High Genetic Diversity and Low Differentiation in Colonized Coyote Populations Across South Carolina

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Abstract - *Canis latrans* (Coyote) is a highly mobile, generalist species that occupies a wide variety of habitats and ecoregions across North and Central America. Assessments of population genetic diversity and structure among the recently colonized populations of Coyotes in the eastern US have been conducted at broad spatial scales, revealing genetic structure concordant with immigration routes and high genetic diversity, or at local spatial scales, usually finding genetic panmixia. However, few studies have assessed eastern Coyote genetics at an intermediate spatial scale (i.e., state-level), which may be commensurate with the population dynamics of this mobile animal. We sampled 10 study areas across South Carolina during summer 2019–2020 using noninvasive genetic sampling and 10 microsatellite loci to identify individuals. We assessed genetic diversity and pairwise relatedness as well as genetic structure across our populations to determine whether there were landscape-level differences in genetic differentiation and the scale of Coyote population structure across the state. We found high levels of observed heterozygosity (0.599–0.872) and allelic richness (3.99–5.12) across our sites but low levels of relatedness. We observed low genetic differentiation (pairwise F_{ST} = 0.010–0.066) and insignificant isolation by distance with limited evidence of genetic structure. Coyote populations seem to be operating at a spatial extent greater than South Carolina, and gene flow is likely maintained by unrelated, transient individuals. Future research and management of Coyotes in the heterogeneous landscapes of the southeastern US should account for the geographic scale that populations encompass.

Introduction

Population-level genetic structure can be maintained by extrinsic barriers to movement such as geographic delineations and habitat clines, or intrinsic processes such as morphological or behavioral limits to home-range size and dispersal (Mills and Allendorf 1996, Wright 1943). Generally, at least 1 immigrant per generation is required to maintain genetic similarity between populations and frequent genetic exchange can result in panmixia (Mills and Allendorf 1996). For instance, low genetic structure and panmixia as a result of high dispersal have been documented in *Odobenus rosmarus divergens* (Illiger) (Pacific Walrus; Beatty et al. 2020), *Eptesicus fuscus* (Palisot de Beauvois) (Big Brown Bat; Richardson et al. 2021), and *Canis latrans* (Say) (Coyote; Heppenheimer et al. 2018a, Kierepka et al. 2017). Understanding the extent and scale of gene flow among populations is necessary

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for defining some aspects of wildlife management and understanding demographic processes such as population dynamics and dispersal.

Coyotes offer an opportunity to investigate population connectivity through genetics due to their behavioral plasticity, rapidly expanding range, and ability to travel long distances (Hinton et al. 2012, Hody and Kays 2018). Past research in the species' endemic range of the western US has shown natal-biased dispersal by Coyotes that encourages genetic structure along habitat-specific interfaces (Sacks et al. 2004, 2005). Recent studies have documented genetic structure within densely populated urban settings, likely due to anthropogenic barriers to movement, genetic bottlenecking, and genetic drift (Adducci et al. 2020, Bird et al. 2023, Damm et al. 2015, DeCandia et al. 2019, Henger et al. 2020, Rashleigh et al. 2008, Riley et al. 2006). However, Coyotes have dramatically increased their historical range by colonizing the eastern US over the last century (Hody and Kays 2018), and studies have documented dispersal movements >300 km (Hinton et al. 2012). As such, recent investigations of genetic diversity in colonizing populations of Coyotes have shown weak signals of substructure in the eastern US at broad spatial scales. Population genetic structure generally adheres to historic colonization routes at large spatial scales east of the Mississippi River (Heppenheimer et al. 2018a, b; Hinton et al. 2019), while genetic panmixia is evident across localized spatial scales (Bohling et al. 2017, Damm et al. 2015, Heppenheimer et al. 2018a, Kierepka et al. 2017).

Furthermore, Coyotes exhibit a dual life-history strategy of residency and transiency, which may also impact local population genetic structure. Resident individuals hold relatively stable territories, while transient individuals disperse in search of available space and resources (Hinton et al. 2015, Morin and Kelly 2017) thereby potentially increasing gene flow. Efforts to lethally control local populations of Coyotes have resulted in mixed success, with evidence of rapid back-fill of habitat by transient individuals from outside the area of management (Gulsby et al. 2015, Kilgo et al. 2014). Williams et al. (2003) and Kierepka et al. (2017) observed little change in genetic structure after Coyote-removal efforts, likely due to compensatory reproduction and immigration from genetically similar populations. High rates of gene flow, therefore, should play an important role in maintaining genetic diversity while minimizing structure in Coyote populations of the eastern US. However, most investigation of population genetics in eastern Coyotes has occurred at either spatial scales greater than typical Coyote movements (Heppenheimer et al. 2018a, b; Hinton et al. 2019; Monzón 2014; vonHoldt et al. 2016a, b) or locally constrained at scales well within the dispersal capabilities of transient individuals (Damm et al. 2015, Kierepka et al. 2017). Two studies, one in West Virginia and Virginia (Bohling et al. 2017) and another in New York (Berkman et al. 2019), studied Coyotes at a state-wide scale and found evidence of panmixia and high gene flow due to dispersal. However, genetic substructure was observed across permeable habitat clines at an intermediate spatial scale in California (Sacks et al. 2004). Assessing genetic structure across several ecoregions, such as within a state, may provide insight into the extent that Coyote population dynamics operate across the heavily forested landscape of the southeastern US.

Our objective was to assess Coyote population genetics across the state of South Carolina. We predicted that genetic diversity would be high due to gene flow and rapid population expansion (Heppenheimer et al. 2018b). We also assessed relatedness within Coyote populations across South Carolina to determine kinship dynamics within each of our study sites due to the potential influence of transiency on local genetic structure. We predicted that kinship would be highly variable due to the presence of resident and transient individuals. Finally, we sampled populations throughout 3 major ecoregions to determine if there were landscape-level differences in genetic structure similar to those found in other portions of the Coyote range (Sacks et al. 2004, 2005). We predicted that population structure would be low (Heppenheimer et al. 2018a) as a result of Coyotes' generalist behavior, high dispersal capabilities, and permeability of regional habitat clines. Our research bridges the gap between previous population-structure studies of eastern Coyotes by assessing genetic characteristics across an intermediate spatial scale and informs our understanding of Coyote populations and their management in the southeastern US.

