2015

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Abstract - *Canis latrans* (Coyotes) are a management concern in the southeastern US because of their potential impacts on agriculture, other wildlife species, and human health and safety. This region is part of a recent range expansion by Coyotes, and information about their population structure in the southeastern US is lacking. In this study, we used microsatellite DNA to assess genetic diversity and population structure among Coyotes in east-central Alabama. We detected high genetic diversity (H_E = 0.78) and no population structure across the total sampling area. Additionally, we investigated population structure between urban and rural groups. We detected low but significant population structure between these groups, which may be biologically meaningful. We discuss the implications of this result in the context of potential management strategies. Overall, our study sought to provide information about the molecular ecology of Coyotes within a region of recent range expansion.

Introduction

Historically, *Canis latrans* Say (Coyote) was native to the Central Plains region of the US, including Texas, Oklahoma, Kansas, and Nebraska (Nowak 1978, Parker 1995, Young and Jackson 1951). Within the last 200 years, Coyotes have expanded their range first into the western US, followed by an eastward expansion predominantly occurring over the last century (Brady and Campbell 1983, French and Dusi 1979, Gipson et al. 1974, Hill et al. 1987, Parker 1995, Wooding and Hardinsky 1990). The Coyote's successful range expansion has likely been facilitated by its behavioral plasticity and capacity for high reproduction (Bekoff 1978). Colonization of the southeastern US by Coyotes began in the early 1960s, with the range-expansion front crossing over southern portions of the Mississippi River, and moving east. This trajectory of Coyote population expansion throughout the Southeast occurred mainly within in the last 30 years (Parker 1995). Coyotes are a management concern within this region for many reasons. However, there is a lack of information regarding the population structure of Coyotes in the southeastern US (Mastro et al. 2012) that could limit the development of effective strategic management plans for the species.

Management concerns regarding Coyotes in Alabama are representative of problems observed across the southeastern US. Issues requiring management

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Manuscript Editor: Michael Conner

of the species within this region include depredation on livestock, damage to crops, perceptions of competition between Coyotes and hunters for game resources including potential effects of Coyotes on *Odocoileus virginianus* Zimmermann (White-tailed Deer) fawn recruitment, and threats to human and aircraft safety at airports (Houben 2004; Howze et al. 2009; Jones 1987; Kilgo et al. 2012, 2014; USDA 2002). Damage to livestock and crops by Coyotes has been documented in the state (Armstrong and Walters 1995, Connolly 1992, Dunn and Smith 2011, Philipp and Armstrong 1995, USDA 2014), and Armstrong and Smith (2014) reported that the number of damage complaints from Coyote activities has risen sharply with the increase in Coyote numbers in Alabama. Recent studies have suggested that Coyote depredation has contributed to reduced White-tailed Deer fawn recruitment in portions of Alabama (Jackson and Ditchkoff 2013, VanGilder et al. 2009). Coyote impacts are not limited to rural Alabama; Coyotes have also become a problem in urban areas. Complaints from the public concerning Coyotes have shifted in recent years from primarily reports of agricultural damage in rural areas, to urban-specific issues, such as attacks on pets and other negative human interactions (Armstrong 2011). In December 2013, the city of Auburn, AL, entered into an agreement with USDA-Wildlife Services (USDA-WS) to reduce a Coyote population around a city park, which included trapping efforts, habitat reduction, and public education (USDA 2013). In addition, USDA-WS has carried out management efforts to reduce the potential human safety threat posed by Coyotes at several Alabama airports (W. Gaston, USDA/APHIS/WS, Auburn, AL, pers. comm.). Overall, Coyotes constitute a significant wildlife management and damage issue within the southeastern US, including Alabama.

