Genetic Analysis of Dispersal and Population Dynamics of the Southeastern Coyote (*Canis latrans*)

by

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Abstract

Two different types of genetic analyses, phylogeography and population genetics, were completed on coyotes (*Canis latrans)* across the Central Plains, Midwestern, and Southeastern United States. The first goal of this study was to infer historical dispersal patterns out of the presumed historical ranges of the Great Plains into the eastern U. S. Phylogeographic analyses using the control region of the mitochondrial genome, including a maximum likelihood tree and median-joining network, in addition to genetic diversity and differentiation indices were employed. The second goal of this study was to assess population structure of coyotes in order to identify possible management units of coyotes in Alabama. We examined patterns of gene flow of coyotes both within a 100 km radius of the Auburn/Opelika Metropolitan Statistical Area and across an urban to rural gradient created in ArcGIS using microsatellite DNA markers.

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CHAPTER I: INTRODUCTION

Originating from Texas, and being raised on both farms and ranches, I was introduced to coyotes at an early age. It seemed as though my family and I were always at war with this infamous species over the cattle, the sheep, or the watermelon crops. Prior to beginning this project, I thought that seeing coyotes on a daily basis was a common event for everyone. However, once I moved to Alabama, I quickly learned that not everyone was as accustomed to this mammal as I was. I have heard numerous accounts from individuals in the area over the past three years describing their first encounters with a coyote, most of which occurred only within the last few decades. The fact that coyotes were a relatively recent addition to the landscape of the southeastern region of the United States was one of the most intriguing factors that lead me, and my committee, to the research questions I addressed throughout this thesis.

Coyotes are endemic to North America, and have inhabited this continent for approximately 1 million years (Kurtén and Anderson 1980). However, the more recent expansion of the species from its historic range within the Great Plains occurred only within the last 150 years (Parker 1995). I conducted phylogeographic analyses of coyotes across the central plains, midwestern, and southeastern regions of the United States to assess dispersal patterns of coyotes within these regions. This portion of the project was conceptualized based on the book, "The Eastern Coyote: The story of its success" by G. Parker (1995). In his book, Parker hypothesized routes of dispersal efforts of the coyote

over the last century. He also highlighted that there had been little, if any, molecular research to assess these proposed dispersal patterns and called for further research using genetic data within wild canids. In addition, the southeastern United States was proposed as one of the last hypothesized regions for coyote range expansion. As such, relatively small amounts of research have been collected on coyotes specifically in these states. Chapter II of this thesis deals with the testing of two hypotheses of dispersal patterns of the coyote out of its presumed historical range. The first hypothesis, called the 'Parker' hypothesis, was based on Parker's (1995) summary of documented sightings of coyotes by people over the last century. The second hypothesis, referred to as the 'Geographic' hypothesis, considered geographic features identified within the study area (i.e. Ohio and Mississippi Rivers) that could have influenced historical coyote dispersal patterns. Additional inferences were made specifically about coyotes sampled within Kentucky and Tennessee since the origins of individuals in this area was previously unknown.

In one of my first conversations with Dr. Armstrong during the development stage of my thesis project, he mentioned that when he first began work at Auburn, he received numerous calls about coyotes in rural areas. Now, twenty years later, he says that the majority of calls concerning coyotes come from more urbanized localities. Although coyotes have historically been more of a rural species, urban landscapes have created a whole new realm of resources of which coyotes have begun to take advantage. Further, increased habituation of coyotes to humans has resulted in coyotes more readily occupying urban areas than they have in the past. Little is known behaviorally and biologically about coyotes that are able to frequent suburban and urban areas, and even less is known about the occurrence of coyotes permanently establishing residence within

city limits. No genetic study had been done on coyotes in Alabama prior to this project, which further validated the need for research in this area. Chapter III of this thesis presents our assessment of the structure of coyote populations' within a 100 km radius of the Auburn/Opelika Metropolitan Statistical Area (MSA) in east-central Alabama. Analyses were completed both within the total sampled area, and across an urban to rural gradient centered on the Auburn/Opelika MSA. The main goal for this portion of the project was to attempt to identify appropriate management units based on genetic structuring (i.e. populations) of which could assist future management strategies.

Genetics has become an extremely useful technique in the field of wildlife sciences in that it allows us to make inferences about dispersal patterns, hybridization among species, parentage and relatedness, abundance, population units and demographics, etc. (DeYoung and Honeycutt 2005). Within this study, we utilized genetics in two main ways: (1) to assess historic and contemporary dispersal patterns across a larger scaled sampling regime crossing 12 states using mitochondrial DNA (mtDNA); (2) to investigate population dynamics on a much finer in east-central Alabama using nuclear DNA (i.e. microsatellites). Mitochondrial DNA is used to assess more historical relationships over a broader spatial context. In addition, mtDNA is maternally inherited, meaning that it only represents the lineage of the mother. This mode of inheritance is important in assessing introgression events as seen in Chapter II. In contrast, microsatellite markers (i.e. nuclear DNA), as used in this study, can allow you to infer about more recent timeframes on the population level of a species. This type of genetic material is inherited biparentally, meaning that both the maternal and the paternal genomes are represented within the data. Such an inheritance pattern allows for a more

well-rounded assessment of population dynamics. Information gathered and discussed in Chapter III using the microsatellite markers includes relatedness, current population diversity and structure, and differentiation between groups of individuals. The differences in these two types of genetic material are paramount in the information that can be gathered from them through genetic analyses as is apparent when comparing Chapters II and III of this thesis.

Coyotes truly are fascinating mammals in my mind solely based on their ability to change and adapt to altered habitats and introduced anthropogenic factors. I feel that assessing historical demographics of the sampled individuals was extremely interesting and will be helpful in the grand scheme of understanding what has made a terrestrial species such as the coyote so successful in expanding its range across much or North America. Furthermore, I believe that gaining a better understanding of the population structure of coyotes, both in rural and more urban localities, of east-central Alabama will assist in the determination of possible management units for wildlife management strategies. This study affords both broad and fine scaled perspectives of coyote genetics, and as such is a valuable addition to the realm of canid biology.

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CHAPTER II: TRACING DISPERSAL OF COYOTES (CANIS LATRANS) FROM THE CENTRAL PLAINS INTO THE MIDWESTERN, AND SOUTHEASTERN REGIONS OF THE UNITED STATES USING PHYLOGEOGRAPHY

ABSTRACT

Coyotes (*Canis latrans*) were present in North America as far back as the Pleistocene era, but only began to disperse out of their limited historical range in more recent times. Hypothesized routes of dispersal of the coyote have traditionally been made based on documented sightings and releases, and to date have not been evaluated using genetic techniques. Our objective was to test two hypotheses of historical dispersal patterns out of the Central Plains into the Midwestern and Southeastern United States using phylogeographic analyses of mitochondrial DNA. The first hypothesis was based on a summary of observed coyote distribution, and the second attempted to take into account geographic features that might have influenced dispersal efforts of the coyote. A maximum likelihood phylogenetic tree and median-joining network were constructed to assess relationships amongst sampled individuals. In addition, diversity indices were estimated to evaluate trends in historical genetic diversity among hypothesized groups. Results supported the hypothesis that the states of Texas, Oklahoma, North Dakota, Wyoming, Wisconsin, and possibly Indiana were part of the coyotes' historical range. Alabama, Mississippi, Tennessee and Kentucky had the lowest genetic diversity supporting our hypothesis that they represent a more recent area of range expansion.

Also, we found evidence to suggest haplotypes sampled in Kentucky and Tennessee originated from lineages to the south. This finding also suggested that the Ohio River could have been a formidable barrier to coyote dispersal from the north. Overall, we rejected both tested hypotheses, and submitted a new alternative hypothesis (i.e. the phylogram presented by this study) for future analyses.

INTRODUCTION

The historical (pre-settlement) distribution of the North American coyote was primarily in the Great Plains area, encompassing most of Texas, Oklahoma, Kansas, Nebraska, and the Dakotas (Fig. 2.1, Young and Jackson 1951; Nowak 1978; Parker 1995). It has been hypothesized that the range of the coyote was restricted to this historical distribution mostly due to the presence of wolves (*C. lupus* and *C. rufus*) to the east and west (Parker 1995). With the introduction of European settlers, lands were converted to agriculture and wolf populations were reduced as people moved westward, both of which favored coyote dispersal (Gier 1975; Parker 1995). In addition to westward movements, coyotes dispersed northward through Canada, finally reaching Alaska by the late 1800s. This movement was facilitated by gold rush events, which resulted in trails of human waste and land clearings that fueled coyote populations (Parker 1995).

Around the turn of the century, coyotes began dispersing into the eastern United States. Parker (1995) reviewed and summarized hypotheses of dispersal patterns and timelines of this eastern expansion. He hypothesized that the eastern dispersal of coyotes occurred in two separate events comprised of a ‘Northern Front’ and secondarily, a ‘Southern Front’

(Fig. 2.1). Under this scenario, coyotes dispersed from their historic range within the Great Plains into the Midwestern states of Iowa, Minnesota, and Wisconsin around 1900, although it is thought that a low density of coyotes could have existed in these areas during more historic times (Parker 1995). Coyotes continued to disperse along this route until they reached the eastern U.S. coast. A road-killed coyote documented in Delaware in 1993 confirmed the completion of this trajectory. Following the 'Northern Front' dispersal event, coyotes are believed to have begun to disperse out of Texas and Oklahoma eastward into Arkansas and Louisiana around 1940, this being considered the 'Southern Front' (Fig. 2.1; Gipson et al. 1974; Parker 1995). The existence of the red wolf in southeastern states is thought to have served as a formidable obstacle, hindering further dispersal of coyotes for close to two decades (Hall and Newsom 1978). Once the red wolf was largely extirpated from its historic range in Mississippi and Alabama, along with extensive clearing of the land and human habitation, coyotes were able to expand their range (Hall and Newsom 1978; Parker 1995). In the 1960s, coyotes spread throughout Mississippi and into Alabama (French and Dusi 1979; Jones and Hill 1985; Woodling et al. 1985). They are believed to have crossed into Georgia during the 1970s, and into the states of Florida, South Carolina, North Carolina, and Virginia in the 1980s (Brady and Campbell 1983; Wooding and Hardisky 1990; Parker 1995). Also during the period of 1960-1980, coyotes are hypothesized to have filtered into the region of Kentucky and Tennessee (Kennedy 1989; Parker 1995). This area was considered a 'buffer zone' by Parker (1995) because the origin of the individuals was not certain. Parker hypothesized that dispersal into Kentucky and Tennessee could have been part of either the 'Northern Front' or the 'Southern Front'. It is important to note that several

translocations into the southeastern states were documented between the years of 1924 and 1981, which accounts for several earlier documented sightings that preceded the hypothesized eastern dispersal of the coyote (Parker 1995). Translocations are currently reported to occur in the southeastern United States (F. Boyd, USDA/WS, *personal communication*).

The hypothesized dispersal routes of Parker (1995) have yet to be empirically tested. In his book, Parker (1995) identified the need for a more robust geographic sampling and application of genomic analysis to help better understand coyote origins. In our study, mitochondrial DNA was amplified and sequenced from coyotes collected from twelve states across the central, midwestern, and southeastern regions of the United States. Samples were considered to belong to 5 groups based on the 'Parker' hypothesis to evaluate whether the dispersal patterns proposed by Parker (1995) were plausible. These groups include: (1) Parker Historic (PH) – Illinois, North Dakota, Oklahoma, Texas; (2) Parker West (PW) – Wyoming; (3) Parker Northeastern (PNE) – Indiana, Wisconsin; (4) Parker Southeastern (PSE) – Alabama, Louisiana, Mississippi; (5) Parker Buffer Zone (PBZ) – Kentucky, Tennessee. Based on these hypothesized groups, we anticipated phylogeographic relationships such that haplotypes collected within PH, and possibly PNE based on their theorized pre-settlement era low density (Parker 1995), to be basal (ancestral) to the rest of the groups. In addition, based on Parker's hypothesis we predicted that haplotypes from PW would be more ancestral to the eastern regions and that PSE and PBZ would be more derived since they represent the area of the most recent range expansion (Fig. 2.2).