Study Area

We assessed Coyote population genetic structure across 10 study sites in South Carolina (Fig. 1). Study sites were a mixture of private and public lands located in

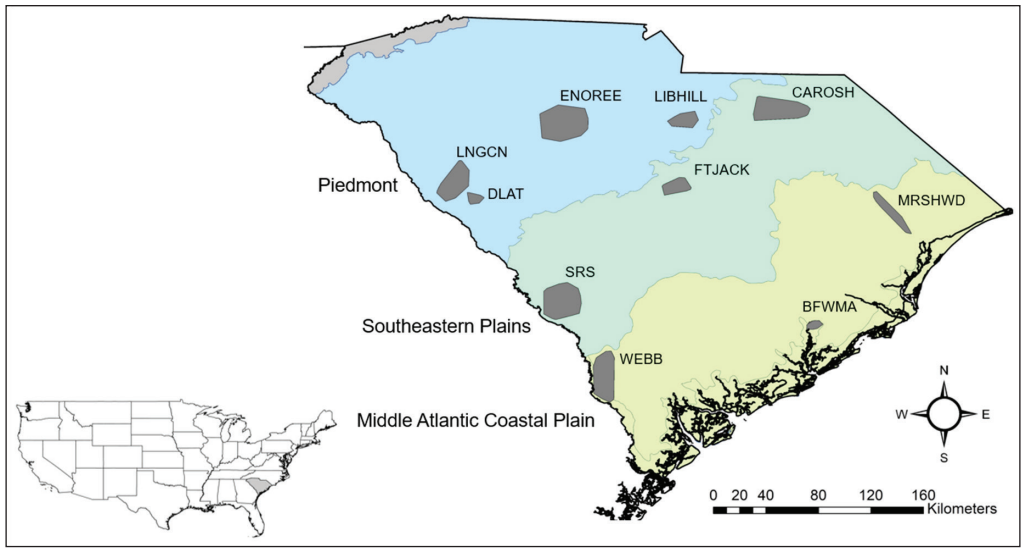

Figure 1. Study sites across South Carolina used to assess *Canis latrans* (Coyote) population genetics across 3 major ecoregions during July–August 2019–2020. LNGCN = Long Cane Ranger District Sumter National Forest, DLAT = Davis Land and Timber, ENOREE = Enoree Ranger District Sumter National Forest, LIBHILL = Liberty Hill Wildlife Management Area and private lands, SRS = Savannah River Site, FTJACK = Fort Jackson, CAROSH = Carolina Sandhills National Wildlife Refuge and State Forest, WEBB = Webb Complex, BFWMA = Bonneau Ferry Wildlife Management Area, and MRSHWD = Marsh and Woodbury Wildlife Management Areas.

the Piedmont, Southeastern Plains, and Middle Atlantic Coastal Plains ecoregions across South Carolina. The Piedmont was historically dominated by *Quercus–Carya–Pinus* (Oak–Hickory–Pine) forests, while the Southeastern Plains and Middle Atlantic Coastal Plains were mostly covered by *Pinus palustris* Mill. (Longleaf Pine) forest prior to timbering and cultivation (Griffith et al. 2002). While each ecoregion currently exhibits a mosaic of cultivated crops and pasture, much of the forests have been converted to planted or regenerated pine stands (Griffith et al. 2002). Although definitive ecoregion distinctions are difficult, and production pine is a primary landcover feature, the Piedmont is generally typified by forest cover of mixed pine and hardwood species, the Southeastern Plains exhibit sandy soils and increased agriculture, and the Middle Atlantic Coastal Plains contain lowlands of estuaries, marshes, and swamps. Mean annual temperatures are ~15–16 °C with a mean annual precipitation of 1229–1358 mm (Wiken et al. 2011). Our study sites within the Piedmont included the Long Cane and Enoree Ranger Districts of the Sumter National Forest (NF), and the Davis Land and Timber property. In 2020, we also sampled the Liberty Hill Wildlife Management Area (WMA) and surrounding private lands. Our study sites within the Southeastern Plains included the Savannah River Site (a US Department of Energy National Environmental Research Park), Fort Jackson, and the Carolina Sandhills National Wildlife Refuge (NWR) and Sandhills State Forest (SF) complex. Our study sites in the Middle Atlantic Coastal Plain included the complex of private and state-owned public lands around the Webb Center and WMA, the Marsh and Woodbury WMA complex, and the Bonneau Ferry WMA.

Methods

Sampling methodology

We sampled transects for Coyote scat at each site across a 2-week period during July and August of 2019 and 2020 except for at Bonneau Ferry WMA where tissue samples were taken from trapped and euthanized Coyotes. We conducted an initial sweep of each transect at the beginning of the sampling period to remove all accumulated scat present from before the start of the sampling session. We then sampled transects every 3 days for a total of 4 sampling occasions in 2019 and 5 sampling occasions in 2020. We drove transects at ~10 km/hr, using the edges of the road as the boundary of our transect sampling area. We collected scat samples using either a wooden popsicle stick, which was discarded after each sampling, or forceps that were sanitized with alcohol wipes and a butane lighter. We collected 0.4 mL of the outer portion of scat and placed it into a 2-mL tube containing 1.6 mL of DETs (DMSO/EDTA/Tris/salt) buffer (Frantzen et al. 1998, Stenglein et al. 2010a). The remainder of the scat was sealed in a Ziploc freezer bag and stored on ice in the truck before being transferred to a freezer for storage at -20 °C. Whole scat was collected as a backup in case of DNA extraction failure with the DETs-preserved samples. We recorded the GPS coordinates of each sample along with the general appearance of the scat and any pertinent information concerning the location and condition of the sample.