Information about the ecology of Coyotes is essential to gain a better understanding of the species and to develop effective management strategies. Traditionally, management units, commonly defined as demographically autonomous groups, have been based on elements like administrative or geographic barriers, habitat characteristics, and geographic distribution of a species (DeYoung and Honeycutt 2005, Lackey 1998, PalsbØll et al. 2006, Wallace et al. 2010). The identification of management units is a crucial component of conservation and management plans, because they define a discrete section for focusing monitoring and management actions (Moritz 1994, PalsbØll et al. 2006, Schwartz et al. 2007). More recently, management units have been identified as groups that exhibit significant genetic differentiation (Moritz 1994, PalsbØll et al. 2006). However, meaningful management units can be difficult to define for Coyotes because of their high capacity for dispersal, migratory tendencies, and contiguous distribution across their range (DeYoung and Honeycutt 2005, Diniz-Filho and Telles 2002). Further, Coyotes are characterized as habitat and foraging generalists (Bekoff 1978), which allows them to thrive in diverse environments, and may also limit characterization of practical management units. Nonetheless, Coyote populations can be influenced by many factors including fragmentation of the landscape and habitat, inter-specific competition, and natural geographic and man-made barriers, including increased levels of urbanization (Arjo and Pletscher 1999; Atwood et al. 2004; Berger and Gese 2007;

Gehrt et al. 2009; Randa and Yunger 2006; Rashleigh et al. 2008; Riley et al. 2006; Sacks et al. 2004, 2005). Modern genetic methods to assess population structure can be a useful tool for wildlife biologists studying a highly adaptable species, such as the Coyote, and can be used to provide information to assist in defining management units (DeYoung and Honeycutt 2005, Honeycutt 2000).

Several studies using DNA to investigate population structure of Coyotes have identified significant population differentiation. Williams et al. (2003) detected low levels of genetic structure between Coyotes grouped by age after a transition from selective to non-selective removal-based management practices in northern California. Other studies identified Coyote population structure related to the presence of a major freeway or based on microhabitat breaks and other habitat-specific delineations in California (Riley et al. 2006; Sacks et al. 2004, 2005, 2008). Monzón (2014) detected population structure within eastern Coyotes at what was considered a "contact zone" between 2 fronts of colonization. Coyotes sampled in New York showed significant population structure, which could be due to deer densities and human land-use (Monzón 2014). Another study detected genetic differentiation at a broader geographic scale between eastern and western Coyote populations (Way et al. 2010). Rashleigh and others (2008) conducted a study around the Cleveland, OH, area where they detected population differentiation among groups separated by the downtown area and 2 major interstates. To date, no study employing genetic data to examine Coyote genetic population structure has been completed in the southeastern US.

This study addressed the evident need for information regarding the molecular ecology of Coyotes within the southeastern US. Our goal was to use nuclear DNA (i.e., microsatellites) to assess genetic diversity and population structure among Coyotes in east-central Alabama. We hypothesized that we would detect high levels of genetic diversity and low levels of population structure among these Coyotes due to the biological profile of the species (i.e., high mobility and reproductivity, and continuous dispersal). In addition to our main objective, we also investigated population structure between urban and rural groups. Other studies (Atwood et al. 2004; Gehrt et al. 2009, 2011; Randa and Yunger 2006; Riley et al. 2003) have found population differentiation between urban Coyotes and surrounding populations. Therefore, we chose to examine if Coyotes captured within, and in close proximity to, the city of Auburn, AL, experienced reduced gene flow with Coyotes from surrounding rural areas. Overall, our study sought to provide basic information about the molecular ecology of Coyotes within a region of recent population expansion for this species, as well as to provide information that could inform development of management strategies.

Field-site Description

Our study area encompassed a 100-km radius around the Auburn/Opelika Metroplex Statistical Area (MSA) in east-central Alabama. We collected samples from Chambers, Coosa, Lee, Macon, Montgomery, Russell, and Tallapoosa counties. With a population of 130,516 people in 2008 (first year of our study), the Auburn/Opelika

MSA was considered the fastest-growing metropolitan area in Alabama since 1990 (US Census Bureau 2001). The landscape directly adjacent to metropolitan sections is a mixture of agricultural, forested, ranching, and farming lands.