Parker's (1995) hypothesized dispersal routes were based mainly on historical documentation and sighting reports, with little emphasis placed on possible geographical features that might have affected dispersal patterns. Several studies have been completed in North America on various species of carnivores showing how geographic barriers such as mountain ranges and rivers can influence dispersal movements. Studies completed in southern Florida have identified the Caloosahatchee River, approximately 250 meters wide, as a possible dispersal barrier to both bobcats (*Lynx rufus*) and Florida panthers (*Puma concolor coryi*, Maehr 1997; Maehr et al. 2002). In addition, Johnson and colleagues (2010) found evidence to suggest dispersal of juvenile bobcats (*Lynx rufus)* was limited by the Ohio River, which ranges from approximately 400 meters up to 1.5 kilometers in width. Movements of American black bears (*Ursus americanus*) were found to be influenced by the Mississippi River, which is greater than 1500 meters in width (White et al. 2000). Within canids, Harrison and Chapin (1998) suggested that the St. Lawrence River, which lies between southeastern Canada and the northeastern U.S., could be a dispersal barrier hindering the movement of Canadian wolves into the northeastern United States. A study conducted on the Penobscot River in Maine, which is greater than 150 meters in width, showed that it served as a significant barrier to the dispersal of juvenile individuals (Harrison 1992). Within our sampling area, the Ohio River and the Mississippi River were identified as possible barriers of dispersal. Thus, an alternative hypothesis, based solely on potential geographic barriers, was tested in contrast to the 'Parker' hypothesis by assigning samples to 4 geographical groups, the 'Geographic' hypothesis (Fig. 2.3). The geographical groups for the 'Geographic' hypothesis are as follows: (1) Geographic Historic (GH) – Louisiana, North Dakota,

Oklahoma, Texas, Wyoming; (2) Geographic North (GN) – Illinois, Indiana, Wisconsin; (3) Geographic South (GS) – Alabama, , Mississippi; (5) Geographic Buffer Zone (GBZ) – Kentucky, Tennessee. Based on our phylogeographic hypothesized groups, we predicted that GH would be most ancestral, and that GS and GBZ would be more derived showing evidence of recent expansion (Fig. 2.3).

In addition to analyses assessing phylogeographic relationships, genetic diversity indices can be very informative in regards to discerning between areas that have been occupied for a greater length of time in comparison to other localities. For instance, greater measures of genetic diversity are expected to be observed within more ancestral ranges of a species (Hewitt 1996; Hewitt 2000) as compared to regions of a more recent range expansion. The use of both types of analyses within this study helped to better understand the historic dispersal patterns of the coyote.

The overall goal of this study was to infer dispersal patterns of coyotes out of their historical range (i.e. PH and GH) into regions of the eastern U.S., using mitochondrial DNA (mtDNA). Two separate hypotheses of coyote dispersal ('Parker' and 'Geographic') were tested using both phylogeographic and genetic diversity analyses. We predicted low resolution of relationships among samples collected within the region of recent range expansion due to the relatively short amount of time since establishment of those populations. In addition, the origin of coyote range expansion specifically into the 'buffer zone' states of Tennessee and Kentucky (i.e. PBZ and GBZ) was investigated. Finally, tested solely within the 'Geographic' hypothesis, we predicted that geographic

barriers, such as rivers and mountain ranges, would best explain dispersal patterns of coyotes.

MATERIALS AND METHODS

Sample Collection and Preservation

We sampled coyotes at twelve localities across the central United States (Fig 2.4): Alabama (*n* = 77); Illinois (*n* = 10); Indiana (*n* = 7); Kentucky (*n* = 9); Louisiana (*n* = 13); Mississippi (*n* = 10); North Dakota (*n* = 7); Oklahoma (*n* = 11); Tennessee (*n* = 19); Texas (*n* = 20); Wisconsin (*n* = 13); Wyoming (*n* = 13); total (*n* = 209). These localities were selected based on hypothesized dispersal scenarios (Parker 1995). We collected tissue samples from both live captures and deceased animals from the ear of each individual using a commercial grade ear-notcher. Directly following sampling efforts, we stored the tissue in an EDTA/DMSO buffer solution saturated with NaCl for preservation (Seutin et al. 1991). We extracted DNA from each sample using a DNeasy® Tissue Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. All collection protocols were approved by Auburn University Institutional Animal Care and Use Committee (Protocol# 2007-1244).

Laboratory Protocol

We ran polymerase chain reactions (PCRs) to amplify the first hypervariable segment of mtDNA control region. Each reaction was accomplished using PCR water to total volume of 24 µl, 5.0 µl of 5x Buffer C with $MgCl_2$ (Invitrogen, Co., Carlsbad, California), 2.5 µl of dNTP (Promega; 10 mM), 2.5 µl of both primers (L15926, H16340; 1 uM

concentration), and 0.2 µl of GoTaq® Flexi DNA Polymerase (Promega; 5 u/ul). The PCR amplification included an initial denaturation cycle of 4 minutes at 94°C. Thirty-five cycles were carried out under the following profile: 94°C for 30 seconds, 46°C for 30 seconds, 72°C for 1 minute. Final extension was accomplished in a one 7 minute cycle at 72°C (Vila et al. 1999*a*; Adams et al. 2003*b*). Once completed, the reaction remained at 4°C until removed and further processed.

Once viable PCR product was detected via gel electrophoresis, each sample was purified using 1µl of the ExoSAP-IT (USB, Affymetrix, Inc, Cleveland, Ohio) per reaction. The ExoSAP-IT reaction was incubated for 15 minutes at 37˚C, and then for another 15 minutes at 80˚C. Following clean-up, each sample was cycle sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California). We purified each cycle sequenced product using the PrepEase® Sequencing Dye Clean-Up Kit (USB, Affymetrix, Inc, Cleveland, Ohio).

We visualized sequences at the Wildlife Genetics Lab at the USDA/APHIS/WS National Wildlife Research Center in Fort Collins, Colorado using an ABI 3130*xl* genetic analyzer (Applied Biosystems Inc., Foster City, California). We aligned and edited sequences using Sequencher 4.9 (Gene Codes, Co., Ann Arbor, Michigan) and exported for statistical analysis. We submitted the total control region sequences sampled from all 12 states (n = 209) to GenBank.

Phylogeographic Analyses

Unique haplotypes collected from each sampled state were detected using the DNA to haplotype collapser and converter tool within FABOX 1.35 (Villesen 2007). We included these non-redundant haplotypes in the remaining phylogeographic analyses to facilitate a more clear presentation. We 'blasted' all haplotypes on the NCBI website (Johnson et al. 2008) to confirm species identity. Some of our haplotypes were identified as gray wolves (*C. lupus*) and dogs (*C. familiaris*), which was not unexpected because coyote sequences have been found to be identical to gray wolf and dog haplotypes previously (Vila et al. 1997; Vila et al. 1999*a*, *b*). Originally, golden jackal (*Canis aureus*) and Himalayan wolf (*Canis himalayensis*) sequences were used as outgroups to root the phylogenetic tree. However, since gray wolves and dogs are also sister taxa to coyotes, we used the coyotes haplotypes that were identical to wolf and dog haplotypes detected within our own data set as outgroup taxa in the phylogeographic analysis. We constructed a maximum likelihood phylogram of the unique haplotypes using the program RAxML 7.0.4 (Stamatakis et al. 2006; Stamatakis et al. 2008). We used the general-time reversible (GTR) model of nucleotide evolution (Tavaré 1986) with gamma (Γ) correction (Yang 1996) as suggested in the RAxML 7.0.4 manual (Stamatakis et al. 2006; Stamatakis et al. 2008). Statistical support of nodes was assessed by bootstrap analyses using 5000 replicates. In addition, we constructed a median-joining (MJ) network from mtDNA sequences of all haplotypes that formed a large polytomy in the phylogram (Clade B, see results) using NETWORK 4.5.1 (Bandelt et al. 1999) to provide better resolution of those relationships.

Sequence Diversity

For the purposes of genetic diversity analyses, we included all 209 mtDNA sequences, because not including all the samples could bias some diversity estimates. Groups selected to test the 'Parker' hypothesis were as follows: (1) PH – Illinois, North Dakota, Oklahoma, Texas; (2) PW – Wyoming; (3) PNE – Indiana, Wisconsin; (4) PSE – Alabama, Louisiana, Mississippi; (5) PBZ – Kentucky, Tennessee (Fig. 2.2). It is important to note that Parker's conclusions were not clear on whether Illinois and parts of Indiana were to be considered as part of the historical range of the coyote. Thus, for this study, we conservatively grouped Illinois with PH and Indiana with PNE. The geographical groups for the 'Geographic' hypothesis were as follows: (1) GH – Louisiana, North Dakota, Oklahoma, Texas, Wyoming; (2) GN – Illinois, Indiana, Wisconsin; (3) GS – Alabama, Mississippi; (5) GBZ – Kentucky, Tennessee (Fig. 2.3). We included Wyoming with the GH group, within the 'Geographic' hypothesis, because all samples were collected east of the Rocky Mountain range. In addition, we also included Louisiana in the GH group because all the samples collected in this state were west of the Mississippi River. Another difference between the 'Parker' hypothesis and the 'Geographic' hypothesis is the placement of Illinois, which we grouped within the GN group in the 'Geographic' hypothesis since it lies on the east side of the Mississippi River. The buffer zone (PBZ and GBZ) remained the same for both hypotheses since our goal was to determine origins of these individuals.

We employed the program DnaSP v5.0 (Librado and Rozas 2009) to measure genetic diversity within each group using haplotype diversity (H_d) and the number of pairwise

differences within groups considering gaps as a fifth state. We also used ARLEQUIN 3.1 (Excoffier et al. 2005) to calculate the number of polymorphic sites (PS) within each group, pairwise F_{ST} measurements, and run tests of selective neutrality. Pairwise F_{ST} values were estimated to evaluate genetic differentiation between the groups for each hypothesis. Both Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) was used to test for selectively neutrality of mutational change within the sequences and changes in population demography.

RESULTS

Phylogeographic Analyses

A 439 base pair (bp) portion of the mtDNA control region was sequenced for a total of 209 individuals. Forty-three unique haplotypes were detected and used to construct the maximum-likelihood tree. Two of the 43 haplotypes were identical to taxa other than the coyote. There was 1 haplotype, represented in 3 states, that was identical to a gray wolf haplotype. Another haplotype was revealed to be identical to a domestic dog. These 2 haplotypes had an average of 5.49% sequence divergence from the most ancestral coyote haplotype (Fig. 2.5). The remaining haplotypes were all found to be identical to coyote control region haplotypes. Six of these haplotypes grouped together with significant bootstrap support, longer branch lengths, and greater within group sequence divergence than among the rest of the coyote haplotypes, thus it was considered to represent a more ancestral lineage (Fig. 2.5). For discussion purposes, two groups of coyote haplotypes were identified in the maximum-likelihood tree amongst: Clade A, encompassing the six previously mentioned coyote haplotypes and Clade B, included the remaining haplotypes,

which formed a polytomy (Fig. 2.5). Clade A included haplotypes sampled from Alabama, Indiana, North Dakota, Oklahoma, Texas, and Wisconsin, and had nearly twice the sequence divergence (2.2%) than detected within the derived Clade B (1.31%). The more derived haplotypes were found in all 12 sampled states. Low levels of resolution characterized Clade B, which formed a polytomy, thus a median-joining network was assembled. We used the 124 individual sequences of which were represented within the 35 nonredundant haplotypes constituting Clade B to further assess relationships based on individual mutations (Fig. 2.6) The resulting MJ network consisted of two star-like clusters (Cluster 1 and Cluster 2), which were separated by two individuals who shared a single haplotype sampled from Texas. Cluster 1 included individuals from all sampled states with the exception of Kentucky and Tennessee. Cluster 2 was comprised of sequences collected from all 12 sampled states.

Sequence Diversity

Genetic diversity estimates calculated for both hypotheses were summarized in Table 2.1. For the 'Parker' hypothesis groups, the greatest haplotype diversity was observed in the PH and PW groups. The lowest haplotype diversity was found in the PSE group, even though its sample size was much larger than any of the other tested groups. Similar to the 'Parker' hypothesis, the 'Geographic' hypothesis showed the greatest haplotype diversity within the GH group and lowest within the GS group.

Pairwise F_{ST} estimates and pairwise differences within groups were summarized in Table 2.2. For the 'Parker' hypothesis, most groups were significantly differentiated from one

another based on the pairwise F_{ST} measurements. The comparisons that were not significantly differentiated included PH versus PW, PSE versus PW, and PSE versus PBZ. Within group pairwise differences were greatest in both the PH and the PSE groups, 14.73 and 11.14, respectively. The only pairwise F_{ST} that was not significant within the 'Geographic' hypothesis groups was between GS and GBZ. Pairwise differences were highest in the GH group (15.64). None of the groups within either hypothesis had significant values for either Tajima's D or Fu's F (Table 2.3).

DISCUSSION

Phylogeographic Analyses

Even though coyotes are endemic to North America, and have inhabited this continent for approximately 1 million years (Kurtén and Anderson 1980), the more recent expansion of this species out of their historic range within the Great Plains has occurred only within the last 150 years (Parker 1995). Due to the recent time scale of these dispersals, we expected low resolution of relationships among samples from the recently invaded regions inferred from phylogeographic analyses (i.e. maximum likelihood tree). Indeed, most relationships among these samples lacked resolution. This is not unexpected given the rate of evolution of the mitochondrial control region. Compared to portions of the nuclear genome, the mitochondrial control region accumulates genetic changes at a slower rate requiring a greater length of time in order to observe significant divergence between groups. Further, coyotes are annual breeders and have relatively long generation times (Bekoff 1978), which also means a significant amount of time is needed to see divergences between various lineages of this species. In addition to an insufficient time of

evolution, the lack of resolution within Clade B (i.e. the polytomy) suggests the possibility of multiple rapid invasions into newly expanded ranges. Despite low resolution in portions of the phylogeographic analyses, strong statistical support in some regions of the maximum likelihood tree did provide for inferences about coyote dispersal.