Laboratory methodology

We extracted DNA from each sample using the Qiagen Mini Stool Kit (Qiagen, Valencia, CA), following the manufacturer's protocol with the exception that we filtered the eluted product a second time through the final filtration step to maximize the concentration of DNA. Samples were amplified using a 10-primer microsatellite multiplex described in Stenglein et al. (2010b) with the addition of 2 sex primers (Seddon 2005). We followed the run specifications laid out in Stenglein et al. (2010b) and Morin et al. (2016) with the exception that we reduced the number of PCR cycles to 30 repetitions because we found that our samples were amplifying too strongly to be easily scored (J.L. Youngmann, unpubl. data). The Stenglein et al. (2010b) lab protocols were developed using scat samples exhibiting a range of degradation and, therefore, required a high number of cycles to produce satisfactory amplification. Our sampling methodology ensured that samples were no more than 3–4 days old and, therefore, contained high levels of quality DNA. We included a negative control on each 96-well PCR plate to detect cross-contamination.

We used the multi-tube approach to run 4 separate replicate PCRs on each sample (Taberlet 1996). PCR products were prepared with formamide and LIZ500 size standard and analyzed with 3730xl capillary machines (Applied Biosystems, Inc., Foster City, CA) at either the University of Georgia Genomics and Bioinformatics Core or the Cornell University Institute of Biotechnology Genomics Core. We observed no difference in PCR replicates between the 2 laboratories. We scored each sample using Geneious 2022.2.2 (Boston, MA; online at https://www.geneious.com/) and confirmed consensus genotypes using 'ConGenR' in Program R v.4.2.2 (Lonsinger and Waits 2015; R Foundation, Austria, Vienna). Heterozygous loci were required to be observed in 2 separate replicates, while homozygous loci were required to be observed in 3 separate replicates (Taberlet 1996). We determined matching genotypes using a threshold of 7 loci matches to address the probability of identity based on likelihood of siblings. Finally, we used 'ConGenR' to identify recaptures across all sites and years.

We additionally screened all samples with a 6-primer, species-specific mitochondrial DNA (mtDNA) control-region multiplex to identify the species of each scat (De Barba et al. 2014). We used the PCR specifications outlined in De Barba et al. (2014) and visualized results using Geneious 2022.2.2. Several *Urocyon cinereoargenteus* (Schreber) (Gray Fox) samples amplified at 7 loci or more, but we removed these samples.

Genetic analysis

We used 8 known Coyotes trapped from the Bonneau Ferry WMA and 25 known *Canis lupus familiaris* L. (Domestic Dog) to run an additional genetic assignment test on all canid samples using Structure 2.3.4 (Falush et al. 2003, Pritchard et al. 2000). We assumed 2 populations and ran 10 iterations with a burn-in of 50,000 repetitions followed by 150,000 repetitions. We then removed all individuals identified as Domestic Dog through Structure 2.3.4.

Genetic diversity. We assessed allelic richness (A_R), which standardizes estimates of allelic diversity by sample size, using the package 'hierfstat' (Goudet and Jombart 2022) implemented in program R. We estimated observed (H_O) and expected (H_E) heterozygosity as well as linkage disequilibrium for each study site in Arlequin v.3.5.2.2 (Excoffier and Lischer 2010) using 10,000 permutations to measure linkage disequilibrium and tested significance using Bonferroni's correction for multiple comparisons ($P \leq 0.001$). We used Genepop (version 4.7.5; Rousset 2008) to estimate inbreeding coefficients (F_{IS}) for each study site. Finally, to estimate relatedness within study sites, we used EIMIDB9 (version 1.0.0.0; Wang 2022). EIMIDB9 uses a joint-likelihood estimator to reduce biases associated with small sample sizes and high numbers of closely related individuals (Wang 2022).

Genetic structure. We calculated linearized pairwise F_{ST} (Reynolds et al. 1983) among study sites using Arlequin (version 3.5.2.2; Excoffier and Lischer 2010) and estimated significance over 10,000 permutations using Bonferroni's correction for multiple comparisons ($P \leq 0.001$). We also used Arlequin to assess genetic differentiation among and within the 3 major ecoregions using an analysis of molecular variance (AMOVA) and 10,000 permutations. We used the 'near' function in ArcGIS to produce a pairwise geographic distance matrix and then used Genepop v. 4.7.5 to perform a Mantel test estimating isolation by distance (IBD) between F_{ST} and geographic distance using 10,000 permutations to test for significance.

We used Sturcture 2.3.4 to assess genetic structure through a Bayesian assignment methodology. We modified the default settings to account for uneven sampling among our study sites by employing an initial admixture coefficient of 0.1 (1/10 populations) and uncorrelated allele frequencies (Wang 2017). We then ran 10 iterations with a burn-in of 50,000 followed by 150,000 repetitions. We assessed the most likely number of groups (K) using Evanno's DK (Evanno et al. 2005) implemented in Structure Harvester (Earl and vonHoldt 2012). However, in cases of negligible population structure, DK is unable to find a best fit for K = 1, or panmixia, because it relies on the rate of change in the likelihood distribution. Therefore, we also used the mean estimate of likelihood for each K value to assess a best fit at K = 1. We visualized Structure 2.3.4 results in Clumpak (Kopelman et al. 2015).

Finally, we conducted a discriminant analysis of principal components (DAPC; Jombart et al. 2010) using the package 'adegenet' (Jombart 2008). While Structure 2.3.4 and estimates of genetic differentiation use assumptions about ancestry, admixture, and population genetic theory such as Hardy–Weinberg equilibrium and linkage disequilibrium, DAPC does not make assumptions based on population genetic principles, serving as a complement to traditional analyses of genetic structure. We used the 'find.cluster' function to initially identify the number of principal components (PCs) to retain and conducted a DAPC using our sample sites as a prior because simulation studies have shown de novo grouping of individuals in populations with high migration rates to be inaccurate (Miller et al. 2020). For the initial DAPC run, we retained 100 PCs and 10 DAs. However, care must be taken to not overfit the data by retaining too many PCs (Jombart and Collins 2022). Therefore,

we used the function 'optim.a.score' to verify the optimal number of retained PCs for discriminating groups without artificially inflating among-group differences or overfitting the data. We then ran a second DAPC using the optimal number of retained PCs and reported those results.