Methods

Sample collection and DNA extraction

With assistance from USDA-Wildlife Services (Auburn, AL) and a host of local volunteers, we opportunistically collected samples (n = 74) from live-captured, targeted/hunter-harvested, and vehicle-killed animals from April 2008 to May 2009. We obtained tissue samples from live-captured individuals during a telemetry study concurrently conducted at Auburn University (Jantz 2011). We sampled tissue from the ear of each live Coyote using a commercial-grade ear-notcher. We collected ample skin or muscle tissue as available from deceased Coyotes. We stored tissue samples 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, CXX140, FH2054, FH2010, FH2159, CX2235, FH2100, FH2062; Breen et al. 2001; Francisco et al. 1996; Ostrander et al. 1993, 1995) using 3 multiplexed polymerase chain reactions (PCRs; Table 1). We ran each reaction with optimized amounts of PCR water, GeneAmp 10X PCR Buffer II (Applied Biosystems, Inc., Foster City, CA), 25 mM $MgCl_2$ (Panel A: 1.0 µl, Panel B: 0.8 µl, Panel C: 0.7 µl; Applied Biosystems, Inc.), 1.0 µl dNTP (Promega, Madison, WI; 10 mM), primers (Table 1; 1 µM), 0.1 µl Amplitaq Gold (Applied Biosystems, Inc.; 5 U/µL), and 0.4 µl 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 amplification products to the Wildlife Genetics Lab at the USDA-WS National Wildlife Research Center in Fort Collins, CO, for visualization on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc.). We binned the visualized data using GeneMapper Software v4.0 (Applied Biosystems, Inc.) 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 analysis software.

Population assignment

We categorized individuals into 3 groups (i.e., urban, rural, and buffer/interface) to investigate the existence of population structure among Coyotes sampled in this

study. We created a point shapefile within ArcGIS (Esri) from coordinates taken at the location where we obtained each Coyote sample. We made the assumption that the location where an individual was sampled corresponded to the habitat/landscape type with which they were most likely to be associated the majority of the time. We 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 US Census Bureau (US Census Bureau 2002). The US Census Bureau classifies any census-block group having a population density of at least 1000 people per square mile with surrounding blocks having at least 500 people per square mile as urban. We defined sites outside of those constraints as rural. We performed zonal statistics using the Spatial Analyst Tools in ArcGIS (Esri) across the total study area 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) 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 4.22-km buffer to the urban polygon, the approximate diameter of a rural Coyote home range calculated for the study area (Jantz 2011). We excluded any Coyote falling within this "buffer/interface" area from the urban/rural comparison analysis in an attempt to eliminate individuals that could not be assigned definitively to either the urban or rural group as defined within this study. We deemed any Coyote sampled at a point that was within the urban polygon to be an urban Coyote. Lastly, we classified all individuals not categorized as urban and not collected within the buffered interface area as rural (Fig. 1). Final sample sizes for each population were: urban (n = 8), buffer/interface (n =16) and rural (n = 50).

Genetic statistical analyses

We completed statistical analyses using the following datasets: (1) total dataset (n = 74; total number of individuals), (2) total urban dataset (n = 8; all assigned urban individuals), (3) total rural dataset (n = 50; all assigned rural individuals), and (4) iterative rural datasets of randomly subsampled individuals (n = 8; from the total rural dataset).

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. We utilized the program FSTAT 2.9.3 (Goudet 2001) to examine the microsatellite loci for linkage disequilibrium. We tested for violations of Hardy-Weinberg equilibrium and assessed genetic diversity and allelic richness in ARLEQUIN 3.1 (Excoffier et al. 2005). We performed sequential Bonferroni tests for Hardy-Weinberg equilibrium estimates across loci to correct for biased significance of data within tables (Rice 1989). We performed all of these tests using the total dataset.

We used BAPS 5.2 (Corander et al. 2008), a Bayesian clustering program, to test for genetic differentiation using the total dataset without a priori population-

membership information using the spatial clustering of individuals algorithm. BAPS works by assigning individuals into population clusters (K) based on genetic structure detected from allelic frequency data and spatial proximity. These tests detect population differentiation among all individuals; we performed 10 iterations for each of K = 1–10. We also used BAPS, incorporating both genotypic and GPS-point data, to assess spatial clustering of groups over 10 iterations of both K = 1 and K = 2 to determine if differentiation between the a priori selected total urban and total rural datasets could be detected.