Of the two clades that were supported within the phylogeny (Fig 2.5), we hypothesized that Clade A was more ancestral due to the longer branches, greater within clade sequence divergence, and higher statistical support based on bootstrap values. Further, Clade A included haplotypes detected within Texas, Oklahoma, and North Dakota, which were included in the historical groups (i.e. PH and GH) for both hypotheses. This finding is evidence that the historical range did include the Central Plains region. However, the presence of haplotypes from Indiana, Wisconsin, and Alabama was not expected within this same clade. Both the 'Parker' Hypothesis and the 'Geographic' Hypothesis include Indiana and Wisconsin in their respective GN/PNE groups. In his book, Parker (1995) was unclear on the existence of coyotes in both Indiana and Wisconsin during pre-settlement times, suggesting the possibility of only low densities of individuals across that range. It is parsimonious to conclude, since both Wisconsin and Indiana lie directly adjacent to the proposed historical range and they both fall in with the more ancestral clade, that they could have been part of the historical range. This conclusion does not suggest a complete dismissal of the 'Parker' Hypothesis, but is evidence refuting the 'Geographic' Hypothesis, since Indiana falls far to the east of the Mississippi River. Finally, the haplotype from Alabama within Clade A was unexpected. This Alabama haplotype was only found in one individual (AL071). One explanation was that this

haplotype is of shared ancestry, suggesting it could have dispersed into the southeast from the historic range many years ago. Another reason for a haplotype sampled in Alabama to appear within an ancestral lineage is that it is identical in state, and not of true ancestral lineage. Lastly, this haplotype could be a result of a translocation event, meaning that it could have occurred in Alabama due to artificial dispersal. Other than the occurrence of an Alabama haplotype detected within Clade A, we conclude that the lineages represented within the group are likely to be of more historical ranges.

Unlike Clade A, Clade B (the polytomy) was characterized by much less within clade sequence divergence and bootstrapping support which suggested individuals detected within Clade B underwent a more recent radiation. In hopes of gaining a better understanding of the relationships amongst individuals within the polytomy (i.e. Clade B), a MJ network was constructed. The MJ network revealed 2 star-like clusters which suggested two separate, recent radiations across the sampling area. Furthermore, a connection between the two clusters, characterized by longer branches, was revealed through a single Texas haplotype. We could not infer directionality, but it is notable that Texas, part of the historical range for coyotes, was a link between the two clusters evident in the network.

Both the wolf and dog haplotypes identified in our coyote samples have been documented previously. The gray wolf haplotype detected was collected during this study in Texas, Oklahoma, and Louisiana. A previous study (Adams et al. 2003*a*) detected the same haplotype, Cla12, in Texas. The gray wolf haplotype in coyote populations has been

hypothesized to be a result of either a gray wolf escaping from captivity, or a remnant of domesticated pets of gray wolf descent (Adams et al. 2003*a*) that successfully bred with a coyote. Although Adams et al. (2003*a*) also mention historical hybridization as a possible explanation, they felt it was less likely because they only detected one sample out of seven. We found the same gray wolf haplotype in 10 coyotes, which constituted 25% of the total Texas sample, 9% of the total Oklahoma sample, and 31% of the total Louisiana sample. Such findings would suggest historic introgression of gray wolf mtDNA through the successful breeding of a male coyote with a female gray wolf, followed by the assimilation of the female offspring back into the coyote population.

The domestic dog haplotype detected in this study was also identified in another study (Adams et al. 2003*b*), referred to as la24, in Virginia, West Virginia, North Carolina, and Florida. Our study marked the first time this haplotype had been reported in Tennessee, Kentucky, and Alabama. Interestingly, the dog haplotype identified in Alabama was detected within 6 individuals, representing 8% of the total Alabama sampling. The collection locations of these individuals ranged across 3 different Alabama counties (i.e. Montgomery, Tallapoosa, and Lee); Lee and Tallapoosa counties are adjacent to one another, while neither border Montgomery County. The distribution of these individuals could suggest that the haplotype may not be new to the area, because it was not isolated in a single locality. The domestic dog haplotype within coyote populations could be a result of a historical, successful reproductive event between a male coyote and a female dog. Further, the southeastern region of the United States was hypothesized to be part of the most recent coyote dispersal, and as such might have facilitated increased

reproductive opportunities for coyotes to breed with dogs due to the shortage of coyotes at the front of the dispersal effort (Adams et al. 2003*b*). Successful interbreeding of coyotes with domestic dogs has been previously documented by the presence of 'coydogs' in the wild (Cook 1952; Mengel 1971). Also, it was important to acknowledge the possibility that red wolves could have also interbred with coyotes since they are reported to have had areas of overlap during coyote dispersal (Paradiso and Nowak 1972, Parker 1995, Kelly et al. 1999). However, there were no matches of any haplotypes sampled within this study to red wolf haplotypes, which suggested that either we did not sample an individual that had resulted from introgression with a red wolf, or that such an individual does not exist within the areas that we sampled.

Testing Hypotheses of Dispersal Patterns

In addition to the phylogeographic analyses, we utilized genetic diversity indices to evaluate trends in historical genetic diversity among hypothesized groups. One would expect to see greater measures of genetic diversity within an ancestral range (Hewitt 1996; Hewitt 2000) than areas of most recent range expansion. The results showed the greatest level of haplotype diversity (h_d) within the GH group under the 'Geographic' hypothesis. The next highest diversity was found in both the PH and PW groups under the 'Parker' hypothesis. These findings supported the proposed regions of historic and western descent within both hypothesis, which was also evident from the phylogeographic analyses. In addition, PH and PW were not significantly different based on pairwise F_{ST} , which suggests that the area sampled in Wyoming (PW) should be considered part of the historical range within the 'Parker' hypothesis. PH and PW were

combined under the 'Geographic' hypothesis to form GH. Measurements of polymorphic sites and the number of pairwise differences were highest in the presumed historical range (PH, PW and GH), and also the southern groups (PSE and GS). This was interesting because we would have expected to see some of the lowest genetic diversity indices in the southeastern region, as observed with measures of haplotype diversity, since the area was presumed to have been invaded most recently. This finding suggested that the southern regions might not be an area of most recent expansion, which would contradict both hypotheses tested in this study. However, the increased polymorphic site and pairwise differences measurements detected in the southern regions might be due to the higher sample size from Alabama which could have allowed for sampling of a higher number of haplotypes. The trends in diversity show similar evidence to what was found in the phylogeographic analyses supporting the historical groupings in both hypotheses.

Tests of neutrality were utilized both to test for mutational selection and to infer about indications of population contraction, expansion, or stability. For both hypotheses, Fu's F_S and Tajima's D values were not significant, indicating that selection was not driving differences between sequences analyzed. For population demography, we would have expected to see evidence of stable populations within the historic range (i.e. Texas, Oklahoma, North Dakota) and evidence of population expansion in the recently occupied areas such as the southeastern and buffer zone states (i.e. Tennessee and Kentucky). The non-significant Tajima's D and Fu's F_S measured across all groups suggested longstanding population demographic stability (Finn et al. 2009). This evidence of stability was contrary to what was expected in the more recently inhabited areas and was

likely due to the low sample sizes used within the study, which can greatly decrease the power of the tests (Simonsen et al. 1995; Fu 1997; Ramos-Onsins and Rozas 2002). No inferences were made regarding either hypothesis based on the tests of neutrality due to the lack of signal.

As part of this study, we also investigated the origins of coyotes that dispersed into the area referred to as the 'buffer zone' by Parker (1995). This included coyotes from Kentucky and Tennessee, and individuals from this region were grouped together in both tested hypotheses (PBZ and GBZ) in order to assess genetic diversity and origins of coyotes sampled in these states. The 'buffer zone' exhibited lower genetic diversity than the historic regions and fell within the derived clade in the phylogeny, evidence that supported both hypotheses, which predicted it was an area more recently occupied by coyotes. The origin of coyotes in both Tennessee and Kentucky was not well-understood because the area is between the northern and the southern dispersal routes (Kennedy 1989; Parker 1995). Interestingly, under both hypotheses we found significant differentiation between the northern groups (i.e. PNE and GN) and the 'buffer zone' (PBZ and GBZ) groups, whereas there was not significant genetic differentiation between the 'buffer zone' groups and the southern groups (i.e. PSE and GS). Thus, we concluded based on our findings that coyotes haplotypes sampled within this study radiated from the south into Kentucky and Tennessee.

Lastly, the examination of geographic features, specifically the Mississippi and the Ohio Rivers, as possible barriers to dispersal of coyotes was performed. The Mississippi River

is more than 1500 meters in width on average, while the Ohio River can range from approximately 400 meters up to 1.5 kilometers in width. Other studies have suggested that both rivers (i.e. the Ohio and the Mississippi) are wide enough to serve as dispersal barriers to many terrestrial mammalian species (Harrison 1992; Maehr 1997; Harrison and Chapin 1998; White et al 2000; Maehr et al. 2002). Significant differentiation between northern coyotes and coyotes from Kentucky and Tennessee based on the pairwise F_{ST} measurements showed evidence that the Ohio River may have served as a formidable barrier for coyote dispersal. However, there was little evidence based on our results to suggest that the Mississippi River also greatly influenced dispersal patterns. Louisiana samples were included within the historic group (GH) for the Geographic hypothesis since the state lies to the west of the Mississippi River, but they were grouped with the southern states (PSE) for the 'Parker' hypothesis. When comparing statistics for both GS (Alabama and Mississippi) and PSE (Alabama, Mississippi, and Louisiana), PSE had increased genetic diversity, indicating that samples collected from Louisiana have a higher degree of genetic diversity and as such could have been part of the historical range. Also, PSE and PW were not found to be significantly different based on pairwise F_{ST} results, which further implies that Louisiana shares genetic diversity with other states within the known historical range. Illinois was also tested in groups on either side of the Mississippi River (i.e. PH and GN). No significant patterns based on results from Illinois samples were detected to suggest the Mississippi River influenced the movements of coyotes into the Midwestern area. Whether or not the Mississippi River did influence the dispersal of coyotes into the eastern United States needs to be further investigated and include sampling within all states along both banks of the Mississippi River. Based on

our results, we fail to reject the ‘Geographic’ hypothesis based on the evidence suggesting the Ohio River could have been a barrier to coyote dispersal.

In conclusion, due to the extensive dispersal abilities of coyotes, the possibility of translocations, and their current continuous distribution, the assessment of genetic origins and dispersal patterns of the coyotes was quite complex. We were able to infer based on our samples that coyotes within the ‘buffer zone’ appear to have radiated up from the southern region more than down from the northern states. Also, we were able to detect both gray wolf and domestic dog haplotypes within the coyote populations where they have not previously been discovered. Further, evidence from this study supported some portion of each of the proposed hypotheses (i.e. ‘Parker’ and ‘Geographic’). However, there were also results to suggest that both portions of each hypothesis could be rejected, and as such we could not completely accept either. Instead we put forth the phylogeography from this study as a new alternative hypothesis of past coyote dispersal to be tested in the future. A greater sampling effort is needed across the United States to construct a more robust phylogeography to infer a more complete picture of coyote dispersal across the U.S. Furthermore, a greater number of samples per region are needed to address whether or not rivers serve as barriers to gene flow and dispersal for coyotes. The research conducted within this study was one of the first steps in discerning both the historical and the more recent spread of the coyotes throughout the eastern United States. It is our hope that these findings will assist in the continued understanding of coyote range expansion.

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Table 2.1: Haplotypic data

	Parker Hypothesis					Geographic Hypothesis			
	PH	PW	PNE	PSE	PBZ	GH	GN	GS	GBZ
N	48	13	20	100	28	64	30	87	28
H	23	8	9	17	9	29	13	12	9
h_d	0.94	0.91	0.86	0.85	0.86	0.95	0.88	0.81	0.86
PS	56	15	38	60	46	56	43	56	46

Sample size (N), Number of haplotypes (H), Haplotype diversity (h_d), Polymorphic sites (PS).

Table 2.2: Pairwise F_{ST} and pairwise differences. The diagonal values are pairwise differences within each population; and below the diagonal are Pairwise F_{ST} measures.

(a) Parker Hypothesis

	PH	PW	PNE	PSE	PBZ
PH	14.73				
PW	0.03	5.31			
PNE	0.08*	0.09*	6.66		
PSE	0.02*	0.04	0.07*	11.14	
PBZ	0.05*	0.08*	0.07*	0.01	8.97

* Denotes significance at α = 0.05.

(b) Geographic Hypothesis

	GH	GN	GS	GBZ
GH	15.64			
GN	0.09*	6.28		
GS	0.06*	0.05*	9.26	
GBZ	0.06*	0.07*	0.01	8.97

* Denotes significance at α = 0.05.