Results

We collected and extracted a total of 539 scat samples and 8 tissue samples. From the scat samples, we identified 382 as Coyote or Domestic Dog, 30 samples as *Lynx rufus* (Schreber) (Bobcat), 40 samples as Gray Fox, and 2 samples as *Vulpes vulpes* (L.) (Red Fox) using mtDNA. We were unable to distinguish between multiple species for 2 samples and unable to confirm species identity for 83 samples, for a total species identification success rate of 84.60%. We documented average dropout rates of 4.10% and average false allele rates of 0.93%, with consensus genotypes obtained for 313 Coyote or Domestic Dog samples (81.9% individual identification success rate). Using genetic assignment, we then removed 2 individuals that clustered with known Domestic Dogs. After accounting for recaptures, we used 204 Coyote individuals across our 10 study sites, with sample sizes varying from 4 to 69 Coyotes per site (Table 1). No individuals were sampled in more than 1 sample site.

Coyote populations exhibited high genetic diversity with allelic richness varying from 3.99 (Bonneau Ferry WMA) to 5.12 (Carolina Sandhills) and observed heterozygosity varying from 0.599 (Bonneau Ferry WMA) to 0.872 (Liberty Hill; Table 1). We observed F_{IS} from -0.065 (Liberty Hill) to 0.252 (Enoree NF) and significant linkage disequilibrium at 0–8 pairwise loci (Davis Land and Timber). Estimates of kinship showed right-tailed relatedness distributions for all of our sites (Fig. 2). Pairwise relationships were predominantly skewed towards 0, meaning that Coyotes within sites were largely unrelated to each other.

Table 1. Summary statistics for genetic diversity of *Canis latrans* (Coyote) across 10 study sites in South Carolina during July–August 2019–2020 including allelic richness (A_R) rarefied at 8 alleles, observed (H_O) and expected (H_E) heterozygosity, number of pairwise loci in linkage disequilibrium (LD), and inbreeding coefficient (F_{IS}). n = number of Coyotes.

Study site	n	A_R (8 alleles)	H_O	H_E	LD*	F_{IS}
Carolina Sandhills	25	5.12	0.789	0.856	0	0.079
Enoree NF	7	5.06	0.634	0.845	0	0.252
Fort Jackson	18	4.90	0.831	0.836	1	0.001
Liberty Hill	9	4.95	0.872	0.825	0	-0.065
Long Cane NF	18	4.85	0.765	0.834	4	0.082
Marsh Woodbury	4	5.00	0.850	0.836	0	-0.020
Savannah River Site	69	4.93	0.724	0.824	7	0.116
Stephen Davis	32	5.03	0.764	0.843	8	0.093
Webb Groton Westervelt	14	4.81	0.707	0.812	1	0.127
Bonneau Ferry WMA	8	3.99	0.599	0.743	3	0.194

*Significant with a Bonferroni correction of $P \leq 0.001$ for multiple comparisons