We employed ARLEQUIN 3.1 (Excoffier et al. 2005) to calculate genetic diversity measures and pairwise F_{ST} (Weir and Cockerham 1984) for the total urban and total rural datasets. However, the unequal sample sizes produced by categorizing individuals as either rural (n = 50) or urban (n = 8) in ArcGIS (Esri) were a concern because pairwise F_{ST} does not perform well with unequal sample sizes (Cockerham 1973). Thus, to calculate pairwise F_{ST} (Weir and Cockerham 1984) using equalized sample sizes, we constructed the iterative rural datasets by randomly sampling, with replacement, 8 individuals from the total rural dataset 100 times. Then, we calculated pairwise F_{ST} between each iterative rural dataset (n = 8) and the total urban dataset (n = 8) using ARLEQUIN 3.1 (Excoffier et al. 2005). We also subsampled

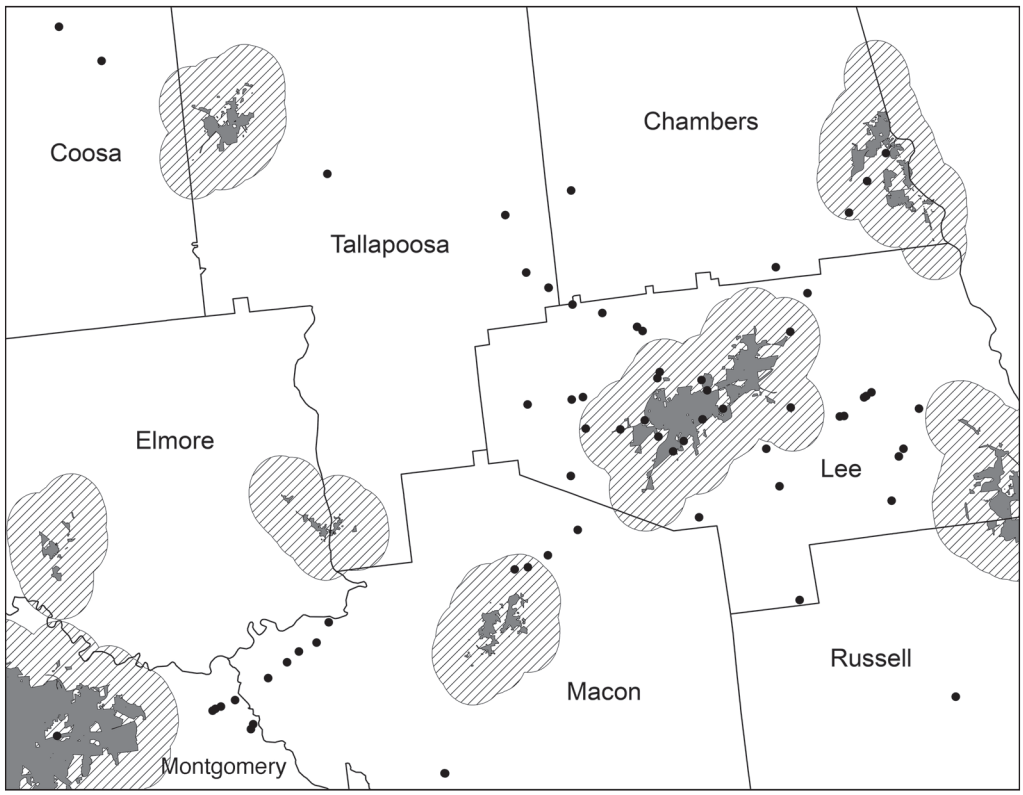

Figure 1. Sampling classifications within the Alabama study site: urban = grey, buffer/interface = simple hatch, and rural = white. The black dots represent the locations of sampled Coyotes.

10 of the 100 randomly selected iterative rural datasets to examine possible sampling bias in measures of heterozygosity.

We used ARLEQUIN 3.1 (Excoffier et al. 2005) to calculate expected (H_E) and observed (H_O) heterozygosity measures for the 10 randomly selected iterative rural datasets to compare to the total urban dataset. We then plotted heterozygosity measures graphically to assess whether the heterozygosity estimates from the total rural dataset were within the same distribution as 10 of the iterative rural datasets. If the estimates fell within the same distribution, we concluded that no sampling bias existed in heterozygosity measures, and thus comparisions of the total urban and total rural datasets was not biased by unequal sample sizes.

