170	178	178	189	193	107	115	170	172
NCMt11	NCM	156	158	107	107	130	148	126	132	154	174	180	182	187	191	107	115	162	172
NCMt12	NCM	150	158	111	117	130	138	120	126	170	172	180	182	187	189	107	117	162	172
NCMt13	NCM	150	150	107	111	144	148	124	132	156	176	178	184	193	195	103	115	164	170
NCMt14	NCM	150	154	109	111	132	152	120	126	170	172	176	178	191	193	107	115	154	172

Sample	Population	G10B		G10C		G10L		G10M		G10P		G10U		G1A		G1D_FIm+Rm		Mu23	
NCMt15	NCM	150	150	105	107	138	150	126	132	154	176	182	182	0	0	103	115	158	170
NCMt16	NCM	146	146	107	109	132	148	128	130	168	170	182	184	191	193	103	107	154	174
NCMt17	NCM	150	154	107	113	148	148	124	126	168	170	182	182	189	191	101	111	172	172
NCMt18	NCM	150	158	0	0	148	148	126	132	170	170	178	184	0	0	109	115	154	174
WV01	WV	148	150	101	101	142	148	124	126	168	168	182	184	191	191	107	113	154	158
WV02	WV	150	152	107	107	150	150	126	132	166	168	180	182	187	191	101	107	158	170
WV03	WV	152	158	107	109	144	148	128	132	168	168	180	184	191	193	109	109	168	170
WV04	WV	150	154	105	111	142	150	120	120	172	172	178	182	189	191	0	0	154	162
WV05	WV	154	158	101	101	134	150	120	124	160	160	182	182	187	187	107	117	171	172
WV06	WV	154	156	0	0	142	154	124	126	164	176	180	180	0	0	107	115	154	171
WV07	WV	150	156	105	109	144	156	122	122	168	168	180	182	187	191	107	109	154	168
WV08	WV	150	154	107	109	132	148	122	126	164	164	180	182	189	193	107	117	168	172
WV09	WV	152	154	105	105	142	150	130	130	168	172	182	182	191	193	107	109	0	0
WV10	WV	0	0	107	111	130	148	126	126	164	168	180	180	189	191	107	107	154	154
WV11	WV	150	150	0	0	130	152	120	128	164	164	178	182	0	0	117	117	154	170
WV12	WV	152	154	105	107	142	142	120	124	164	166	180	184	187	193	107	109	154	154
WV13	WV	154	154	107	111	142	148	120	130	164	164	180	182	0	0	115	115	154	154
WV14	WV	154	154	105	107	144	150	126	128	164	168	176	178	187	189	105	107	154	172
WV15	WV	152	154	0	0	142	144	120	128	168	168	178	182	0	0	105	107	154	172
WV16	WV	150	160	105	109	148	148	120	122	156	160	182	184	0	0	0	0	154	170
WV17	WV	150	152	107	111	148	150	120	130	164	176	176	180	0	0	107	109	154	156
WV18	WV	148	150	107	107	144	144	126	128	168	168	180	184	191	193	103	107	164	171
WV19	WV	152	152	107	111	130	142	126	130	164	168	180	182	0	0	107	117	154	170
WV20	WV	152	152	0	0	148	152	126	132	164	166	178	182	191	191	103	117	158	164