Table 2.3: Tests of Neutrality

	Parker Hypothesis					Geographic Hypothesis			
	PH	PW	PNE	PSE	PBZ	GH	GN	GS	GBZ
TD	0.29	0.44	-1.20	-0.31	-0.97	0.85	-1.33	-0.71	-0.97
FS	-0.76	-0.55	0.90	5.12	3.63	-1.52	-0.74	6.92	3.63

Tajima's D (TD), Fu's F_S (FS).

Figure 2.1: General theorized pattern of coyote dispersal: (1) to the West; (2) Northeastern expansion; (3) Southeastern expansion. The hypothesized historical range of the coyote is in grey (Adapted from Parker 1995).

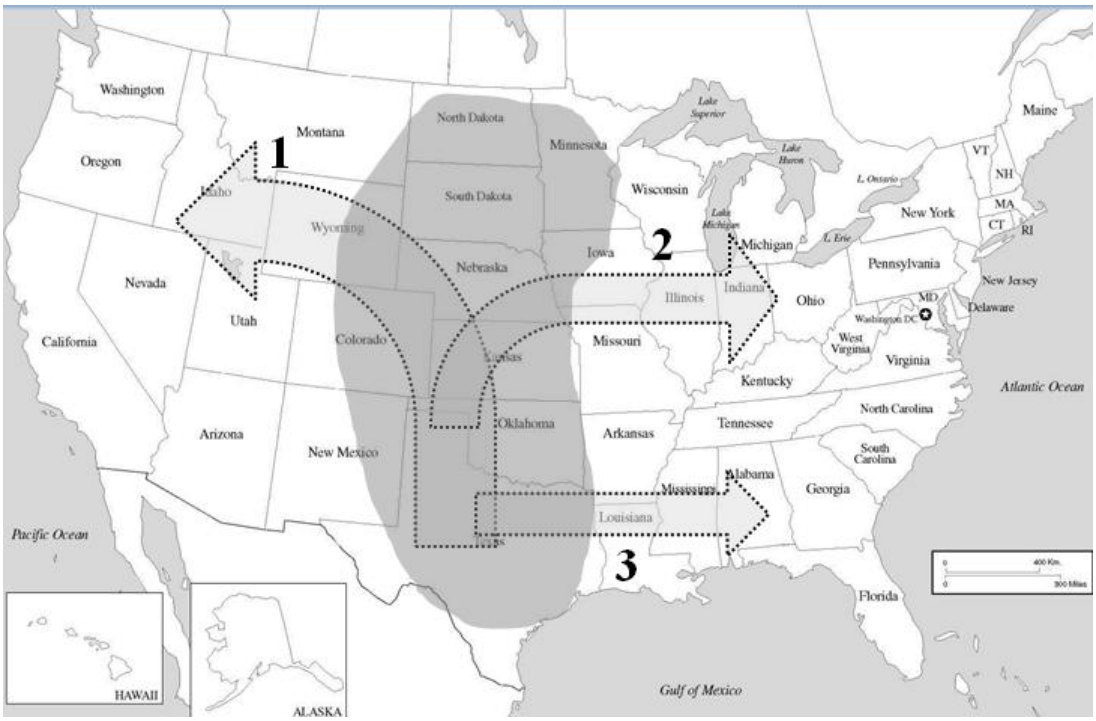


Figure 2.2: ‘Parker’ Hypothesis (Parker 1995)

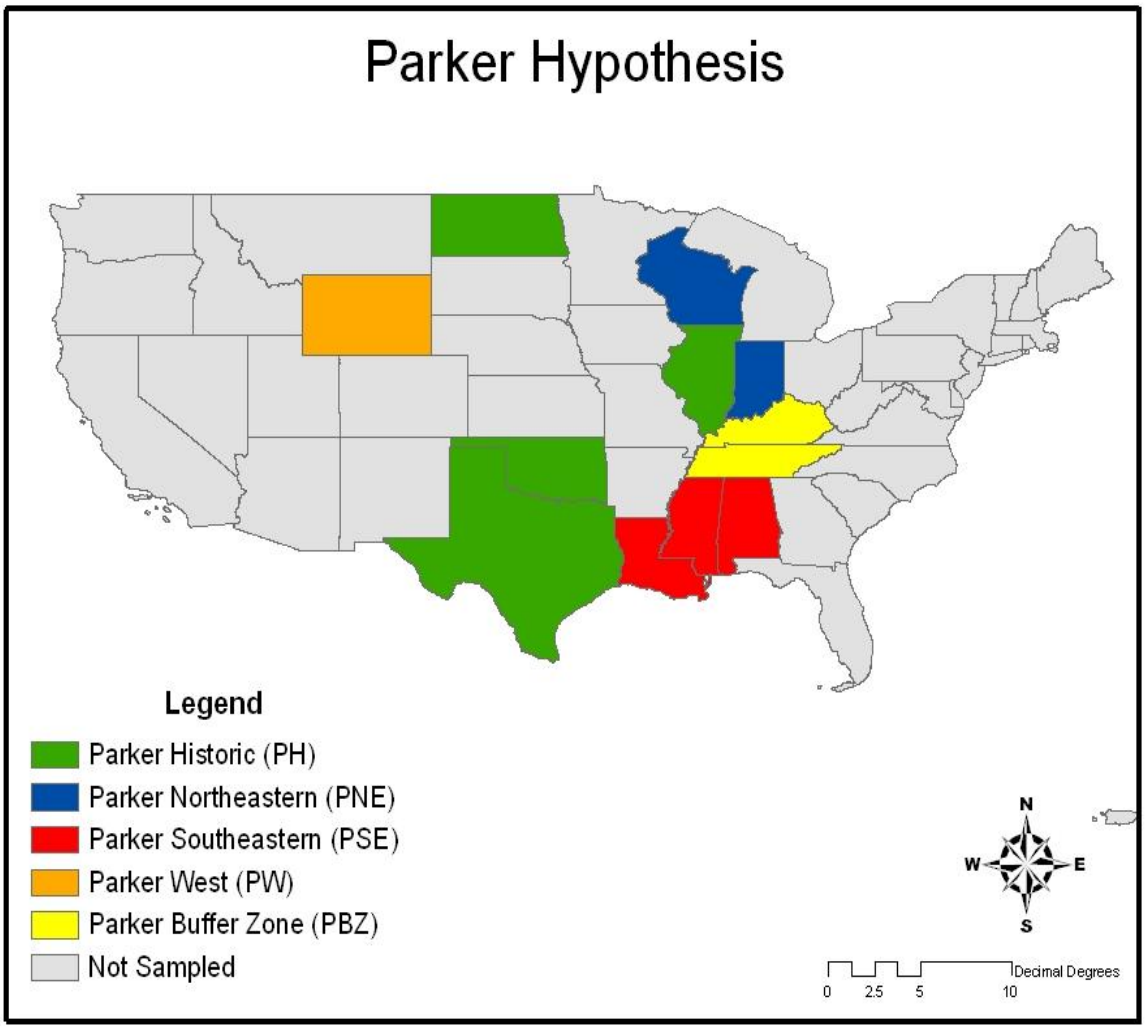


Figure 2.3: ‘Geographic’ hypothesis

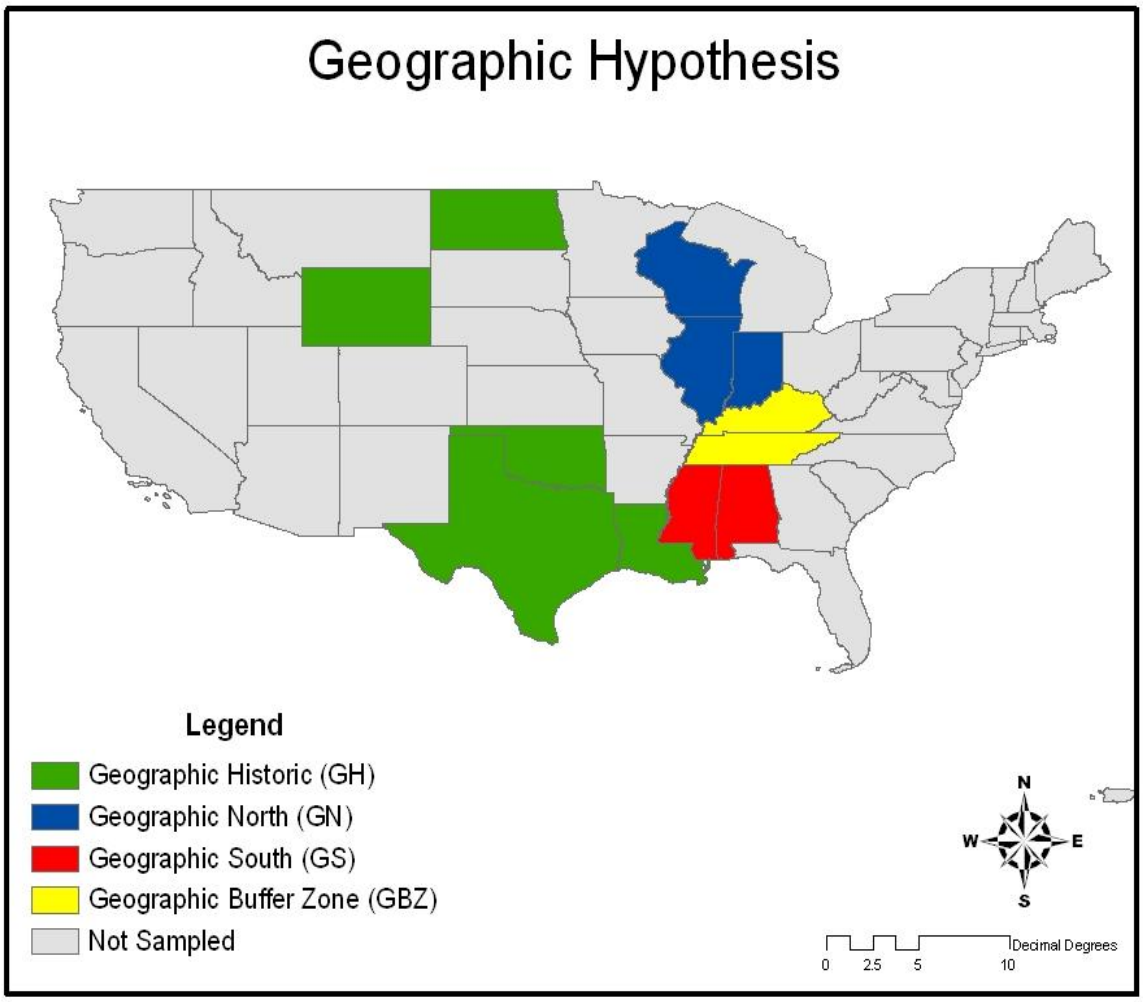


Figure 2.4: Map of sample collection. Note: Red areas represent each county that a coyote was sampled within.