Results

We successfully genotyped 74 individuals sampled within the study area. Of the 74 total Coyotes sampled, 30 (40.5%) were either captured or targeted, and 44 (59.5%) were vehicle-killed. We recognize the potential for bias if a large portion of the Coyotes assigned to the urban and rural groups were sampled during dispersal season when Coyotes were more transient and moved through the landscape. However, only 38% (n = 28) of the Coyotes within the total dataset were sampled during a common dispersal time period (i.e., 1 September–31 December) (Holzman et al. 1992). We categorized 17 Coyotes sampled during a dispersal time period as rural and 4 as urban. The remaining 7 Coyotes collected during this time period fell within the buffer/interface area, and were excluded from the urban/rural comparison. Of the 21 Coyotes that we sampled during dispersal season and categorized as either rural or urban, 12 were vehicle-killed and 9 were captured/targeted.

One microsatellite marker, CX2235, showed evidence of null alleles at $P \leq 0.05$, which was a concern because null alleles can be evidence of reduced primer annealing, competition among target alleles of various lengths during amplification, or poor template quality (Dakin and Avise 2004, Wattier et al. 1998). However, we retained CX2235 in the study because the occurrence of null alleles at this locus was <0.20, which is considered uncommon or rare (Dakin and Avise 2004). All 10 loci were used in the analyses, with no loci having more than 5% missing data. We did not detect linkage disequilibrium across loci over all samples. All loci were polymorphic with 1 locus, CXX140, violating Hardy-Weinberg equilibrium (Table 1). This locus had a significantly lower H_O (0.82) than H_E (0.83) after sequential Bonferroni corrections (P = 0.001), which suggested significant homozygosity. This result might indicate the presence of allelic dropout, null alleles, linkage of alleles, or inbreeding. However, we detected neither null alleles nor allelic dropout. 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, we would have expected to observe such a phenomenon at many or all loci and not just at a single locus (Selkoe and Toonen 2006). It is important to note the difference between the observed and expected values of heterozygosity is quite low (0.01). Three of the 9 total alleles detected at CXX140 represent 68.92% of the total allelic frequency for the locus, thus demonstrating a

deficiency of heterozygotes in the population at this locus. We retained the locus in the study because it was variable and did not seem to suffer from linkage disequilibrium or null alleles. Expected heterozygosity among all samples was 0.78, and allelic richness ranged from 5 to 22 alleles per locus (Table 1), indicating that genetic diversity was high.

Without an a priori population designation, BAPS 5.2 (Corander et al. 2008) detected a single genetic cluster among all individuals (K = 1), which suggested no differentiation among individuals within 100 km of the Auburn/Opelika MSA. We further assessed genetic differentiation using datasets (i.e., total urban, total rural, and iterative rural) comprised of rural and urban populations assigned a priori. The clustering program BAPS 5.2 (Corander et al. 2008) detected no genetic differentiation between the total urban and total rural datasets. An estimate of pairwise F_{ST} between the total urban and total rural datasets showed significant genetic differentiation (F_{ST} = 0.03; P = 0.01). Additionally, estimates of pairwise F_{ST} between the total urban and iterative rural datasets indicated genetic differentiation between the groups. Sixty-five percent of the 100 iterative pairwise F_{ST} comparisons were significant at $P \leq 0.05$ (Table 2). All significant F_{ST} estimates were low (0.01–0.06; $P < 0.05$).

Heterozygosity measures for the total rural dataset fell within the distribution of the values generated from the 10 randomly selected iterative rural datasets; thus, we concluded that there was no sampling bias in heterozygosity measures. Thus, we report the gene diversity estimates for both the total rural (0.78) and the total urban (0.71) datasets (Fig. 2).