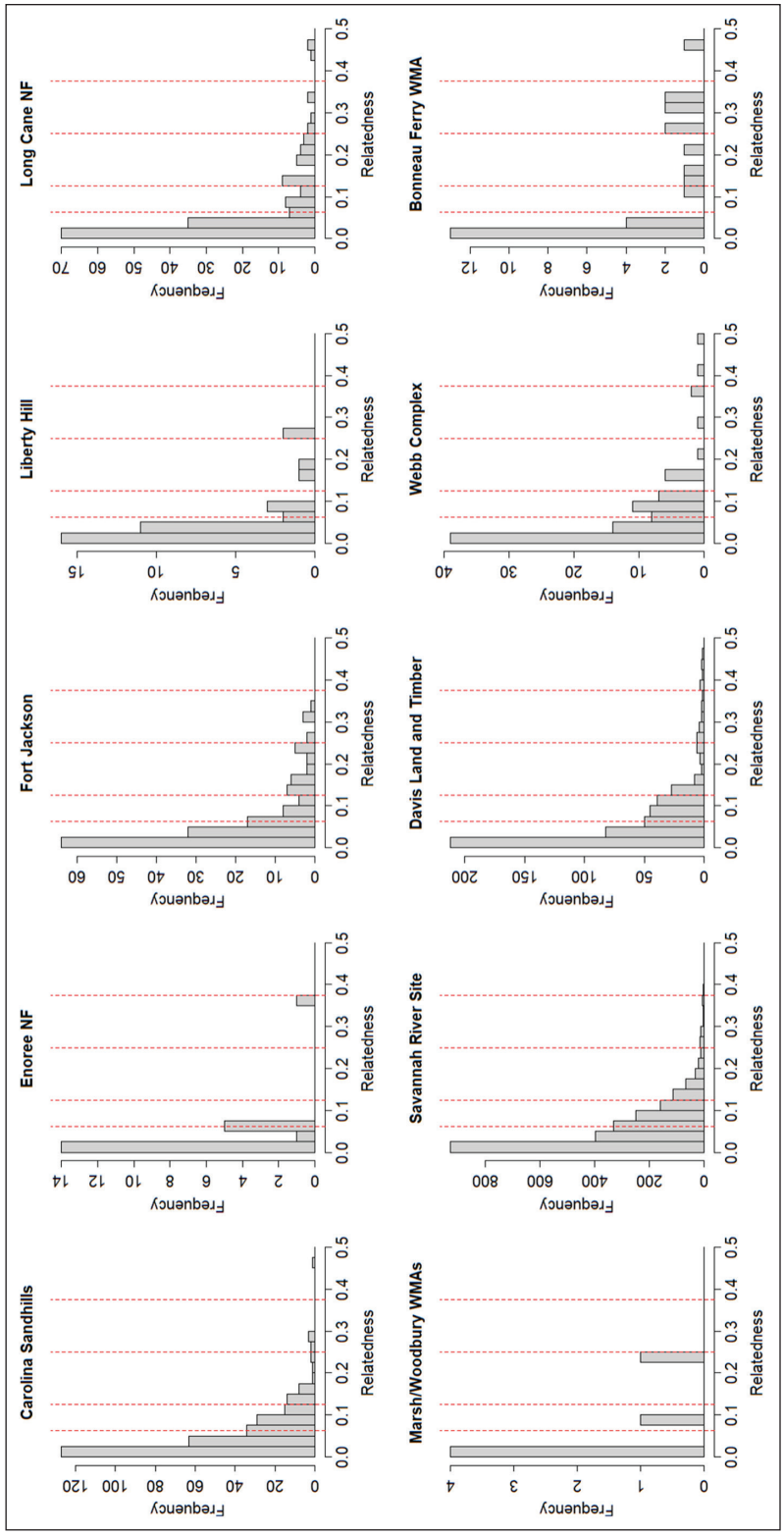

Figure 2. Pairwise estimates of relatedness among *Canis latrans* (Coyote) at 10 study sites across South Carolina during July–August 2019–2020. Vertical dotted lines indicate threshold values for unrelated individuals ($r = 0$), full cousins ($r = 0.0625$), half siblings ($r = 0.125$), full siblings or parent/offspring ($r = 0.25$), and full siblings whose parents are also full siblings ($r = 0.375$).

Genetic structure

We observed low population structure among ecoregions and among our study sites. F_{ST} varied from 0.010 (Liberty Hill to the Carolina Sandhills) to 0.066 (Bonneau Ferry WMA to Long Cane NF) (Table 2 [see following page]). Estimates of molecular variance revealed that 97.30% of the genetic variation was found within individual study sites, whereas differences between study sites within ecoregion accounted for only 3.08% of the variation (Table 3). Variation among ecoregions was -0.38%, meaning that there was more similarity among ecoregions than differences among study sites or individuals. Similarly, IBD revealed positive but insignificant genetic differentiation as a function of geographic distance ($P = 0.167$; Fig. 3). Bayesian clustering analysis revealed a best fit of K = 8 using Evanno's DK followed by K = 2 (Fig. 4). However, estimates of mean likelihood probability

Table 3. Analysis of molecular variance (AMOVA) comparing genetic variation of *Canis latrans* (Coyote) from 10 study sites across 3 ecoregions in South Carolina during July–August 2019–2020.

Source of variation	df	Sum of squares	Variance of components	Percentage of variation
Among ecoregions	2	16.07	-0.02	-0.38
Among study sites within ecoregions	7	57.57	0.12	3.08
Within study sites	398	1568.41	3.94	97.30
Total	407	1642.05	4.05	

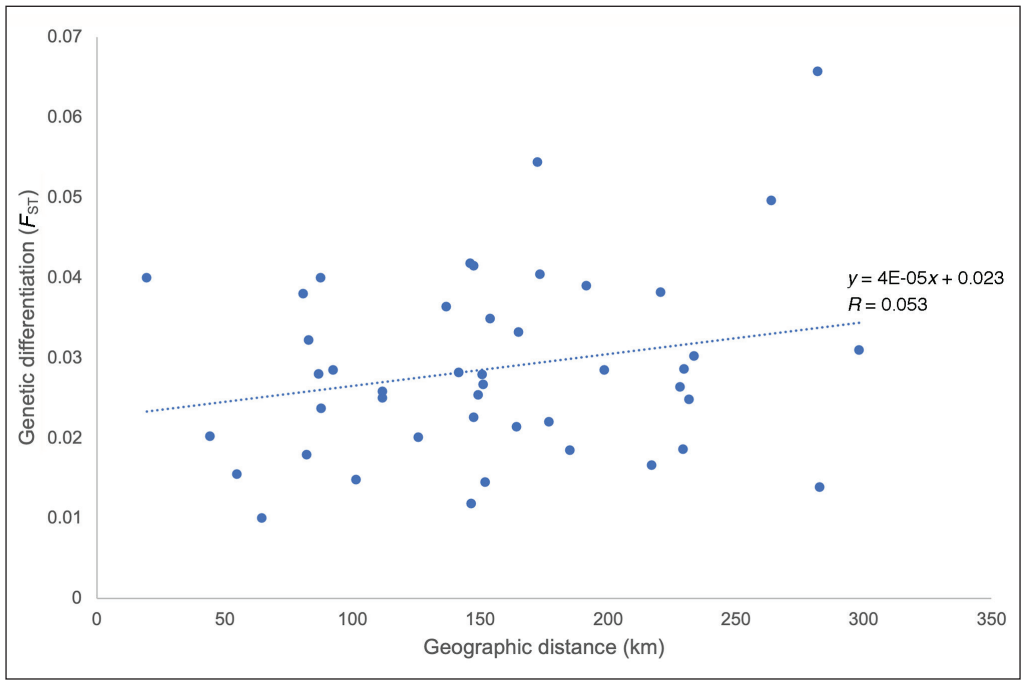

Figure 3. Genetic isolation by distance showing the correlation between geographic distance and genetic differentiation among *Canis latrans* (Coyote) sampled at 10 study sites during July–August 2019–2020 across South Carolina.

Table 2. Pairwise estimates of genetic differentiation (F_{ST}) among *Canis latrans* (Coyote) sampled across 10 study sites in South Carolina during July–August 2019–2020.