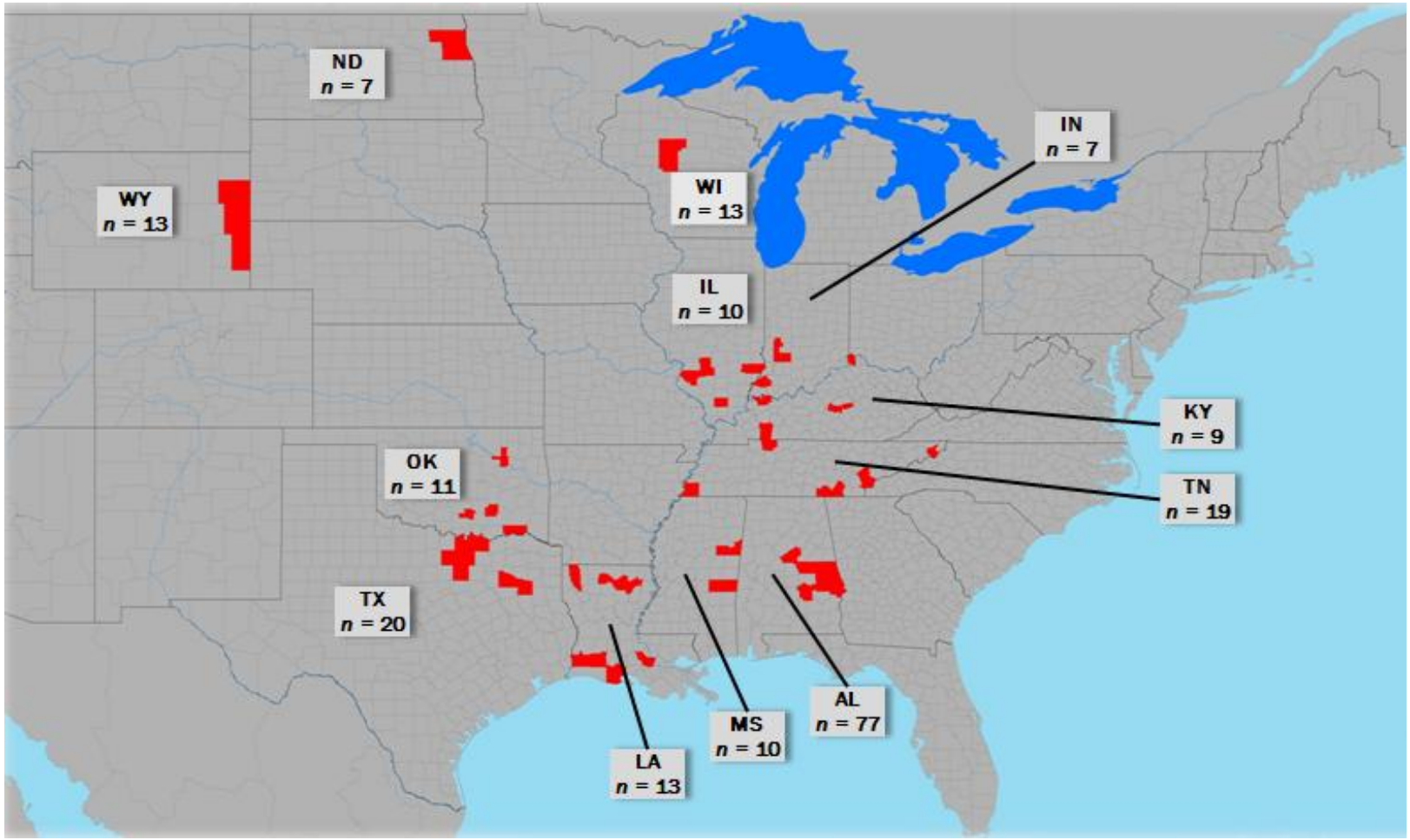


Figure 2.5: Maximum-likelihood phylogenetic tree of coyote mitochondrial control region haplotypes using the GTR+G model of sequence evolution, including wolf (W), dog (D), and coyote (C) haplotypes. Bootstrap support values are indicated at the nodes. The two coyote groups are color coded: Clade A (i.e. 'ancestral') in red and Clade B (i.e. 'derived') in blue.

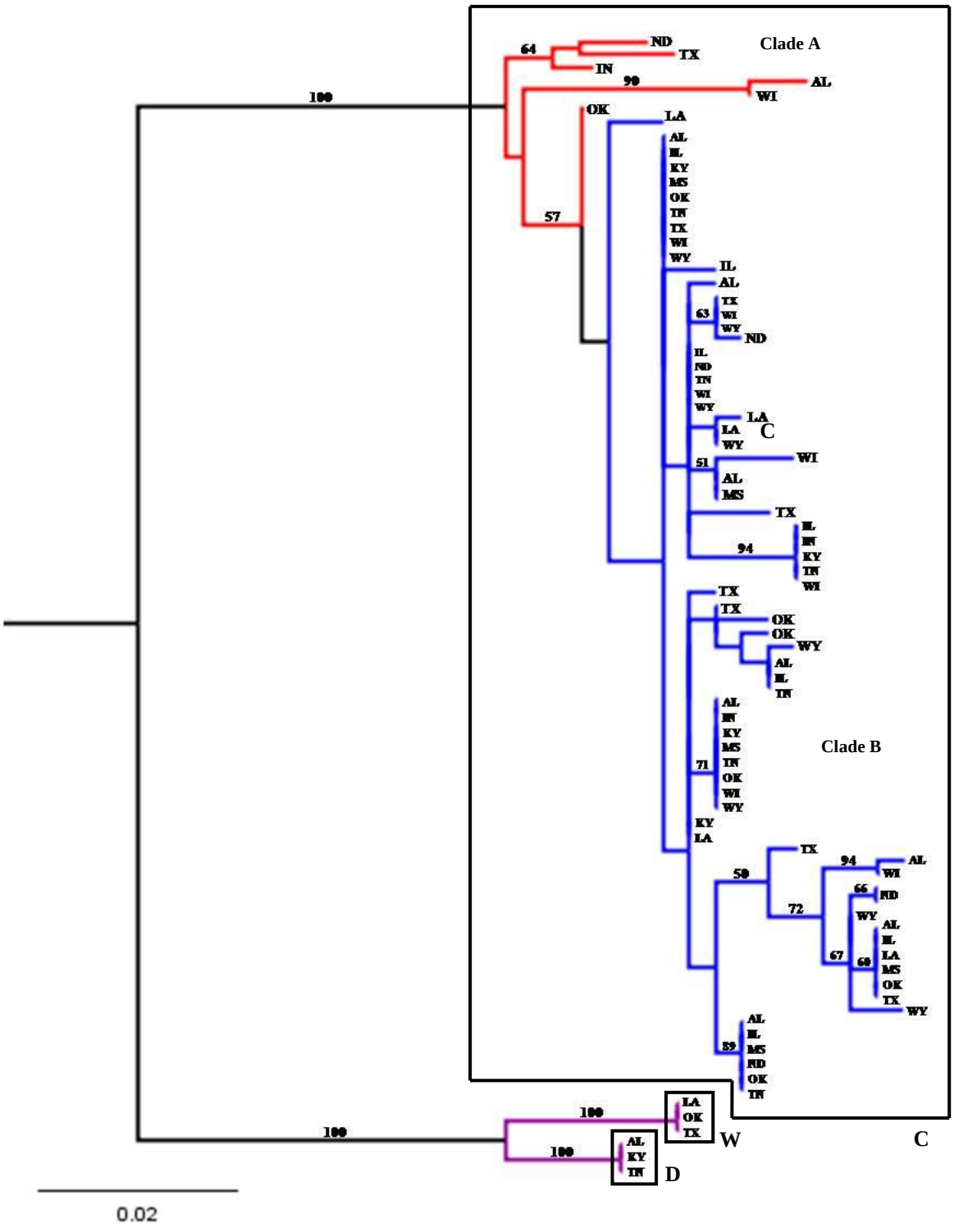
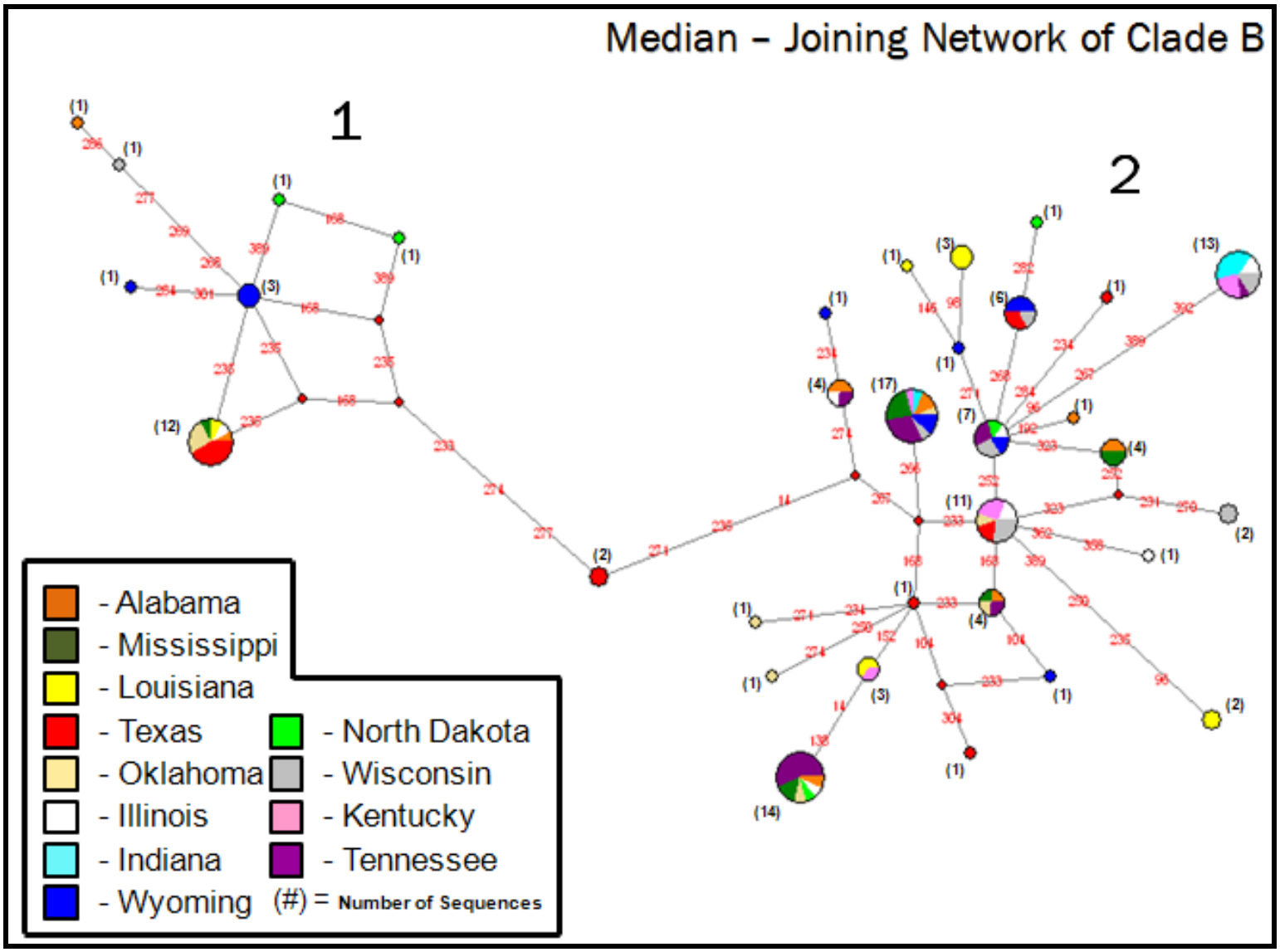

Clade A
Clade B
C
W
C
D
0.02

Figure 2.6: Median-joining network comprised of the ‘derived’ individual sequences. Clusters are marked ‘A’ and ‘B’. The red numbers denote mutated positions, while the smaller red nodes represent hypothesized sequences that were not sampled.


Median – Joining Network of Clade B
1
2
- Alabama
- Mississippi
- Louisiana
- Texas
- Oklahoma
- Illinois
- Indiana
- Wyoming
- North Dakota
- Wisconsin
- Kentucky
- Tennessee
(#) = Number of Sequences

CHAPTER III: POPULATION STRUCTURE ANALYSES WITH AN ASSESSMENT OF GENE FLOW BETWEEN URBAN AND RURAL COYOTES IN EAST-CENTRAL ALABAMA

ABSTRACT

Coyotes (*Canis latrans*) have generally been considered a pest species due to their adaptive ability, high reproductivity, and impact as a top predator on commercial agricultural business. Population dynamics of coyotes is still poorly understood, yet such knowledge could be beneficial to improving coyote population management practices. The two main goals of this study were to determine population structure of coyotes using microsatellite DNA markers, both (1) within a 100 km radius of the Auburn/Opelika Metropolitan Statistical Area and (2) across an urban to rural gradient. Investigating genetic differentiation (i.e. population structure) within coyotes was tested as a method of outlining management units in Alabama. Bayesian clustering analysis was used to incorporate spatial data with genotypes to identify population boundaries. High genetic diversity (H_E= 0.78) and no population differentiation (K=1) were detected across total sampled individuals within Alabama. However, some genetic differentiation was measured between the urban and rural groups, which may be a consequence of increased urbanization and anthropogenic influences in the area. Thus, we concluded that urban coyotes might be a basis for delimiting individuals into management units. We encourage

managers to consider various data including genetic information, landscape ecology, anthropogenic effects, and urbanization practices for a more robust assessment of urban coyote management units.

INTRODUCTION

Coyotes (*Canis latrans*) are a wildlife pest management issue across the United States due to their impact as a top predator on commercial agricultural business and range expansion aided by the species' adaptive ability and high reproductivity (Bekoff 1978, Knowlton et al. 1999, Bodenchuk et al. 2000). Historically, the coyote was native to the Central Plains region of the United States, including Texas, Oklahoma, Kansas, and Nebraska (Young and Jackson 1951; Nowak 1978; Parker 1995). Although coyotes have naturally increased their current range to include the eastern and western U.S. states, many consider the species to be invasive (Cunningham and Dunford 1970; Brady 1983; Hill et al. 1987; Wooding and Hardinsky 1990; Schmitz and Brown 1994; Parker 1995).