Discussion

The main goal of this study was to evaluate population structure and genetic diversity of Coyotes sampled within a portion of east-central Alabama. Our effort

Table 1. Per-locus information: microsatellite panel/primers information and genetic diversity indices (allelic richness, heterozygosity) for total sample of Coyotes (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	10	0.76	0.70
A	FH2096	Hex	Tetra	89–109	5	0.60	0.57
A	FH2137	Ned	Tetra	158–194	14	0.89	0.88
A	CXX140	Hex	Di	130–154	9	0.83	0.82
B	FH2054	Ned	Tetra	135–175	9	0.76	0.85
B	FH2010	Hex	Tetra	221–237	5	0.74	0.66
B	FH2159	Fam	Tetra	155–206	22	0.94	0.91
C	CX2235	Fam	Tetra	136–176	8	0.81	0.72
C	FH2100	Hex	Tetra	142–176	5	0.72	0.72
C	FH2062	Ned	Tetra	129–145	6	0.76	0.73
				Mean	9.3	0.78	0.76

Table 2: Pairwise F_{ST} values with *P*-values calculated using a subsample ($n = 8$) of the rural Coyote population against the total urban population ($n = 8$) near Auburn, AL. * denotes $P \leq 0.05$.

Iteration	F_{ST}	*P*-value	Iteration	F_{ST}	*P*-value
1*	0.03	0.01	51	0.02	0.06
2*	0.03	0.01	52*	0.03	0.02
3*	0.06	0.00	53*	0.04	0.01
4	0.02	0.09	54*	0.03	0.02
5	0.01	0.17	55*	0.04	0.01
6*	0.04	0.01	56*	0.04	<0.001
7*	0.03	0.03	57*	0.05	<0.001
8*	0.04	0.01	58	0.02	0.06
9*	0.05	0.01	59*	0.03	0.04
10*	0.03	0.02	60	0.01	0.14
11*	0.03	0.02	61	0.02	0.07
12*	0.02	0.04	62*	0.02	0.04
13	0.02	0.08	63*	0.04	<0.001
14*	0.03	0.02	64*	0.03	0.02
15*	0.05	0.01	65	0.01	0.18
16	0.02	0.06	66*	0.04	0.01
17*	0.03	0.03	67*	0.03	0.03
18	0.03	0.07	68*	0.04	0.02
19*	0.06	<0.001	69*	0.05	<0.001
20	0.02	0.10	70*	0.04	0.01
21	0.02	0.09	71*	0.04	0.02
22*	0.04	<0.001	72*	0.04	0.01
23*	0.04	0.01	73*	0.03	0.03
24*	0.03	0.03	74*	0.03	0.03
25	0.02	0.10	75*	0.03	0.03
26*	0.04	0.02	76	0.02	0.08
27*	0.05	0.01	77	0.02	0.11
28*	0.04	0.03	78*	0.05	<0.001
29	0.02	0.06	79*	0.05	0.01
30	0.02	0.07	80*	0.03	0.04
31*	0.03	0.04	81*	0.03	0.02
32*	0.03	0.04	82*	0.04	0.01
33	0.02	0.09	83*	0.03	0.03
34	0.02	0.06	84	0.03	0.06
35	0.02	0.07	85*	0.03	0.02
36	0.02	0.08	86*	0.03	0.02
37*	0.03	0.02	87	0.02	0.08
38	0.02	0.06	88	0.02	0.07
39*	0.04	0.01	89	0.02	0.08
40*	0.05	<0.001	90*	0.03	0.04
41	0.03	0.06	91	0.01	0.09
42*	0.04	0.01	92*	0.03	0.04
43*	0.05	0.00	93	0.02	0.05
44*	0.03	0.04	94	0.01	0.13
45*	0.03	0.02	95*	0.03	0.04
46*	0.03	0.01	96*	0.03	0.05
47	0.02	0.07	97*	0.05	0.01
48	0.02	0.07	98*	0.03	0.04
49*	0.04	0.01	99	0.02	0.08
50	0.02	0.08	100	0.02	0.06

provided baseline genetic information about Coyotes within an area of recent range expansion for this species (Parker 1995). Coyotes across the study area (i.e., total dataset) showed high levels of genetic diversity and no significant population structure. This finding is consistent with other studies completed across the US and Canada using autosomal microsatellite DNA, which have shown that Coyotes maintain a high level of genetic diversity and gene flow across their range (Riley et al. 2006; Roy et al. 1994; Sacks et al. 2004, 2008).