	Carolina Sandhills	Fort Jackson	Savannah River Site	Enoree NF	Liberty Hill	Long Cane NF	Davis Land and Timber	Marsh Woodbury WMAs	Webb Groton Westervelt	Bonneau Ferry WMA
Carolina Sandhills	-	-	-	-	-	-	-	-	-	-
Fort Jackson	0.024*	-	-	-	-	-	-	-	-	-
Savannah River Site	0.029*	0.025*	-	-	-	-	-	-	-	-
Enoree NF	0.012	0.028	0.020	-	-	-	-	-	-	-
Liberty Hill	0.010	0.020	0.028	0.018	-	-	-	-	-	-
Long Cane NF	0.026*	0.027	0.026*	0.040	0.021	-	-	-	-	-
Davis Land and Timber	0.017	0.036*	0.029*	0.032	0.035	0.040*	-	-	-	-
Marsh/Woodbury WMAs	0.015	0.023	0.025	0.029	0.015	0.031	0.014	-	-	-
Webb Complex	0.038*	0.028	0.016	0.022	0.019	0.033*	0.042*	0.019	-	-
Bonneau Ferry WMA	0.025	0.041	0.039*	0.030	0.040	0.066*	0.050*	0.038	0.054	-

*Significant with a Bonferroni correction of $P \leq 0.001$ for multiple comparisons

distributions for each value of K showed support for a highest likelihood at K = 1 (Fig. 4). Although K = 2 revealed heterogeneous ancestry within each Coyote study site, K = 8 showed no population structure (Fig. 5). Assessment of the number of

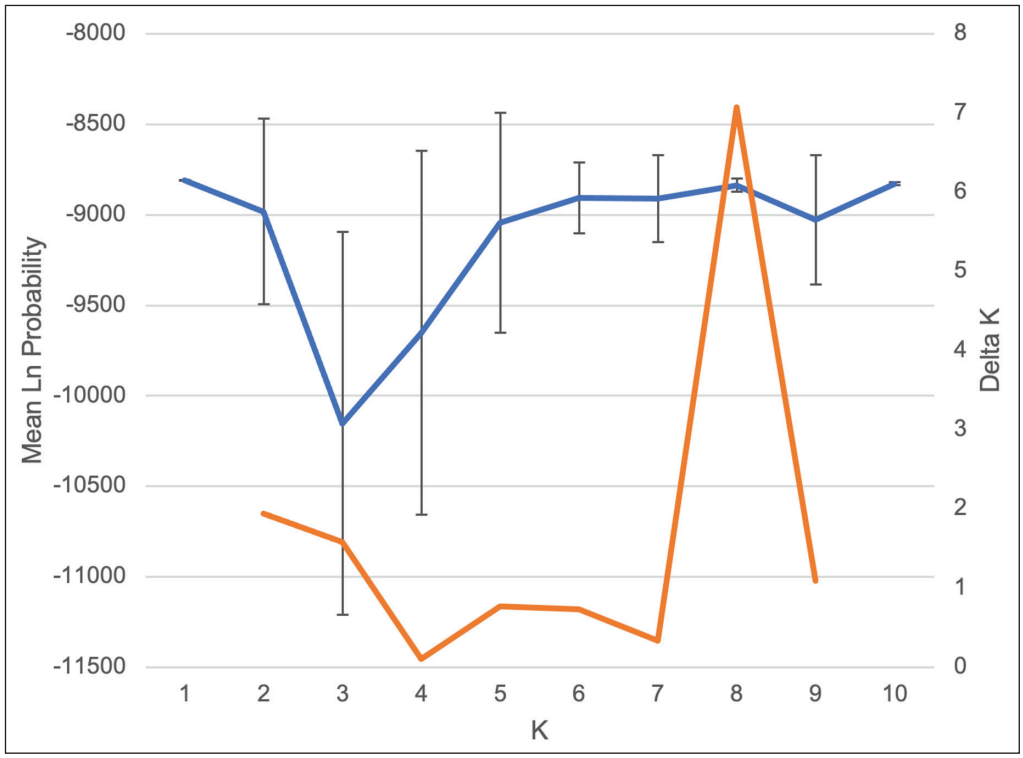

Figure 4. Mean likelihood (Ln) probability (blue line) and Delta K (orange line) estimates for best fit of purported clusters (K) in Sturcture's Bayesian clustering among *Canis latrans* (Coyote) sampled from 10 study sites in South Carolina.

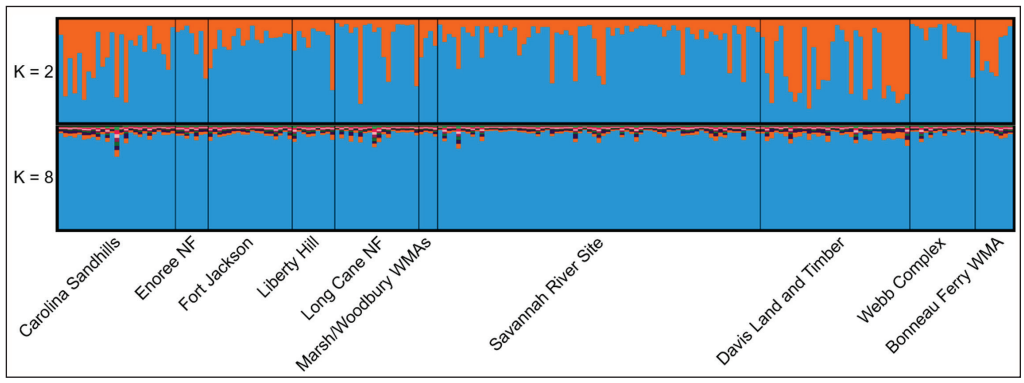

Figure 5. Estimates of ancestry coefficients among *Canis latrans* (Coyote) individuals from 10 study sites sampled across South Carolina during July–August 2019–2020 using the program Structure 2.3.4. Estimates are broken into 2 purported groups (K = 2, top) and 8 purported groups (K = 8, bottom) as indicated by a best fit of Evanno's delta K. Vertical bars represent individual Coyotes, while colors indicate purported ancestry groups.