Most recently, coyotes have become a management concern in more urbanized areas due to their increasing prevalence within more heavily developed and human inhabited localities. Many studies nationwide have shown an increase in the reported number of coyote sightings (Baker and Timm 1998; Timm et al. 2004; Carrillo et al. 2007). Even more disturbing is the number of these reports that include recognition of a coyote's decreased fear of humans in conjunction with an increased aggression towards humans and pets. Victims of coyote attacks are frequently children because they are smaller and thought to be more easily mistaken as appropriate prey (Baker and Timm 1998; Timm et

al. 2004; Schmidt and Timm 2007; Stull and Mengak 2009). Ample anthropogenic resources such as trash, pet food, small pets, and feral cats increase the coyote's ability to readily inhabit urbanized areas. In addition, landscaping can serve as excellent habitat for rodents and other small mammals, another prey group , which can draw coyotes into more urbanized areas. Overall, the habitat created in the urban environments, leads to less energy expense and lack of competition to attain needed resources, and is a factor promoting sustained coyote presence (Baker and Timm 1998). The present successful infiltration of coyotes specifically into more urbanized localities has justified the need for developing urban management plans in order to help decrease undesirable interaction between coyotes and humans and other domestic and wildlife species.

Management plans are usually based on a management unit, an assemblage of demographically autonomous groups of a species (Palsbøll et al. 2006). In the case of coyotes, management units are difficult to define because of their high capacity for dispersal, migratory tendencies, and continuous distribution across their range (Diniz-Filho and Telles 2002; DeYoung and Honeycutt 2005). Further, coyotes are characterized as habitat and foraging generalists (Bekoff 1978), which allows them to thrive in a diverse environments, and also limits our ability to define practical management units. Due to coyotes' dispersal abilities, identifying groups (i.e. management units) based on natural barriers is typically not plausible. However, several studies on coyotes utilizing molecular techniques have detected genetic differentiation (restricted gene flow) between coyote populations, which can be partially explained by the existence of human developments (Sacks et al. 2004; Riley et al. 2006; Sacks et al. 2008). To date, no study

has been completed in the southeastern U.S. employing genetic data to examine genetic structure and other population dynamics of the coyote.

The main goal of this study was to assess population structure by testing for genetic differentiation among coyotes in east-central Alabama using molecular data (i.e. microsatellite DNA). Population differentiation was examined using two different methods: (1) without *a priori* population assignment; and (2) with assignment of individuals to urban and rural populations. We hypothesized that high levels of genetic diversity and low levels of population structure would be detected within both methods due to the biological profile of the coyote (i.e. increased mobility, high reproductivity, and continuous dispersal). Further, we hypothesized coyotes sampled within urban and rural localities would not exhibit genetic differentiation. The identification of genetic differentiated populations that could serve as possible management units could assist in the adaptation of management plans for this species.

STUDY AREA

Our study area encompassed a 100 km radius of the Auburn/Opelika Metroplex Statistical Area (MSA) in east-central Alabama. The area included Autauga, Bullock, Chambers, Chilton, Clay, Coosa, Elmore, Lee, Macon, Montgomery, Randolph, Russell, Shelby, Talladega, and Tallapoosa counties (Fig. 3.1). With a total population of 130,516 people in 2008, the Auburn/Opelika MSA is considered the fastest growing metropolitan area in Alabama since 1990 (U.S. Census Bureau 2001). The landscape directly adjacent to metropolitan sections is a mixture of agricultural, ranching and farming lands.

METHODS

Sample Collection and DNA Extraction

We collected samples (n = 74) from both live captures and road-kill animals in concert with other federal and state wildlife management efforts from April 2008 to May 2009. We obtained tissue samples from live captures in conjunction with a telemetry study conducted by a fellow colleague at Auburn University. We sampled tissue from the ear of each individual using a commercial grade ear-notcher. Directly following sampling efforts, we stored the tissue in an EDTA/DMSO buffer solution saturated with NaCl for preservation (Seutin et al. 1991). We extracted DNA from each sample using a DNeasy® Tissue Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. All collection protocols were approved by Auburn University Institutional Animal Care and Use Committee (Protocol# 2007-1244).

Laboratory Protocol

We amplified 10 microsatellite markers (FH2001, FH2096, FH2137, CX140, FH2054, FH1010, FH2159, CX2235, FH2100, FH2062; Ostrander et al. 1993; Ostrander et al. 1995; Francisco et al. 1996; Breen et al. 2001) using three multiplexed polymerase chain reactions (PCRs: Table 3.1). Each reaction was run with optimized amounts of PCR water, GeneAmp 10X PCR Buffer II (Applied Biosystems Inc., Foster City, California), $MgCl_2$ [Panel A: 1.0µl, Panel B: 0.8µl, Panel C: 0.7µl] (Applied Biosystems Inc., Foster City, California; 25mM), 1.0µl dNTP (Promega; 10mM), primers (Table 3.1; 1µM), 0.1µl Amplitaq Gold (Applied Biosystems Inc., Foster City, California; 5 U/µL), 0.4µl BSA (Promega; 10 mg/ml). The multiplexed PCR amplification process included an

initial denaturation cycle of 10 minutes at 95°C followed by 52 cycles of 94°C for 30 seconds, panel specific annealing temperatures for 30 seconds (Panel A = 51°C, Panel B = 50°C, Panel C = 59°C), and extension at 72°C for 45 seconds. A final extension was accomplished in one 7-minute cycle at 72°C.

We sent the amplification products to the Wildlife Genetics Lab at the USDA/APHIS/WS National Wildlife Research Center in Fort Collins, Colorado for visualization on an ABI 3130 Genetic Analyzer (Applied Biosystems Inc., Foster City, California). We binned the visualized data using GeneMapper Software v4.0 (Applied Biosystems Inc., Foster City, California) and exported it using GMConvert (Faircloth 2006). We employed CONVERT v1.31 (Glaubitz 2004) to transform the raw data files into the proper input files for various downstream statistical analyses software.

Population Assignment

We created a point shapefile within ArcGIS (ESRI) to represent the location of each coyote that was sampled. We then assigned each point to a category of either urban or rural based on Alabama Gap Analysis Project (AL-GAP) landcover data (Kleiner et al. 2007) and TIGER/Line census block data from the 2000 U.S. Census Bureau (http://www.census.gov/geo/www/tiger/). The U.S. Census Bureau considers any census block group having a population density of at least 1,000 people per square mile with surrounding block having at least 500 people per square mile as being urban. According to this assumption, everything outside of those constraints was categorized as rural. We performed zonal statistics using the Spatial Analyst Tools in ArcGIS (ESRI) for each of

the fifteen representative counties to determine majority landcover type per census block, based on AL-GAP landcover data (Kleiner et al. 2007). We selected landcover types of low, medium, and high intensity development, and open developed areas (i.e. impervious surfaces, golf courses, etc.) and reclassified them as urban. We then performed a spatial query to select attributes from both the census and landcover data layers. We combined all polygons that had been classified as urban based on both census and landcover type into a single urban polygon. We then applied a buffer to the urban polygon equal to 4.22 km, the diameter of a rural coyote home range in the area (H. Jantz, Auburn University, unpublished data), to account for sampling of possible transients between urban and rural selected areas. We deemed any point, which represented the location of a sampled coyote, which fell within the urban polygon an urban coyote. Lastly, we classified all individuals not categorized as urban and not collected within the buffered interface area as rural. The complete sampling regime is shown in Figure 3.2. Final sample sizes for each population were: urban (n = 8), buffer (n =16) and rural (n = 50).

Genetic Statistical Analyses

We completed statistical analyses over three different data sets: (1) total number of individuals; (2) subsampled rural populations versus total urban samples; and (3) rural clusters selected with similar spatial distribution to the urban group. We used the program MICRO-CHECKER 2.2.3 (Oosterhout et al. 2004) to test for evidence of genotyping errors, such as null alleles and scoring errors, using the total sample of 74 individuals (i.e. data set 1). We utilized the program FSTAT 2.9.3 (Goudet 2001) to examine the microsatellite loci for linkage disequilibrium. We performed analyses including allelic

richness for all polymorphic loci, observed and expected heterozygosity to test for violations to the Hardy-Weinberg Equilibrium, and genetic diversity in ARLEQUIN 3.1 (Excoffier et al. 2005). Bonferroni corrections were performed for Hardy-Weinberg Equilibrium estimates across loci to compensate for biased significance of data within tables (Rice 1989).

We utilized BAPS 5.2 (Corander et al. 2008), a Bayesian clustering program, to test for genetic differentiation across the total sampling effort (n = 74) without *a priori* population membership information using the spatial clustering of individuals algorithm. BAPS works by assigning individuals into population clusters (K) based on detected genetic structure and spatial proximity. The test to detect population differentiation amongst all individuals consisted of 10 iterations for each of K = 1 through K = 10.

The unequal sample sizes produced by categorizing individuals *a priori* as either rural (n = 50) or urban (n = 8) in ArcGIS (ESRI) as previously described, were a concern since tests based on distributions, such as the test for F_{ST}, do not perform ideally with unequal sample sizes (Cockerham 1973). Thus, to calculate pairwise F_{ST} (Weir and Cockerham 1984), we randomly selected 8 individuals from the rural population for 100 iterations. We then ran each of these rural subsamples, referred to as the 'reduced' rural populations (i.e. data set 2), against the total urban individuals in ARLEQUIN 3.1 (Excoffier et al. 2005). Evidence of population differentiation as measured by pairwise F_{ST} estimates was considered as a proportion out of 100. We subsampled 10 of the 100 randomly selected rural samples of (n = 8) to examine possible sampling bias in measures of heterozygosity.

We utilized ARLEQUIN 3.1 (Excoffier et al. 2005) to calculate expected (H_E) and observed (H_O) heterozygosity measures for the 10 random rural samples in addition to the total urban sample. We then plotted heterozygosity measures graphically to assess whether this estimate from the total rural samples was different (not within the range of variation distribution) from the 'reduced' rural populations (Fig. 3.3). If the estimate for the total rural samples was included within the distribution of the estimates for the 'reduced' rural populations, we would conclude that no sampling bias existed in the case of heterozygosity measures.

Family structure can serve as a confounding variable in clustering algorithms (Waples 1998), thus we conducted analyses in the program RELATEDNESS 5.0.8 (Goodnight and Queller 2000) to examine presence of first-order and second-order relatives within selected groups. We used ArcGIS (ESRI) to identify 2 clusters of 8 individuals within the rural population that had spatial distributions congruent to that of the urban group (Fig. 3.4). Clustering rural individuals within similar spatial distributions as the urban group was necessary in order to test if coyotes from a spatially restricted area might tend to be more related than samples caught across a broader area. We had this concern because a majority of the urban coyotes (n = 6) were caught in a geographically proximate area and therefore might have been closely related animals causing bias of the BAPS and F_{ST} results. One rural cluster was to the west of the core area of the Auburn/Opelika MSA, so it was referred to as the 'west' cluster, while the other rural cluster being to the east was referred to as the 'east' cluster. We ran pairwise F_{ST} tests in ARLEQUIN 3.1 (Excoffier et al. 2005) between the urban population and each of the rural clusters ('west' and 'east')

to test for genetic differentiation (i.e. data set 3). Lastly, we employed BAPS using both genotypic and GPS point data to run spatial clustering of groups over 10 iterations of both K = 1 and K = 2 to simply determine if differentiation between the *a priori* selected urban and rural groups could be detected. First, we ran the total rural (n = 50) samples against the total urban (n = 8) samples for a total dataset of (n = 58) to determine if these two groups would genetically and spatial cluster differently. Then, under the same conditions, we ran each of the rural clusters ('east' and 'west') against the urban cluster to evaluate possible genetic differentiation.

RESULTS

A total of 74 individuals were genotyped within a 100 km radius of the Auburn/Opelika Metropolitan Statistical Area in east-central Alabama. One microsatellite marker, CX2235, showed evidence of null alleles at $\alpha = 0.05$, which was a concern since null alleles can be evidence of reduced primer annealing, competition amongst target alleles of various lengths during amplification, or poor template quality (Wattier et al. 1998; Daken and Avise 2004). However, if the probability (α) is less than 0.20, it is considered uncommon or rare that a null allele actually exists (Dakin and Avise 2004), thus CX2235 marker was retained in this study. All 10 loci were used in this analysis, with no loci having more than 5% missing data. No significant linkage disequilibrium was detected across loci over all samples. All loci were polymorphic with one locus, CX140, having a violation of Hardy-Weinberg Equilibrium (Table 3.1). This locus had a significantly lower observed heterozygosity (H_O) value (0.82) than expected heterozygosity (H_E) value (0.83) after Bonferroni corrections (P = 0.001). The averaged observed

heterozygosity across all ten loci (0.76) was not significantly different from expected (0.78) after Bonferroni corrections. Allelic richness ranged from 5 to 22 alleles per locus (Table 3.1). Without *a priori* population designation, a single genetic cluster was detected using BAPS 5.2 (Corander et al. 2008) for all sampled individuals in Alabama which suggested no differentiation among individuals within 100 km of the Auburn/Opelika MSA.

Genetic differentiation between the *a priori* assigned 'reduced' rural and total urban populations was detected based on the 100 iterative pairwise F_{ST} estimates. Sixty-five percent of the pairwise comparisons were significant at $\alpha = 0.05$ (Table 3.2). The heterozygosity measures for the total rural population fell within the distribution of the values generated from the 10 'reduced' rural populations, thus we concluded that there was no sampling bias in the case of heterozygosity measures and used the total rural samples versus the urban samples to estimate H_E and H_O. Gene diversity, equivalent to H_E, for the rural population was 0.78, and 0.71 for the urban population. The observed heterozygosity, H_O, for the rural population was 0.74, and for the urban population was 0.84.