We observed weak genetic differentiation (Balloux and Lugon-Moulin 2002, Wright 1978) between the urban and rural groups. Low, but significant, F_{ST} estimates resulted from pairwise comparisons of the total urban and total rural datasets, as well as among a majority of the total urban and iterative rural dataset comparisons. However, we urge caution in considering this evidence of genetic differentiation as being biologically meaningful because the observed F_{ST} values were very low. These results conflict with the BAPS results and the biology of Coyotes, which together suggest a high level of genetic diversity resulting in little or no population structure. Comparisons between the total rural and total urban datasets were extremely skewed with 50 rural individuals compared to 8 urban individuals in the dataset. Further, 35% of the pairwise F_{ST} estimates comparing groups within the total urban and iterative rural datasets were not significant. We acknowledge that the exclusion of the 16 buffer/interface individuals from the urban/rural comparison analysis might have removed some allelic diversity that could have demonstrated a connection between the rural and urban groups tested. This potential elimination of information may have strengthened the results suggesting significant population structure. However, removing the Coyotes that fell within the buffer/interface area was necessary to account for the individuals that could not clearly be assigned to

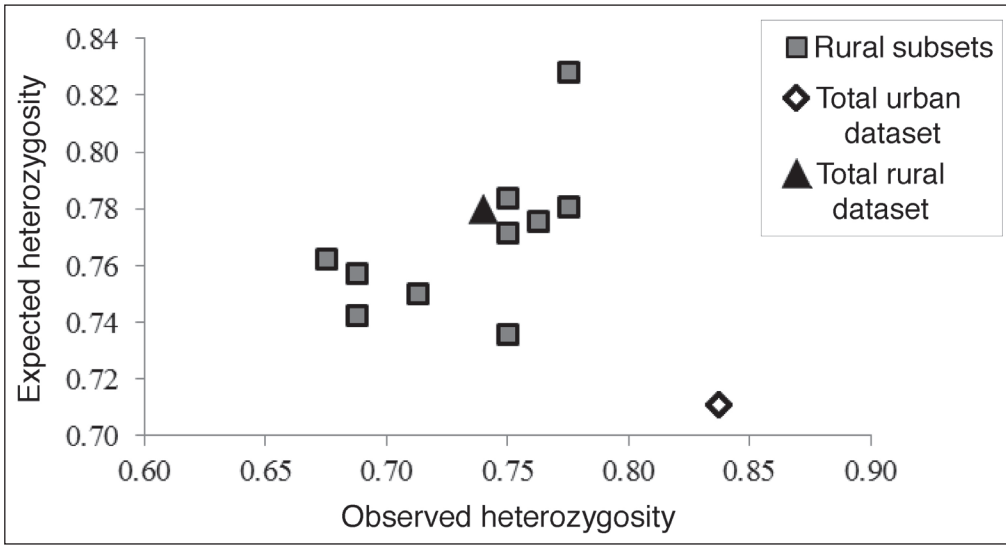

Figure 2. Scatter plot showing results of iterative testing of subsamples (grey squares) from the rural Coyote population compared to estimates for the total rural dataset (n = 50; black triangle) and total urban dataset (open diamond).

either the urban or rural group. It is also possible that our reduction in sample size through subsampling the rural group masked shared allelic diversity between the total urban and iterative rural groups and thus led to low but significant F_{ST} estimates. It is also true that the high rates of mutation that occur in microsatellites can lead to an underestimation of F_{ST} estimates (Balloux and Lugon-Moulin 2002, Hedrick 1999). In a study of Coyote populations in southern California, population differentiation was detected between populations on either side of a major freeway. The F_{ST} estimates (0.03–0.04) that characterized this differentiation were significant and low (Riley et al. 2006), similar to those observed in this study. Further, it has been demonstrated that in some systems, such as fish populations, low but significant F_{ST} reflects biologically meaningful population structure (Knutsen et al. 2011). Therefore, consideration of the relevance of potential population differentiation between urban Auburn, AL, Coyotes and those from surrounding rural areas is warranted.