PCs retained revealed an optimal a-score of 14 PCs (Fig. 6). Similar to our Structure 2.3.4 results, DAPC at 14 retained PCs revealed no apparent differentiation between sample sites, with high levels of overlap between clusters and variation between individuals found at each site (Fig. 7).

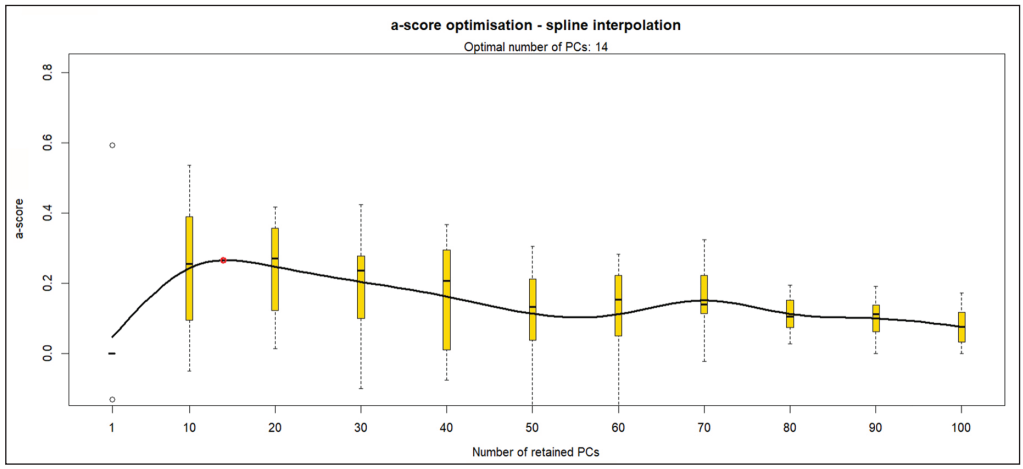

Figure 6. Spline interpolation to optimize the a-score for number of principal components (PCs) retained to differentiation groups of *Canis latrans* (Coyote) across 10 study sites in South Carolina during July–August 2019–2020. Best fit shows an optimal number of 14 PCs retained to be used in downstream discriminant analysis of principal components (DAPC).

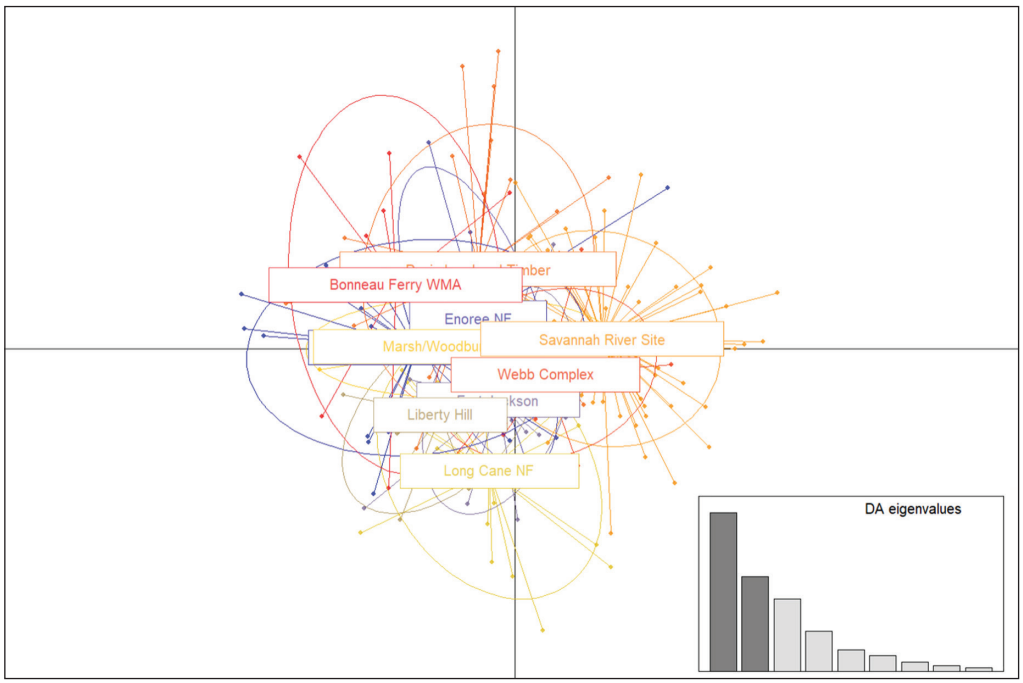

Figure 7. Groups of *Canis latrans* (Coyote) individuals across 10 study sites in South Carolina during July–August 2019–2020, assessed using discriminant analysis of principal components (DAPC).

Discussion

Across sample sites, we observed comparable estimates of allelic richness and heterozygosity to previous studies of eastern Coyotes (Bohling et al. 2017, Heppenheimer et al. 2018b). Coyotes began colonizing South Carolina by the late 1970s and quickly became established across the state by the 1990s (Ruth and Cantrell 2021). Harvest reports of Coyotes killed during the fall and winter deer season in South Carolina showed a steady increase during the 2000s until a peak in harvest around 2014 (C. Ruth, unpubl. data; Ruth and Cantrell 2021). Coyotes have, therefore, experienced rapid population growth across a largely contiguous spatial scale and may have recently stabilized. Population genetic theory stipulates that rapid population growth should mitigate the effects of genetic bottlenecking (Nei et al. 1975), which has been shown in other species including *Odocoileus virginianus* (Zimmermann) (White-Tailed Deer; DeYoung et al. 2003). In the case of eastern Coyotes, previous studies have confirmed low levels of introgression by dog and wolf species (other canid species) along the colonizing front of Coyotes moving eastward (Heppenheimer et al. 2018b; Hinton et al. 2019; Kays et al. 2010; Monzón et al. 2014; vonHoldt et al. 2016a, b; Way and Lynn 2016). Admixture with other canid species likely has contributed to high genetic diversity found in eastern Coyotes. Finally, the mobile nature of Coyotes has undoubtedly aided in high rates of gene flow and contributed to our observations of high heterozygosity and allelic richness across the state. Therefore, a confluence of historical and demographic processes is responsible for the high genetic diversity of Coyotes throughout South Carolina.