With the application of *a priori* population assignment of total rural and urban populations, no genetic differentiation was initially detected in BAPS (Corander et al. 2008). However, we did detect genetic differentiation (K = 2) between rural clusters (i.e. 'east' and 'west') and the urban cluster. Also, significant pairwise F_{ST} were estimated between the urban and the 'east' rural group ($F_{ST} = 0.04$; $P = 0.04$), and between urban

and the ‘west’ rural group (F_{ST} = 0.03; P = 0.04). Since there was significant pairwise F_{ST} estimates detected between the urban and rural populations, tests for relatedness were employed to determine if sampling closely related individuals may have influenced these results. None of the 3 populations showed significant relatedness amongst the individuals.

DISCUSSION

The first goal of this study was to assess population structure without *a priori* population designation of coyotes in east-central Alabama. Further, this study evaluated if genetic groups (i.e. populations) could be identified and therefore represent practical management units. Coyotes across the total study area, based on statistical analyses, showed high levels of genetic diversity and low levels of population structure. In addition, clustering of individuals based on both genotypic and spatial datasets resulted in no significant genetic groupings. Thus, we failed to reject our hypotheses and concluded due to lack of genetic structure in this species, populations based solely on genotypic data do not serve as viable management units.

The high genetic diversity we found within Alabama coyotes has also been found in other studies using autosomal microsatellites DNA data completed across the United States and Canada (Roy et al. 1994; Sacks et al. 2004; Riley et al. 2006; Sacks et al. 2008). From a management perspective, the life characteristics of the coyote (i.e. high reproductivity, continuous distribution, and great dispersal abilities), which lead to the increased levels of genetic diversity and low population differentiation, only serve to complicate the

determination of viable management units when basing such categorization solely on genetics. Despite high genetic diversity, which indicated an abundance of heterozygotes, we did detect one violation of Hardy Weinberg Equilibrium (HWE) within the total population. This discrepancy indicated a deficiency of heterozygotes at locus CX140. Such a finding would suggest issues such as presence of allelic dropout, null alleles, linkage of alleles, or inbreeding. However, null alleles and allelic dropout were not detected. Also, the tests for linkage disequilibrium were not significant suggesting the markers evolved independently within our sample. Lastly, if the violation was a consequence of inbreeding, you would expect to observe such a phenomenon at all loci and not just one (Selkoe and Toonen 2006). It is important to point out that the difference between the observed and expected values of heterozygosity were quite low (0.01), suggesting that the finding of significance is probably not biologically meaningful. Three of the nine total alleles detected at CX140 represent 68.92% of the total allelic frequency for the locus. Such an unequal distribution of alleles could account for the violation by appearing to have a deficiency of heterozygotes in the population. Furthermore, the sample size for this study was low, which could also serve as an explanation of the HWE violation at this single locus within the total sample.

The second portion of this study, used to test for population structure between *a priori* designated populations (i.e. urban and rural) showed evidence supporting genetic differentiation between the two groups. Thus, we rejected our second hypothesis, concluding that genetic differentiation between coyotes inhabiting more urbanized areas may exist on a finer scale due to restriction of movement in areas surrounded by

highways and other aspects of urbanization. We believed, based on the significant pairwise F_{ST} estimates between urban and rural populations when rural population size was made to be equitable to the urban sampling, that the genetic differentiation identified in this study was real, but weak in nature. Manel et al. (1995) found that unless true populations are definitively identified and exhibit strong divergence from each other, any clustering analysis involves a high level of uncertainty. BAPS 5.2 (Corander et al. 2008) has been reported to have the power to provide grouping correctly when F_{ST} is greater than 0.05 (Latch et al. 2006), which is the case in this study. Yet, Waples (1998) warned that the ability of clustering programs to distinguish small departures from panmixia (i.e. random mating) also puts them at risk to confuse minute genetic remnants caused from family structure or non-random sampling. In the case of this study, no relatedness was detected amongst any of the individuals, thus we concluded that remnants of family structure was not an explanation of the weak genetic differentiation detected.

Significant differentiation of coyotes inhabiting more urban areas in this study could be explained in a few ways. Six of the 8 urban individuals were collected around a common landmark, the Auburn-Opelika Robert G. Pitts Airport. Two major highways that intersect in the Auburn/Opelika MSA, Interstate I-85 and Alabama State Highway 280, converge within approximately 2.5 km from this airport (Fig. 3.5). Sacks et al. (2004) commented on the likelihood that highways in urban areas could lead to reduced dispersal of coyotes. Later, Riley et al. (2006) found that freeways in California presented formidable barriers to gene flow between coyote populations. Secondly, commercial development within 2 miles of the airport and other significant urbanization has

drastically changed the face of the landscape in that area over the last few years. Therefore, the majority of our urban coyotes may experience limited interactions with rural coyotes because they are geographically isolated by highways and increased urbanization. This scenario would limit reproductive opportunities between the urban individuals and rural coyotes leading to lower gene flow and greater genetic differentiation. Lastly, increased anthropogenic resources (i.e. trash, pet food, ornamental landscaping, etc.), which is a common characteristic in growing urban areas, could have allowed the urban individuals to reduce their home ranges due to a concentration of resources, resulting in isolation from other coyotes.

MANAGEMENT IMPLICATIONS

Coyotes are of great interest in wildlife management due to their relatively negative reputation, primarily brought on by human dimensions concerns. Management units based on populations of coyotes are difficult to define due to their high dispersal abilities, adaptability of food resources and habitat use, and continuous distribution across their range (Bekoff 1978; Diniz-Filho and Telles 2002; DeYoung and Honeycutt 2005). Genetic analyses conducted in this study in the attempt to identify management units allowed for a less intrusive perspective into movements and associations of individuals that can be much less labor-intensive than other, more traditional methods, such as radio-telemetry and point count protocols. Such technology also offers an alternative methodology of investigating weary or highly secretive species, like the coyote, that are hard to track via visual or direct contact (Scribner et al 2005). We acknowledge that in the field of wildlife management, genetic data may not be readily available for all species

in all regions. However, the acquisition of such data is becoming less and less difficult and more cost-effective with the increase of resources and laboratories with the capabilities of completing such analyses (McKelvey and Schwartz 2004; DeYoung and Honeycutt 2005). Many studies, such as those dealing with wildlife disease and food habits, have protocols that collect tissues and fecal material for their analyses, which may also be useful to isolate genetic information. Thus, we encourage wildlife managers to take advantage of genetics to aid in the assessment and management of wildlife species.

Information gained from this study suggests that a population determined solely by genetic data, without *a priori* grouping of individuals, is not as informative in the identification of viable management unit of coyotes. Further research is needed utilizing a much larger sampling of urban coyotes, and in a much larger metropolitan area where definition between urban and rural coyotes could be more distinct. If similar findings are found in future studies, coyotes within urban settings may constitute a practical management unit for use in management plans. We advise that other influential factors due to elements of landscape ecology, urbanization, and anthropogenic dynamics be combined with genetic data in the assessment of management units within this species.

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Table 3.1: Per-Locus Information - Microsatellite panel/primers information, and genetic diversity indices (allelic richness, heterozygosity) for total sample (n = 74). A_R = Allelic Richness; H_E = Expected Heterozygosity; H_O = Observed Heterozygosity.

Primer/Locus Details						Genetic Diversity Indices		
Multiplex	Locus	Color	Type	Approximate Allele Size Range		A_R	H_E	H_O
A	FH2001	Fam	Tetra	122-158		9	0.76	0.70
A	FH2096	Hex	Tetra	89-109		8	0.60	0.57
A	FH2137	Ned	Tetra	158-194		10	0.89	0.88
A	CX140	Hex	Di	130-154		5	0.83	0.82
B	FH2054	Ned	Tetra	135-175		9	0.76	0.85
B	FH2010	Hex	Tetra	221-237		6	0.74	0.66
B	FH2159	Fam	Tetra	155-206		5	0.94	0.91
C	CX2235	Fam	Tetra	136-176		5	0.81	0.72
C	FH2100	Hex	Tetra	142-176		14	0.72	0.72
C	FH2062	Ned	Tetra	129-145		22	0.76	0.73
					Mean	9.3	0.78	0.76

Table 3.2: Pairwise F_{ST} values with p-values calculated using a subsample (n = 8) of the rural population against the total urban population (n = 8)

Iteration	F_{ST}	p-value	Iteration	F_{ST}	p-value	Iteration	F_{ST}	p-value	Iteration	F_{ST}	p-value
1*	0.03	0.01	14*	0.03	0.02	27*	0.05	0.01	40*	0.05	0.00
2*	0.03	0.01	15*	0.05	0.01	28*	0.04	0.03	41	0.03	0.06
3*	0.06	0.00	16	0.02	0.06	29	0.02	0.06	42*	0.04	0.01
4	0.02	0.09	17*	0.03	0.03	30	0.02	0.07	43*	0.05	0.00
5	0.01	0.17	18	0.03	0.07	31*	0.03	0.04	44*	0.03	0.04
6*	0.04	0.01	19*	0.06	0.00	32*	0.03	0.04	45*	0.03	0.02
7*	0.03	0.03	20	0.02	0.10	33	0.02	0.09	46*	0.03	0.01
8*	0.04	0.01	21	0.02	0.09	34	0.02	0.06	47	0.02	0.07
9*	0.05	0.01	22*	0.04	0.00	35	0.02	0.07	48	0.02	0.07
10*	0.03	0.02	23*	0.04	0.01	36	0.02	0.08	49*	0.04	0.01
11*	0.03	0.02	24*	0.03	0.03	37*	0.03	0.02	50	0.02	0.08
12*	0.02	0.04	25	0.02	0.10	38	0.02	0.06	51	0.02	0.06
13	0.02	0.08	26*	0.04	0.02	39*	0.04	0.01	52*	0.03	0.02

* Denotes significance.

Table 3.2: (Cont.)

Iteration	F_{ST}	*p*-value	Iteration	F_{ST}	*p*-value	Iteration	F_{ST}	*p*-value	Iteration	F_{ST}	*p*-value
53*	0.04	0.01	66*	0.04	0.01	79*	0.05	0.01	92*	0.03	0.04
54*	0.03	0.02	67*	0.03	0.03	80*	0.03	0.04	93	0.02	0.052
55*	0.04	0.01	68*	0.04	0.02	81*	0.03	0.02	94	0.01	0.13
56*	0.04	0.00	69*	0.05	0.00	82*	0.04	0.01	95*	0.03	0.04
57*	0.05	0.00	70*	0.04	0.01	83*	0.03	0.03	96*	0.03	0.05
58	0.02	0.06	71*	0.04	0.02	84	0.03	0.06	97*	0.05	0.01
59*	0.03	0.04	72*	0.04	0.01	85*	0.03	0.02	98*	0.03	0.04
60	0.01	0.14	73*	0.03	0.03	86*	0.03	0.02	99	0.02	0.08
61	0.02	0.07	74*	0.03	0.03	87	0.02	0.08	100	0.02	0.06
62*	0.02	0.04	75*	0.03	0.03	88	0.02	0.07			
63*	0.04	0.00	76	0.02	0.08	89	0.02	0.08			
64*	0.03	0.02	77	0.02	0.11	90*	0.03	0.04			
65	0.01	0.18	78*	0.05	0.00	91	0.01	0.09			

* Denotes significance.

Figure 3.1: Counties included in the study area. Red circle represents total study area where coyotes were sampled in Alabama.

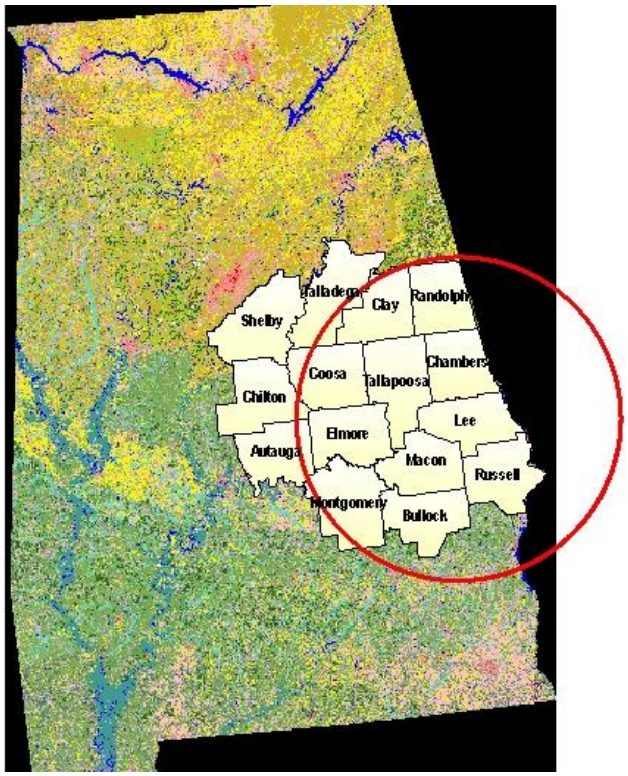


Figure 3.2: Sampling classifications within study site; Urban (Green), Interface (Light Purple), Rural (light yellow). The red dots represent the location of each sampled coyote.