We acknowledge the possibility for bias within the urban/rural comparison analysis introduced by including potentially dispersing Coyotes in the urban and rural populations tested. It could be suggested that vehicle-killed Coyotes might include a large proportion of dispersing animals because they are less familiar with their surroundings during dispersal activities (Bekoff and Gese 2003). We did not take into account whether an individual was sampled as a vehicle-killed or captured/targeted individual when delineating urban and rural Coyotes. However, there was not an overwhelming majority of vehicle-killed Coyotes included within this study, nor was there a majority of the Coyotes sampled during a common dispersal time period.

Of the 8 Coyotes making up the total urban dataset investigated within this study, 6 individuals were sampled in close proximity to the Auburn University Regional Airport (formerly known as the Auburn-Opelika Robert G. Pitts Airport), suggesting that Coyotes may be concentrated within the area. Coyotes have been identified as the most common carnivore species threat to aircraft, and in most cases, the mammalian species second only to deer (predominately White-tailed Deer) (Cleary and Dolbeer 2005, Cleary et al. 2006, Dolbeer and Wright 2009, Dolbeer et al. 2000). Coyotes specifically are drawn to airports because the facilities have ample water sources and large, open grassland areas that are advantageous for hunting prey species (Cleary and Dolbeer 2005, Dolbeer and Wright, 2009, Dolbeer et al. 1993). It may also be that these Coyotes were isolated in the area due to anthropogenic barriers such as highways, and the increased commercial development within 2 miles of the airport that has drastically altered the landscape over the last several years. The 2 major highways that intersect in the Auburn/Opelika MSA, Interstate 85 and Alabama State Highway 280, converge approximately 2.5 km from the airport. Alabama State Highway 280 runs to the east and north of the Auburn University Regional Airport, while Interstate 85 more closely borders the airport to the south. Riley et al. (2006) found that freeways in California served as barriers to gene flow between Coyote populations. We speculate that reproductive opportunities between this subset of urban Coyotes and rural individuals could be limited, thus leading to reduced gene flow and increased levels of genetic differentiation. If this were true, it is possible that

a group of Coyotes, like those observed near the Auburn University Regional Airport, could be considered a viable management unit.

Coyotes exhibit high capacity for dispersal and continuous distribution across their range, are transient in nature, and are habitat generalists (Bekoff and Gese 2003), thus we expected to observe high levels of population diversity and low population structure. Indeed, when examining population diversity across the entire study area we detected high levels of genetic diversity and no population structure. These findings suggest that Coyotes within east-central Alabama move large distances and interact in a way that does not promote population subdivision. We conclude that defining distinct groups, or management units, for Coyotes based solely on genetic data may not be viable at the broader spatial scale used within this study (i.e., 100-km radius of the Auburn/Opelika MSA). Therefore, management strategies should be applied uniformly to all Coyotes being managed within the area investigated in this study. When examining genetic differentiation at a finer scale (i.e., within the Auburn/Opelika MSA) while incorporating landscape and human population data, we may have detected weak population structure between urban and rural Coyotes. To better understand population structure of Coyotes in urban areas of Alabama and across the southeastern US, further research efforts are needed. In fact, the results of this study may differ from what might be observed within other metropolitan areas of Alabama. A larger sample of urban Coyotes within the Auburn/Opelika MSA, as well as a comparison between other small urban areas and larger metropolitan areas within Alabama, and throughout the southeastern US, would likely provide better information to wildlife biologists for use in generating effective management strategies for the species. This study provides baseline information regarding population genetics of Coyotes in east-central Alabama and the utility of using genetic techniques to assist in the delineation of management units, a practice that is becoming more important as we observe an increase in human-Coyote conflicts and thus the need to manage Coyote populations across the southeastern US.

Acknowledgments

We thank The Center for Forest Sustainability and Auburn University for funding this study. We thank the USDA-National Wildlife Research Center's Genetics Lab and the Kenny Brock Lab at the Auburn University School of Veterinary Medicine for support with data collection and analyses. Also, thanks to all of the individuals and agencies that collected carcasses and tissue samples for this project, including Alabama USDA-Wildlife Services. Thank you to Dr. Todd Steury for helping tremendously with statistical analyses, Amy Silvano for assistance with ArcGIS, and Karen Tenaglia-Hoksbergen, Dr. Scott Santos, and Philip Damm for frequent help and guidance. Lastly, we appreciate the comments provided by reviewers of this manuscript during consideration of publication.

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