Estimates of kinship between individuals showed a majority of Coyotes within our study sites were unrelated to each other, with only a few related individuals, usually first sibling and parent–offspring pairs. Way et al. (2001) observed kinship among packs to consist of unrelated breeding individuals and their offspring in the northeastern US following colonization. Williams et al. (2003) found low levels of relatedness in exploited Coyote populations with a high turnover rate. It should be noted that we sampled during July and August, when pups are becoming precocious and detectable using our sampling methodology and population-wide mortality is relatively low (Harrison et al. 1991, Harrison and Gilbert 1985, Sasmal et al. 2019). Pups begin to disperse during the fall (Harrison et al. 1991), concurrent with the onset of hunting season when a majority of Coyote mortality occurs (Margenau et al. 2023, Van Deelen and Gooselink 2006). The resulting transiency and mortality should provide an influx of new individuals into exploited populations (Kierepka et al. 2017, Kilgo et al. 2017, Williams et al. 2003), meaning that our estimates of relatedness during the summer likely represent local kinship at its highest point of the year. As such, predominately unrelated pairwise relationships between Coyotes at each of our study sites speak to high rates of immigration and emigration and the influence of transient individuals on maintaining gene flow.

Our analyses of genetic structure revealed little evidence of differentiation, even between geographically disparate study sites. Our results did not indicate any genetic structure among ecoregions, unlike past studies of Coyotes in California (Sacks et

al. 2004, 2005). Notably, ecoregions within South Carolina represent no functional impediment to Coyote dispersal and may not present distinctive alternatives during natal-biased dispersal as they are predominately forested with a mosaic of agricultural landscapes intermixed. Instead, Coyotes across our study sites exhibited low genetic differentiation and ancestry grouping approaching K = 1. Previous estimates of population structure in the southeastern US has found genetic panmixia across local, intermediate, and broad scales (Bohling et al. 2017, Damm et al. 2015, Hinton et al. 2019, Kierepka et al. 2017). Even at localized spatial scales, we determined that many individuals are unrelated by descent. Previous attempts to lethally control populations of Coyotes in the southeastern US have shown that Coyotes quickly respond to local-scale removal through immigration (Gulsby et al. 2015, Kierepka et al. 2017, Kilgo et al. 2014). Furthermore, Coyotes in the Southeast have been documented to travel >300 km (Hinton et al. 2012), and 30% of Coyotes can be expected to be transient at any given time (Hinton et al. 2015, Ward et al. 2018). Therefore, Coyote population dynamics likely are strong contributing factors in mitigating genetic structure, even across hundreds of kilometers, and the one-immigrant-per-generation rule indicates that Coyote dispersal maintains a shifting mosaic of gene flow across a broad geographic area (Mills and Allendorf 1996). Our findings indicate that Coyote populations may experience frequent gene flow at a scale larger than geographic boundaries of the state of South Carolina.

However, several lines of evidence in our data speak to a subtle presence of genetic structure among our study sites. Although genetic differentiation was generally low ($F_{ST} < 0.07$), 31% of pairwise comparisons were statistically significant. Additionally, isolation by distance was slightly positive, albeit statistically insignificant. Coyotes have fully colonized South Carolina for several decades (Ruth and Cantrell 2021), and limited genetic differentiation may be occurring as populations stabilize. However, the signal in our data is weak and should be approached with caution, especially as the majority of our findings point to high levels of gene flow across the state.

Our study was limited by several factors that make further assessment of Coyote population genetics across our study sites difficult. First, we did not collect samples from outgroups from either the Coyote endemic range of the western US or other states in the eastern US. A lack of genetic outgroups can impact the ability of genetic structure analyses such as Structure 2.3.4 to delineate between ancestry groups and may have led to our observation of a best fit of K = 1. However, lack of outgroups typically leads to inflated signals of genetic structure, perhaps as seen in our marginal support for K = 2. We also observed low sample sizes across several of our study sites, likely as a result of sparse Coyote densities. Low sample sizes may have reduced our ability to accurately describe genetic diversity and could also impact estimates of genetic differentiation and structure. Finally, although we sampled later in the summer to maximize our chances of collecting scat from Coyote pups while avoiding fall mortality during hunting season and juvenile dispersal, we may not have sufficiently obtained samples from young-of-the-year individuals. By sampling only adult and juvenile Coyotes, we may have underestimated

pairwise kinship and missed important genetic structure among Coyote packs. Future research would benefit from sampling during multiple seasons to obtain a representative sample size for each site, as well as collecting samples from other regions of the Coyote range to contextualize genetic structure among populations.

Highly mobile animals are capable of maintaining genetic connectivity over a broad spatial scale. Rapid range expansion also facilitates gene flow, but colonizing populations typically exhibit evidence of genetic bottlenecking and drift due to sparse distribution of individuals along the colonization front (Welles and Dlugosch 2018). Having recently expanded their range throughout the eastern US, Coyotes have established robust populations across eastern North America. Although past assessment of eastern Coyote genetics has revealed population structure along immigration routes and contact zones (Heppenheimer et al. 2018a, Hinton et al. 2019, Monzón 2014), evidence of genetic bottlenecking has largely been relegated to fragmented populations inside urban areas (Adducci et al. 2020, DeCandia et al. 2019, Henger et al. 2020, Rashleigh et al. 2008). Instead, rural populations of Coyotes have been shown to exhibit high allelic richness and heterozygosity (Bohling et al. 2017, Heppenheimer et al. 2018b, Kierepka et al. 2017). Rapid population expansions can result in increased genetic diversity, and it is likely that high rates of gene flow across broad geographic space have resulted in a genetically diverse and generally homogenous population structure within eastern Coyotes. Our findings corroborate these previous reports while further emphasizing the scale at which Coyote populations may be interacting with each other in the southeastern US.

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