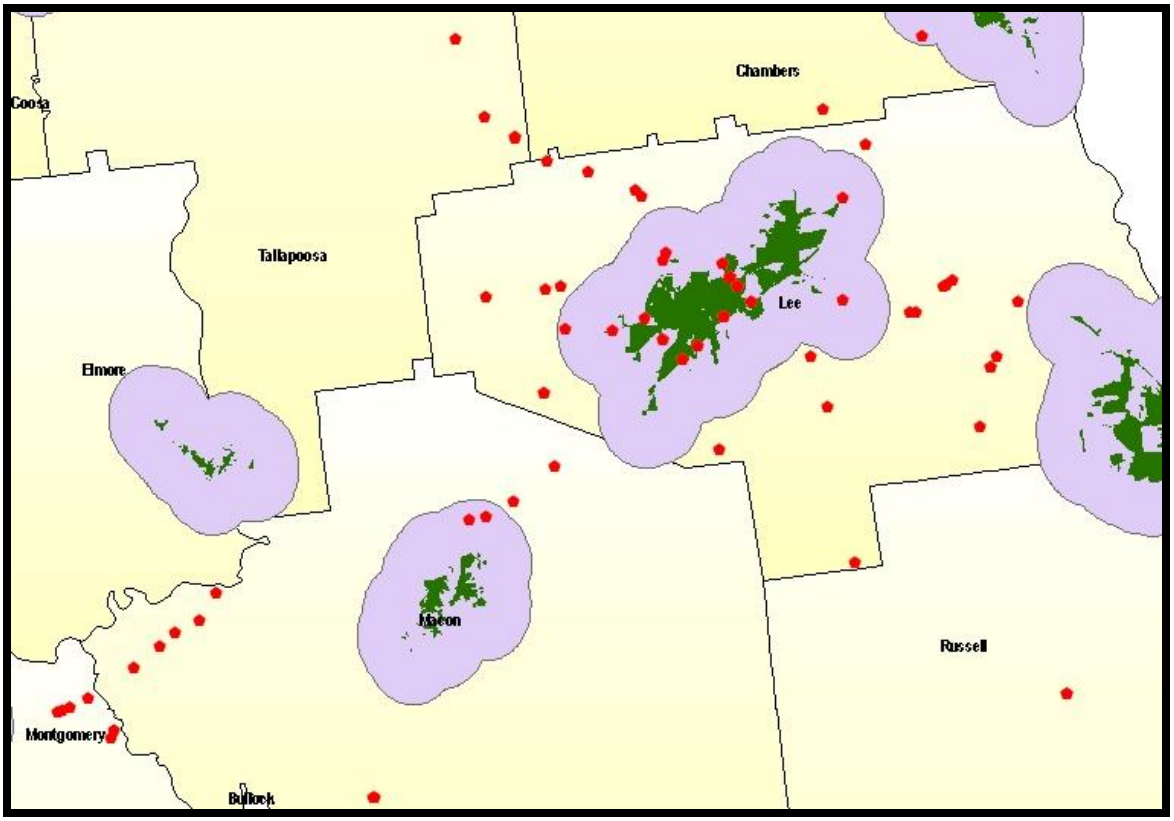


Figure 3.3: Scatter plot showing results of iterative testing of subsamples from the rural population as compared to the estimates for the total rural sample and total urban sample. Note: the triangle denotes the heterozygosity estimates for the total rural. (n = 50) samples.

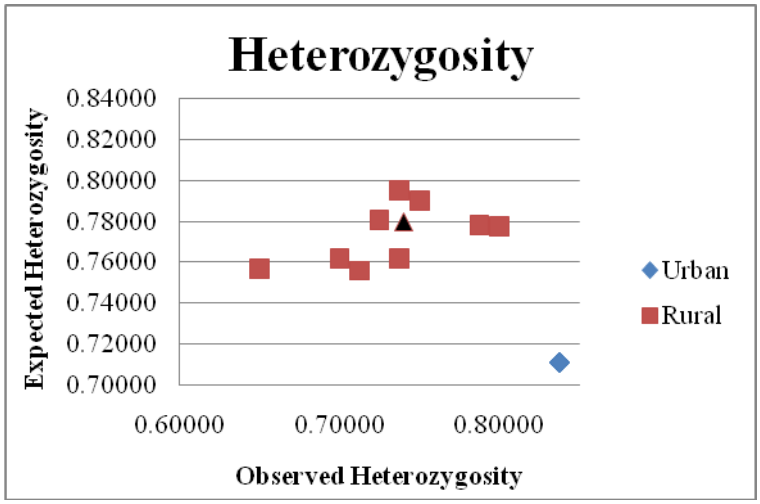


Figure 3.4: Three clusters chosen to compare estimates of relatedness and genetic clustering from congruently sized rural samples to the urban sample.

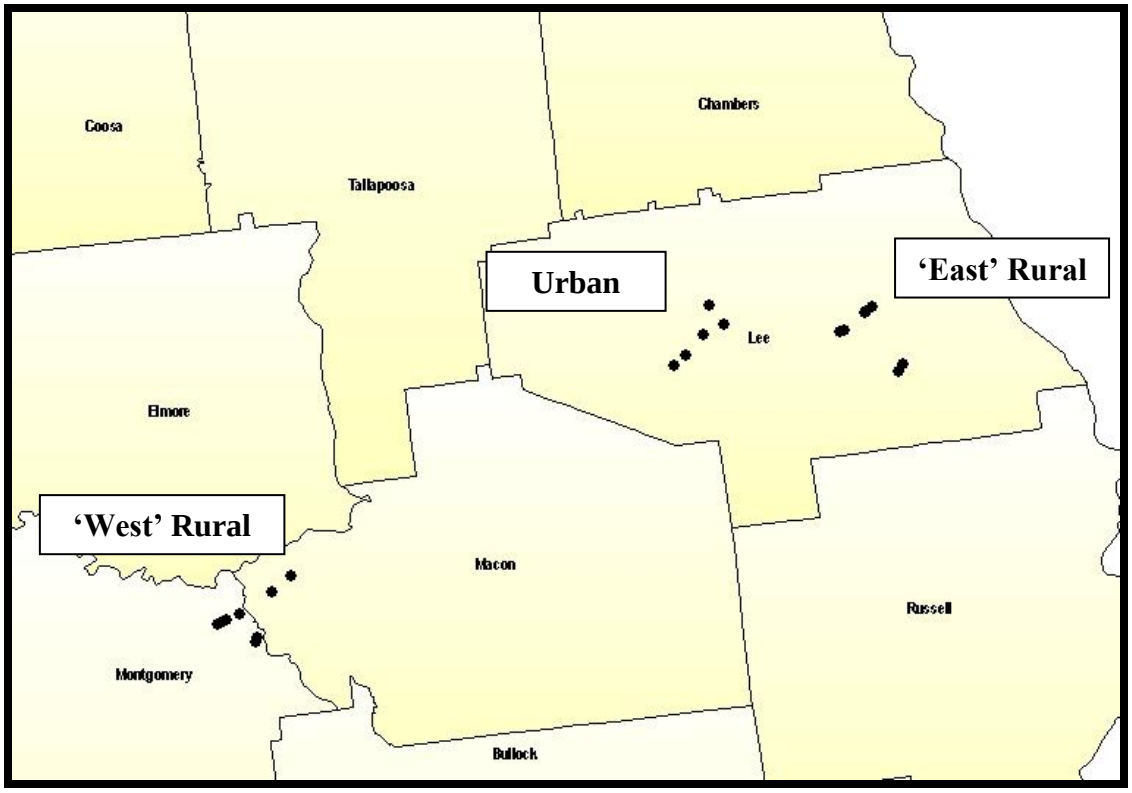
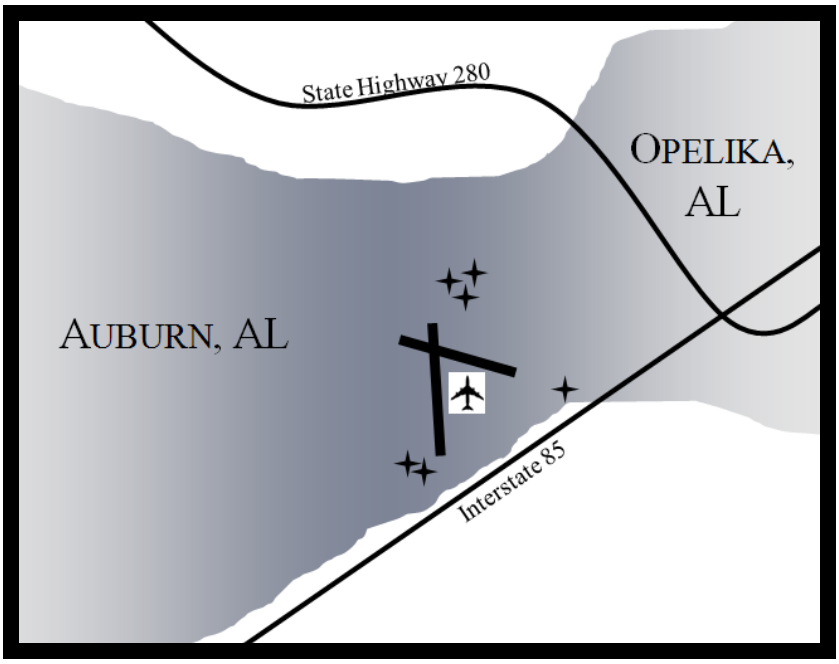


Figure 3.5: Map of where 6 urban individuals were collected. Crosses denote capture localities and plane denotes the Auburn-Opelika Robert G. Pitts Airport. Major highways and urban areas are included.



CHAPTER IV: CONCLUSIONS

Several noteworthy and intriguing findings emerged from this project. Using phylogeographic analyses, we were able to develop a new hypothesis about probable dispersal routes for coyotes during the last 150 years. In addition, we found evidence to suggest that the Ohio River has served as a barrier to dispersal and as such, the previously unknown origins of coyotes within the region including Tennessee and Kentucky appear to have been from the southeastern states (i.e. LA, MS and AL). We rejected both of the proposed hypotheses that were tested in Chapter II, and submitted a new alternative hypothesis that was inferred from the maximum-likelihood phylogenetic tree.

To date, there have been a few attempts to construct canid phylogenies using the mitochondria genome including samples of North American wolves such as the gray wolf (*Canis lupus*), red wolf (*C. rufus*), the proposed Great Lakes wolf (*C. lupus lycaon*), and the Canadian/Algonquin wolf (*C. lycaon*), along with other wolf-like canids such as domestic dogs (*C. familiaris*) and coyotes (*C. latrans*) (Lehman et al. 1991; Wayne 1993; Wayne et al. 1997; Vila et al. 1997; Vila et al. 1999*a*; Vila et al. 1999*b*; Bininda-Emonds et al. 1999; Wilson et al. 2000; Adams et al. 2003; Zrzavý and Řičánková 2004; Koblmüller et al. 2009). Many of the relationships among these canid species has been highly debated over the years. It is our belief that the coyote haplotypes collected from the 12 states sampled within the phylogeographic portion of this project, if supplemented with a greater sampling effort of coyotes across the other unrepresented regions, could be

combined with databases including sequences for the other canid species of interest to construct a robust phylogenetic analyses of the interrelations of wild canids. Such information would be of great importance for issues of species level conservation, taxonomic classification, and historical and modern range expansion patterns.

Coyotes inhabiting more urbanized areas have created a growing wildlife management concern, mostly in the arena of human dimensions. Based on the results of the population genetic analyses (Chapter III), urban coyotes were identified as a basis to begin strategic grouping of individuals into management units. However, we also concluded that additional data should be used in collaboration with genetic information, including elements of landscape ecology, anthropogenic effects, and urbanization practices. Since the sample size within this study was limited, further research must be completed to ascertain if the patterns of genetic differentiation of coyotes sampled in the urban landscapes of the Auburn/Opelika Metropolitan Statistical Area could be detected in other urban areas. The implications of such findings would suggest a management unit that, if removed based on wildlife management plans, could have future effects on the coyote population within the managed area. Manipulation of this species from the genetic perspective could assist in efforts to decrease the amount of negative human-coyote interactions.

Both portions of this project have attributed to a sound foundation for future research within coyote and canid genetics. Throughout this study, data has been collected within two molecular genomes (nuclear and mitochondria) across twelve states. It is my hope that this work will not end with the conclusion of this thesis, but continue to help shed light on the past, present, and future of coyote populations.

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